

**F. N. BRIGGS
P. F. KNOWLES**

INTRODUCTION TO PLANT BREEDING

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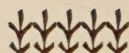
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INTRODUCTION
TO
Plant Breeding

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CONSULTING EDITOR'S STATEMENT

ONE hundred years ago Gregor Mendel quietly conducted the first controlled experiments in heredity which were to become the building blocks of genetics. As crop scientists acquired more knowledge and understanding of the relation of genetics to plants, there developed a subject known as plant breeding. It has been through the unrelenting efforts of plant breeders that American agriculture has attained the high level of proficiency that it enjoys today. As the world's population increases, the demand for food and feed will be unprecedented. Thus, the plant breeder has a major obligation to produce the primary ingredient for an agricultural industry which must achieve a degree of efficiency never before considered possible. Spectacular results have been made through hybrid corn, sorghum and the breaking of wheat yield barriers by the variety Gaines. However, such achievements must be considered as only being the threshold of what will be needed to meet the needs of the future.

The authors of *Introduction to Plant Breeding* have contributed substantially to the science of plant breeding through their own research. They also recognize the importance of training students in plant breeding, both as potential plant breeders and as agricultural scientists in the broadest sense. To this end they have set forth in an orderly and current manner the underlying principles and methods of plant breeding. The student will find the quotations at the beginning of each chapter most significant.

Reinhold is proud to present *Introduction to Plant Breeding* by F. N. Briggs and P. F. Knowles as another of the REINHOLD BOOKS IN AGRICULTURAL SCIENCE, a series dedicated to clearly enunciating the current advances in agriculture and identifying those advances that make this series meaningful.

J. Ritchie Cowan



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INTRODUCTION
TO

Plant Breeding

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PREFACE



This book is designed as a text to be used in an introductory course in plant breeding for advanced undergraduate students. It is assumed that the students will have had courses in general botany, genetics, and other biological sciences and crop production. It is further assumed that most students who use this book will take no further courses in plant breeding, though it is expected that many of them will be in industries depending directly or indirectly on plant breeders. It is hoped, however, that enrollment in a course in plant breeding will encourage some students to choose it as a career.

Every attempt has been made to relate plant breeding to underlying genetic principles. For this reason some genetics has been included, particularly in earlier chapters. As a consequence, it has been necessary to de-emphasize consideration of individual crops. Wheat has been freely used to illustrate breeding principles for self-pollinated crops, and corn for cross-pollinated crops, in both cases because of the abundant information available on these crops. Chapter 6 has been included to illustrate the impact of genetic information on plant breeding.

It is realized that Chapters 5 and 30 will not be used by students who have had courses in statistics. However, the authors have always found

that some students in introductory plant breeding courses have little or no understanding of statistics. The information in these chapters has been used in several laboratory periods devoted to the application of statistics to plant breeding problems.

The senior author spent much of his professional career as a barley and wheat breeder, focusing his efforts strongly on the development of varieties resistant to disease. He used the backcross method extensively, and was widely known as the leading advocate of this method of breeding. His direct involvement in plant breeding was interrupted by administrative responsibilities, first as Chairman of the Agronomy Department and later as Dean of Agriculture. After retirement from the University of California, Dean Briggs, Emeritus, served as a consultant for cotton breeding programs in California. While serving in the latter capacity, he was killed in an automobile accident.

In part because of his professional background, and in part because he had organized and taught for many years an introductory course in plant breeding at the University of California at Davis, Dean Briggs hoped one day to write a textbook on plant breeding. The junior author, who succeeded him as instructor in his course, joined him in this task, and together after his retirement they had finished the book. Following Dean Briggs' death, however, the junior author revised the entire book, at the same time adding a few chapters and most of the illustrations.

The writers are indebted to several of their associates who have reviewed portions of the manuscript. Dr. J. C. Williams, now at the University of Kentucky, made helpful suggestions on chapters dealing with statistics. Dr. R. S. Loomis, University of California at Davis, has read and commented on Chapter 8. Chapter 20 has been revised in part following a review by Dr. R. P. Knowles, Canada Department of Agriculture, Saskatoon, Saskatchewan, Canada. Mr. F. G. Parsons, Secretary-Treasurer, California Crop Improvement Association, made suggestions to improve Chapter 29. In all cases, however, the junior author bears full responsibility for the book in its present form.

The junior author is particularly grateful to his wife, who has typed both the entire manuscript and almost all correspondence relating to publication. In addition she has assisted in proofing the preparation of the book in all its stages.

The authors are indebted to the Literary Executor of the late Sir Ronald A. Fisher, F.R.S., Cambridge, and to Oliver & Boyd, Ltd., Edinburgh, for their permission to reprint Tables Nos. III and IV from their book *Statistical Methods for Research Workers*.

CONTENTS



Preface	ix
1 Perspectives in Plant Breeding	1
Influence on Agricultural Production, 2	
Careers in Plant Breeding, 6	
The Training of Plant Breeders, 6	
Résumé, 7	
References, 7	
2 The Evolution of Cultivated Plants	9
The Neolithic Period, 10	
The Historical Period, 12	
The Modern Period, 13	
The Genetic Basis for the Evolution of Cultivated Plants, 14	
Selection, 18	
Genetic Drift, 20	
Résumé, 20	
References, 20	

3	Plant Introductions	22
	Historical Background, 23	
	Types of Introductions, 25	
	Centers of Diversity, 27	
	Plant Introduction Procedures, 30	
	Preservation of the Germ Plasm, 32	
	Description and Evaluation, 33	
	Résumé, 34	
	References, 34	
4	Mode of Reproduction in Relation to Plant-Breeding Methods	36
	Sexual Reproduction, 37	
	Asexual Reproduction, 42	
	Self-Pollinated Crops, 44	
	Cross-Pollinated Crops, 47	
	Résumé, 50	
	References, 51	
5	Variability in Plants	52
	Scale of Variability, 53	
	Qualitative Variability, 54	
	Quantitative Variability, 56	
	Statistics for a Single Population, 57	
	Comparison of Two Populations for Quantitative Characters, 62	
	Measures of Association, 64	
	Résumé, 68	
	References, 69	
6	Genes and Qualitative Characters	70
	Inheritance of Crop Characters of Economic Importance, 71	
	Measurement of the Value of Genes or Certain Morphological Characteristics, 79	
	Résumé, 82	
	References, 83	
7	Quantitative Inheritance	84
	Terminology of Gene Action, 85	
	Modifying Genes, 90	
	Multiple Factors, 91	
	Geometric Gene Action, 96	
	Analysis by Partitioning, 97	
	Polygenes, 98	

Résumé, 99
References, 100

8 Role of the Environment in Plant Breeding 101

Control and Evaluation of the Environment of a Breeding Nursery,
102
Heritability, 104
Adaptation of Varieties to an Environment, 106
Environmental Modifications of the Genotype, 108
Résumé, 112
References, 113

9 Selection in Self-Pollinated Crops 114

The Pure-Line Theory, 115
Land Variety, 116
Level of Heterozygosity, 117
Mass Selection, 117
Pure-Line Selection, 118
Sources of Variability in Pure Lines, 119
Résumé, 120
References, 121

10 Hybridization and Gene Combinations 122

Factors Affecting Gene Recombinations in F_2 , 123
Generations after F_2 , 127
Hybridization in Cross-Pollinated Crops, 131
The Successful Gene Combination, 131
Résumé, 132
References, 132

11 Breeding Self-Pollinated Crops by Hybridization and Pedigree Selection 133

Objectives, 134
Choice of Parents, 135
Making Crosses, 136
Handling Hybrid Pollinations by the Pedigree Method, 139
Generations of Single-Plant Evaluations (Segregating), 140
Generations of Line Evaluations (Nonsegregating), 143
Modifications of the Pedigree Method, 144
Early Generation Evaluations, 144
Résumé, 145
References, 146

12 Bulk Population Method of Breeding Self-Pollinated Plants	147
Procedures in the Bulk Method, 148	
Role of Natural Selection in Bulk Populations, 150	
Composite Crosses, 154	
Early-Generation Evaluations, 158	
A Comparison of the Pedigree and Bulk Methods, 159	
Résumé, 159	
References, 160	
13 The Backcross Method of Breeding	162
Recurrent Parent and Its Recovery, 164	
Character Under Transfer and Its Recovery, 166	
Examples of Backcross Breeding, 167	
Cross-Pollinated Crops, 170	
Evaluation of the Backcross Method of Breeding, 171	
Résumé, 173	
References, 173	
14 Cross-Pollinated Crops	175
The Gene Pool, 176	
Hardy-Weinberg Law, 177	
Features of Cross-Pollinated Crops, 179	
Résumé, 180	
References, 180	
15 Control of Cross-Pollination	181
Separation of Male and Female Flowers, 182	
Incompatibility, 183	
Male Sterility, 190	
Résumé, 193	
References, 194	
16 Selection in Cross-Pollinated Crops	196
Effects of Selection on One Locus, 197	
Techniques of Mass Selection, 198	
Mass Selection of Corn, 203	
Responses to Selection, 206	
Résumé, 210	
References, 210	

17 Inbreeding and Heterosis	212
Inbreeding, 213	
Heterosis, 216	
Genetic Basis of Inbreeding and Heterosis, 216	
Résumé, 221	
References, 221	
18 Hybrid Varieties	223
Historical Background, 224	
Hybrids from Crosses of Varieties, 225	
Single Crosses, 225	
Double Crosses, 226	
Inbred Lines, 226	
Production of Hybrid Seed, 232	
Hybrid Varieties in Other Crops, 234	
Résumé, 236	
References, 238	
19 Recurrent Selection	240
Recurrent Selection for Phenotype, 242	
Recurrent Selection for General Combining Ability, 244	
Recurrent Selection for Specific Combining Ability, 246	
Reciprocal Recurrent Selection, 246	
Other Crops, 248	
Résumé, 249	
References, 250	
20 Synthetic Varieties	251
Producing a Synthetic Variety, 252	
Predicting the Performance of Synthetic Varieties, 253	
Synthetic Varieties of Corn, 255	
Synthetics of Sunflowers, 256	
Synthetic Varieties of Forage Crops, 256	
Résumé, 263	
References, 263	
21 Autoploidy in Plant Breeding	265
Polyploids, 266	
Induction of Polyploids, 266	
Effects of Autoploidy, 268	
Success with Autoploids in Plant Breeding, 270	

Cytological Behavior of Autoploids, 272
Genetics of Autoploids, 273
Reversion to the Diploid Condition, 276
Breeding Procedures with Autoploids, 277
Haploidy, 278
Résumé, 278
References, 280

22 Allopolyploidy 282

Production of Allopolyploids, 283
Examples of Allopolyploids, 284
Genetics of Allopolyploids, 287
Résumé, 289
References, 290

23 Aneuploids 291

Chromosome Additions, 292
Chromosome Deletions, 298
Chromosome Substitutions, 298
Résumé, 299
References, 300

24 Mutagens and Crop Improvement 301

Mutagenic Agents, 302
Procedures, 305
Effects of Mutagens, 309
Objectives and Achievements in Mutation Breeding, 311
Résumé, 315
References, 315

25 Interspecific Hybridization 317

Reasons for Making Interspecific Hybrids, 318
Overcoming Difficulties in Obtaining F_1 Hybrids, 319
Sterility of F_1 Hybrids, 321
Degeneration in F_2 , 323
Interspecific Hybrids from Parents Differing in Many Genes, 324
Interspecific Hybrids Where the Parents Differ in Genomic Content,
327
Asexual or Long-Lived Perennials, 330
Unexpected Results of Interspecific Hybridization, 331
Résumé, 331
References, 332

26	Interspecific Transfer of Characters	334
	Gene Transfers Between Species with the Same Chromosome Number, 335	
	Gene Transfers Between Species with Different Chromosome Numbers, 336	
	Gene Transfers Between Two Genera, 337	
	Résumé, 339	
	References, 339	
27	Genetics of Resistance to Diseases and Insects	341
	Disease Organisms, 342	
	Insects, 345	
	Genetics of Fungi, 345	
	Genetics of Resistance, 350	
	Gene-Race Relationships, 355	
	Résumé, 357	
	References, 357	
28	Breeding for Resistance to Diseases and Insects	360
	Historical Background, 361	
	Sources of Resistance, 362	
	Managing the Pathogen or Insect, 363	
	Breeding Methods, 364	
	Stability of Resistance, 365	
	Resistance to Stem Rust of Wheat, 366	
	Resistance to the Spotted Alfalfa Aphid (<i>Therioaphis maculata</i>), 370	
	Résumé, 372	
	References, 373	
29	Maintenance and Distribution of Varieties	374
	Historical Background, 375	
	Production of Seed of Varieties of Field Crops, 377	
	Production of Seed of Varieties of Flowers and Vegetables, 381	
	Production of Crops Propagated Asexually, 381	
	Localizing Seed Production, 382	
	Isolation, 383	
	Seed Laws, 384	
	Plant Breeders' Rights, 384	
	Résumé, 385	
	References, 386	

30	Field-Plot Technique and Experimental Design	387
	Field-Plot Technique, 388	
	Experimental Design, 391	
	Résumé, 400	
	References, 401	
	Appendix	403
	Index	409

INTRODUCTION
TO
Plant Breeding

1

PERSPECTIVES IN PLANT BREEDING



Plant breeding is the genetic adjustment of plants to the service of man.

FRANKEL, 1958

PLANT BREEDING is one of the oldest accomplishments of man. It began when he domesticated plants by growing them under controlled conditions and selecting those types that provided a dependable source of food. This early plant breeding, as well as animal breeding, contributed greatly to man's social evolution, for gradually, as the new forms of plants and animals became more productive, groups settled around these sources of supply. As villages and towns developed, the labor for producing these foods became more plentiful and men could find time to innovate arts

and crafts. Thus the domestication of plants and animals marked one of the most important phases in man's progress from a nomadic and highly individualistic life to the organized and cooperative society he enjoys today. Essentially all our food crops as we know them now are the direct results of this primitive husbandry.

This early plant breeding was haphazard and slow. It remained an art, and not a science, until Mendel's laws of inheritance were recognized and applied to plant breeding at the beginning of this century. Despite this transforming influence, plant breeding will always be something of an art, with the breeder trying to mold or create living material to conform to an image or an ideal in his mind. Indeed, this is one of the appeals of plant breeding. As an art, plant breeding is dependent upon a knowledge of the plant itself, its structure, and its responses to its environment.

As a science, plant breeding is heavily dependent on genetic principles. Genetics has explained inheritance, and its laws have given predictability to plant breeding. At first the focus of genetics was on genes with large effects, the genes influencing the qualitative characters such as color, obvious changes in form, and resistance to disease. More recently, genetic studies have inquired into the inheritance of genes with small effects, the genes that determine quantitative characters such as yield, height, and early maturity.

The breeding of plants and animals is a form of evolution, dependent in large part upon the same rules that regulate the evolution of natural species. But there is one important difference—natural selection has been replaced, at least in part, by man's conscious selection.

Influence on Agricultural Production

Productivity. Over the last 40 years there has been a remarkable increase in the productivity of field crops in many countries, much of it

Table 1.1 Average yields in pounds per acre of wheat, corn, oats, soybeans, and tobacco in the United States by ten-year periods from 1915 to 1964.^a

TEN-YEAR PERIOD	WHEAT	CORN	OATS	SOYBEANS	TOBACCO
1915-1924	833	1488	998		788
1925-1934	825	1354	884	805	779
1935-1944	910	1595	977	1085	952
1945-1954	1027	2103	1091	1199	1236
1955-1964	1427	3102	1321	1398	1712

^a From U.S.D.A. Agricultural Statistics, published each year.

due directly to the accomplishments of the plant breeder. Data for five crops in the United States (Table 1.1) will indicate the scale of the increase, varying from 32.4 per cent for oats to 117.3 per cent for tobacco. Similar data could have been produced for other crops. In view of the difference in the value of the increased production and the cost, it would be safe to say that no investment of the American people has returned so high a dividend.

Another example is the increase in wheat production in Mexico achieved by the joint efforts of personnel of the Rockefeller Foundation and Mexican scientists.³ In 1943, when the research program was initiated, Mexico imported half of her wheat. With improved wheat varieties and better knowledge of wheat production, Mexico became self sufficient in 1956 and in 1965 exported a half million tons of wheat. Yields increased from 660 pounds per acre in 1945 to 2340 pounds per acre in 1964.

Plant breeders have striven over the past century to contribute to the improvement and extension of crop production. They have been successful in many different ways, some examples of which follow.

Expansion in the area of production. By changing some characteristic of a crop, its area of production may be extended. For example, the hard red spring wheat industry covering much of the prairies of Canada and the northern Great Plains of the United States stems back to the variety Red Fife. David Fife of Otonabee, Ontario, developed this variety from a single plant found in a lot of winter wheat grown from seed received from a friend in Glasgow, Scotland. The seed had come to Scotland from central Europe.

Earlier maturity will permit a crop to move into a new area. The variety Red Fife was replaced in the early part of this century by Marquis, developed from a cross of Red Fife to Hard Red Calcutta, which was made by Charles Saunders (Fig. 1.1). The variety Marquis matured a few days earlier and extended the hard red spring wheat area even farther. Marquis, of course, has been replaced by other varieties, some of them even earlier.

Drought resistance will aid in the establishment of a crop in some areas. Although most sorghum types have been introduced into the United States only during the past 100 years, they have become extremely important to the agricultural economy of the southwestern Great Plains because of their drought tolerance.

Improved winter hardiness has always meant the extension northward of winter types of all our cereal crops. Flax became a more reliable winter crop in some areas of Texas when a cold-tolerant selection was found in an introduction from Turkey.

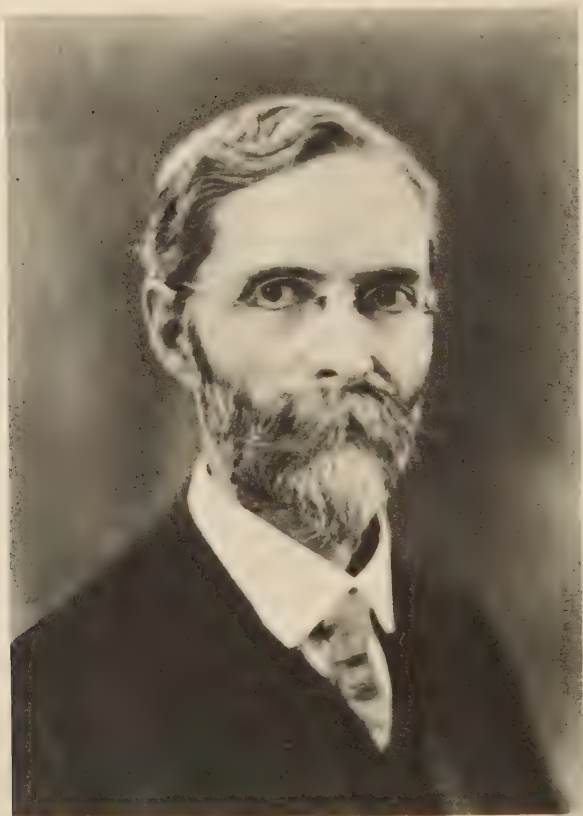


Fig. 1.1 Charles E. Saunders, Dominion Cerealist, 1903 to 1922, breeder of Marquis and other varieties of wheat, for which the British Government honored him with knighthood. (Courtesy W. G. McGregor, Genetics and Plant Breeding Institute, Ottawa, Ontario, Canada.)

Hybrid varieties. The single feature contributing to high yield of many new varieties is their hybrid nature. The most dramatic example is hybrid corn, which was first introduced in significant amounts in 1932 and now makes up about 95 per cent of the acreage of corn in the United States. Hybrid varieties are now available in such crops as sorghum, sunflower, onions, castor beans, and a number of garden flowers and vegetables.

Resistance to disease. Certainly the most significant accomplishment of the wheat breeders during the past 30 years has been the development

of stem-rust resistant varieties. Although the contest is still going on between wheat breeders and disease, the epidemics that destroyed millions of acres have been eliminated. Practically all plant breeders, no matter what crop is involved, have as an objective increased resistance to disease.

Resistance to insects. In a remarkably short period of time, alfalfa varieties have been developed which are able to resist the spotted aphid, a pest that appeared for the first time in the United States in 1954. Alfalfa varieties have also been produced which are resistant to the pea aphid, stem nematode, and the root knot nematode. This built-in resistance is of great importance because it solves the problem of toxic residue in crops that have been sprayed with insecticides.

Quality. New varieties of many of our crops are subjected to exhaustive qualitative tests before they are released for commercial production. Cotton breeders for many years have carefully measured new varieties for fineness, strength, and smoothness of their fibers. They are even more conscious of quality today because cotton must compete with synthetic fibers. A new variety of bread wheat must satisfy a host of standards: As grain, it must have the required bushel weight, color, size, and shape; when milled, it must exceed certain minimum standards in flour yield, flour color, protein content, and protein character; and when baked, it must produce a loaf graded carefully for size, fineness and uniformity of texture, color, and taste. Breeders of fruit and vegetable crops are particularly conscious of quality in their new varieties, not only at the time of harvest, but also throughout processing and shipping.

Adaptability to mechanical harvest. The rapid increase in the importance of sorghum, which has already been mentioned, followed the development of dwarf and double-dwarf types that could be harvested with a combine. Many vegetable crops, including tomatoes, are already harvested by machine; it is imperative that new varieties be adapted to the harvester. More and more, the engineer and plant breeder are working cooperatively.

Other agricultural developments. An improvement in the varieties of crops that are grown is only one of several changes that have made agriculture more productive in the past 100 years. Some other changes are development of new land areas, development and extension of irrigation, increased use of fertilizers, other applications of chemistry to farming, improvements in tillage and cultural practices, and mechanization. This is not the place to discuss the full impact of these developments on agriculture and society, although plant breeding may be involved in each of them.

Breeding, a long-term undertaking. Most new varieties have at least 10 years of work invested in their development, and some have up to 20

years. This means that the returns from plant breeding, great as they often are, cannot be realized in a few years. It means also that the plant breeder must anticipate by several years the needs of agriculture and the rest of society. If he is wrong in his estimations, it will take a long time to recover from his mistake. Occasionally the plant breeder may be wise to work against the recommendations of the market place.

Careers in Plant Breeding

Career opportunities in plant breeding are increasing both in numbers and scope. In part, this is a reflection of shifts in the patterns of plant breeding. One shift, in the United States at least, is an increasing interest by seed companies in the development of varieties, particularly hybrid varieties. At the same time state agricultural experiment stations and the U.S. Dept. of Agriculture are focusing more strongly on the development of germ plasm which can be utilized by other plant breeders; they are also continuing their traditional role of improving plant breeding procedures; and they must remain active in the breeding of crops not receiving attention by commercial companies. Another important development is international in its scope; various organizations are supporting plant breeding programs the world over, realizing that this is an important step in bringing population and food supply into balance. Obviously plant breeding offers careers to persons with different interests and levels of training.

The Training of Plant Breeders

The plant breeder is first of all a biologist. As such, he must have a knowledge of the anatomy and physiology of plants, particularly the plants of direct concern to him. Paramount, however, is a knowledge of genetics, since plant breeding is one field of applied genetics. Actually, a well-trained plant breeder will consider himself both a geneticist and a plant breeder, and will contribute to both fields. Plant breeding may also be considered a form of directed evolution and, for this reason, the plant breeder should be thoroughly familiar with patterns of evolution in natural species.

Not only the crop plant itself, but also other forms of life that will be part of the environment, be they microorganisms or insects, must be considered. So important are these other forms of life to the plant breeder that he is well advised to have some training in plant pathology or entomology.

A plant breeder continually compares populations. These may be progenies of superior selections or new varieties developed to replace others. The branch of mathematics known as statistics has developed

techniques to facilitate these comparisons and to measure the validity of differences. For this reason more and more statistics have been included in the training of professional plant breeders. However, one must always remember that statistics is an important tool of the plant breeder, and not the main objective of his work.

A plant breeder producing varieties of field or horticultural crops will need some knowledge of farming operations, simply because these operations may very well dictate many features of the varieties produced. Farm equipment may work only within certain limits of the structure of a crop plant in terms of such characters as height and branching. Irrigation practices may require specific row spacings. Or the nature of the growing season may put restrictions on the date of flowering and the date of maturity. However, a plant breeder must never let traditional farm practices restrict him completely; he must be alert to the possibilities of simultaneous change in both the plant and its environment that will increase production.

Résumé

It is obvious that the plant breeder will play an important role over the next 100 years in supplying a hungry world with more food and food of higher quality. The trends now under way will continue. There will be increased use of hybrid varieties, perhaps in all crops. The chemical specifications of varieties will be more closely drawn in terms of enzymes, amino acids, fatty acids, carbohydrate characteristics, and cellulose structure.

Plant breeding will be conducted with more precise control of the environment. This will lead to a better understanding of relationships between genes and separate components of the environment. With this understanding will come the simultaneous structuring of varieties and management of the environment to achieve maximum production.

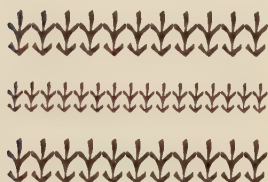
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2

THE EVOLUTION OF CULTIVATED PLANTS



Breeding . . . is evolution directed by the will of man.

VAVILOV, 1935*

ABOUT 50,000 years ago modern man evolved from the prehuman types dating back 500,000 to 1,000,000 years. He sustained himself by hunting and food-gathering but had few implements or weapons to aid him in his struggle for existence. Primitive tools were first developed, perhaps 250,000 years ago, by chipping stone. This period is known as the Paleolithic, or Early Stone Age. No plants or animals had been domesticated, not even the dog.

* N. I. Vavilov. *The Origin, Variation, Immunity and Breeding of Cultivated Plants*. Translated by K. Starr Chester. Copyright 1951. The Ronald Press Company, New York.

The Neolithic Period

The Neolithic or Late Stone Age is identified by the appearance of stone tools produced by grinding. During this period man learned to cook, to make pottery, and to weave wool and plant fibers into cloth. But most important, he domesticated plants and animals. The domestication of plants began at different times in different areas, though the record is only now becoming clear in a few carefully studied locations.

Probably the earliest domestication of plants occurred some 9,000 to 11,000 years ago on the hills above the Tigris River in the area between Iran and northeastern Iraq at the town of Jarmo.³ Here, food-gathering had been intensified, leading to attempts to supplement the supplies of nature. It is not certain whether man first began the cultivation of crops in the exact area where he found them or moved them to an area more convenient for him. In any case, about 7,000 years ago, village farming communities had moved down toward the bottom lands of the Tigris and Euphrates rivers. Farming was so advanced a thousand years later that urban civilizations were developing in southern Mesopotamia. Domestication of animals occurred at about the same time. Summing up the significance of this agricultural revolution, Braidwood³ states:

As man learned to produce food, instead of gathering, hunting or collecting it, and to store it in the grain bin and on the hoof, he was compelled as well as enabled to settle in larger communities. With human energy released for a whole spectrum of new activities, there came the development of specialized nonagricultural crafts. It is no accident that such innovations as the discovery of the basic mechanical principles, weaving, the plow, the wheel and metallurgy soon appeared.*

A study of caves in the Ocampo region of Tamaulipas, Mexico, gives the time scale in crop domestication in one area of the New World (Table 2.1).¹¹ Residues were obtained from different occupational levels and dated with carbon 14. Domestication began about 9,000 years ago, continued for a period of 7,000 years, and then declined. During these 7,000 years there was an increase in the number of varieties of each crop. Corn became steadily more important in the diet. Quality of tools and craftsmanship improved.

About 5,000 years ago in the Old World, and about 3,000 years ago in the New World, essentially all our important food crops had been domesticated, more or less in the form we know them now. A minimum of 1,000 and a maximum of 7,000 years were required for the process. During these years man was close to his crops. He saw, felt and tasted

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the differences among individual plants. His opportunities for selection were much less as he mechanized production, and increased his distance from the plants.

Of the quarter of a million species of higher plants, man has made use of 3,000 species, and 150 have been extensively cultivated. Students of primitive economies feel that the natural curiosity of man, and desperate need when supplies were scarce, led him to evaluate all species.

It appears that the cereal grains were the first crops to be domesticated, at least in the Old World. They were easy to store from one harvest to the next, and simple to process and prepare for food. The largest seeded and most productive species were selected for use. Thus barley, wheat, and millet (probably in that order) became the foundation for the development of the civilizations of southwestern Asia and Europe, rice for southeastern Asia, grain sorghums for Africa, and corn for the western hemisphere. The only other species that provided a possible cereal crop for the Americas was wild rice. Plants producing abundant edible seed such as the legumes probably came next. Vegetable and fiber crops were not far behind. Root, oil, and fruit crops came later. Probably the last plants to be domesticated were drug sources and flowers. Forage crops were not domesticated as a group until the recorded historical period.

Weeds developed during the Neolithic period. Through a process of natural selection some species were modified to occupy the new environment of the fields and about the dwellings. Actually, some crop species may have had a long history as weeds before their domestication; this appears to be the case with both oats and rye. Harlan feels that weedy relatives of crop plants have been important reservoirs of genetic vari-

Table 2.1 Time scale in the first appearance of domesticated plants in the diet of Indians in the Ocampo region of northeastern Mexico.¹¹

ESTIMATED YEARS AGO	CROPS FIRST APPEARING	WAY OF LIVING
9,000	Gourd, pumpkin	Mostly nomadic, collecting wild plants
6,000	Common beans, chili peppers, opuntia, runner beans, and possibly corn	Seminomadic plant collectors
4,300	Squash, <i>Panicum</i> , amaranths, and corn definitely	Seminomadic still
3,800	Cotton	Semisedentary, small villages?
3,400	Sunflower	Villages
1,800	Warty squash, walnut squash, lima beans, <i>Manihot dulcis</i> , tobacco	Circular pyramids, stone buildings

ability and that periodically, through crossing, they have injected some of the variability into the cultivated form.⁸

Some of the species changes¹⁴ that are associated with domestication are: (1) larger plant types, (2) larger seeds, (3) more rapid and uniform germination, (4) nondehiscent fruits and a tougher rachis in cereal crops, (5) unusual colors of seeds and fruits, (6) loss of defensive structures such as hairs, spines, and thorns, (7) double instead of single flowers in ornamentals, (8) seedless fruits in horticultural plants, (9) improvement in palatability of foods and feeds, (10) self-pollination, and (11) annual habit.

The Historical Period

The historical period, beginning between 2,500 and 3,000 years ago, has not contributed many new crops, and certainly very few crops that man uses directly. Mostly, its contribution has been forage crops, fruits, ornamentals, or crops used for industrial purposes. The discovery of the New World some 500 years ago was one of the most significant events during this period.

Both oats and rye were products of this period. Neither was found as a crop in ancient remains, nor mentioned in ancient writings, but both were present at the beginning of the Christian Era. Both probably developed and persisted as successful weeds in fields of wheat and barley, and moved with these crops to many different environments.

Many domesticated species of *Rubus*, such as the dewberry and the blackberry, were developed during the nineteenth century in the eastern part of the United States. One important factor, apparently, in their emergence was the rapid expansion of settlements. As the forests were cleared, *Rubus* species became more prominent along fence rows and margins of the forest. Greater opportunities existed for crossing diverse types and for developing more desirable combinations of characteristics.

More significant than all these developments was the domestication of forage crops during this period. These would include primarily the perennial grasses and legumes, not the cereals or root crops used as human food. The dependence of the agricultural economies of northern Europe and colonies in North America upon pastures and hay crops undoubtedly was a factor in the domestication of forage species. For example, timothy (*Phleum pratense*), a native wild forage species in northern Europe and Asia, was first cultivated in the United States during colonial times.

An important industrial crop which was domesticated within the past century is rubber (*Hevea brasiliensis*).¹⁵ Most rubber trees of Malaya

stem back to 22 seedlings sent to the Singapore Botanic Gardens in 1877. These seedlings were grown from seeds collected in 1876 from wild plants growing in the middle Amazon River basin.

The discovery of the New World had an impact upon the development of crop plants, quite apart from the interchange of crops already domesticated. As mentioned above, the introduction of timothy to cultivation followed its migration to the Americas. The great variability of the pumpkins in Asia Minor led Vavilov²⁰ to believe that they were native to that area, though they actually originated in the New World.

The shift of plants to new environments following the discovery of the New World indicates that a crop may actually prosper more when it is removed from its area of origin. For example, rubber had not been successfully developed commercially in the Amazon River basin where it originated, primarily because of damage by a fungus, *Dothidella ulei*.

But because this fungus did not exist in Malaya and Indonesia, rubber became well established as a crop. Sunflower (*Helianthus annuus*), which was developed in North America, has been severely attacked by many indigenous insects, particularly by the sunflower moth (*Homoeosoma electellum*). This crop's greatest development has been in Russia and Argentina. Both areas are far removed from this insect pest.

The Modern Period

Modern man is in a sense continuing the pattern of the historical period. He is still strongly focusing attention on the domestication of forage crops,⁴ but has given little attention to the domestication of additional species for food. The distinctive features of the modern period are a reflection of man's need for valuable industrial products and pharmaceuticals. In other words, man is inquiring into the domestication of plants that contain specific chemical compounds.

Wild species of *Dioscorea*, a tropical yam, are rich in cortisone; this has prompted efforts by research agencies to attempt to domesticate such species. A vast host of other wild species are being studied as sources of drugs, and species showing promise will follow the development pattern of *Dioscorea*. *Rauwolfia serpentina*, a plant used for centuries in India to treat fevers and snakebite, has been considered for cultivation.

Modern plant breeders are not so interested in the domestication of species as they are in the domestication of genes. Genes of greatest interest in wild species have been those that impart disease resistance. For example, the rust resistance of sunflower varieties traces back to a wild species of northern Texas. Suneson obtained earliness in oats from the wild species (*Avena fatua*) growing in California.¹⁹

The Genetic Basis for the Evolution of Cultivated Plants

If compared from the genetic point of view, plant breeding and the evolution of both natural and cultivated plants have many features in common. All require genetic variability, all require some form of selection, and all require a degree or form of isolation to preserve the characteristics of emergent types. Clausen* suggested four chief methods of transforming cultivated plants from their wild progenitors:

1. Mendelian variation.
2. Interspecific hybridization, including introgression.
3. Autopolyploidy.
4. Allopolyploidy.

It is recognized that these four types of evolution do not exhaust the methods by which plants may be molded to suit the needs of man, nor are they mutually exclusive. Hutchinson has also summarized the genetic bases of crop evolution.⁹

Mendelian variation. A crop plant can be developed within the confines of a species by the accumulation and utilization of gene mutations, a method dependent on Mendelian principles. Under natural conditions most gene mutations produce plants with high mortality, so they disappear or persist at very low frequencies. Under cultivation mutant types have been favored in some cases because they appealed to the curiosity of the primitive farmer or, in other cases, because they were better adapted to the environment provided by man. In Turkey, Harlan observed a great deal of variability in several New World crops, including the cucurbits, corn, tobacco, red pepper, tomato, and sunflower.⁷ He felt that the following factors preserved this variability: (1) A favorable climate; (2) subsistence agriculture, with no rigid market requirements; (3) primitive or nonmechanized husbandry, with planting and harvesting being a long drawn-out affair; (4) extensive culture, with large populations being grown; and (5) historical continuity, with agrarian populations remaining in one location for long periods of time.

Many crops originated by virtue of Mendelian variation, among them corn, sugar beets, flax, soybeans, and various species of other beans. The development of barley (*Hordeum vulgare*) from its wild relative (*H. spontaneum*) will illustrate some of the steps in domestication on this basis.^{10,17} *H. spontaneum* is widespread in southwest Asia, so it is no sur-

* We are indebted to the late Professor R. E. Clausen for the loan of the manuscript of his presidential address on, "The evolution of cultivated plants," delivered at a meeting of the Pacific Division of the American Association for the Advancement of Science in 1956.

prise to find that the barley remains from about 7,000 B.C. at Jarmo in Iraqi Kurdistan resemble the wild species. Significantly, however, the only difference is that the rachis of the spike of the remains appears to be tougher than the one in the wild species, and less likely to shatter seed before harvest. Six-row types came later, and were found in remains from the Nile Valley and Mesopotamian Plain from 5,000 to 4,000 B.C. Natural and artificial selection acted powerfully on other mutations of barley, making it a staple food crop of all Old World agrarian civilizations and an important feed crop at a later date. From southwest Asia barley moved to tropical Africa and to arctic Europe, and from sea level to upper mountain limits of agriculture. A vast array of morphological variability appeared, including the following: naked kernels and kernels of different colors; very dense heads; awns without barbs or modified into hoods; and kernels with a wide array of qualities for making malt. It is clear that Mendelian variation has been a potent force in the domestication of this crop.

Interspecific hybridization. Distantly related species appear to have contributed little to the development of cultivated species through hybridization, except through introgression or when accompanied by allopolyploidy. The reasons are obvious: such species cross with difficulty; the hybrid is usually sterile; and segregation products are often inferior to either parent.

Where species are less distantly related, their hybridization may yield types valuable to agriculture. The best example of this is provided by the garden strawberry (*Fragaria x ananassa*).¹⁸ Actually the first attempts to domesticate the strawberry were made in Europe with the wild species *F. vesca*, *F. viridis* and *F. moschata*. In all these species an attempt was made to exploit Mendelian variation, but without success. Likewise, the eastern American species *F. virginiana* showed little promise when introduced into European gardens in the early seventeenth century. However, when the Chilean or Pacific Coast wild species *F. chiloensis* was brought to Europe, it was crossed by English gardeners to *F. virginiana*, probably during the period 1714 to 1759. From this cross, superior segregation products were obtained, which were developed into the modern strawberry, *F. x ananassa*. Like its parents, *F. x ananassa* has 56 chromosomes. The vigorous growth and the large size of the western American species were combined with the superior fruitfulness, the quality of flesh, and the flavor of the eastern species (Fig. 2.1).

Where species are very closely related, so close that they might properly be called related subspecies, then their hybridization would appear to have influenced crop evolution a great deal. Dent corn is an example of a type that evolved in this way.^{2,13} With the expansion of settlements



Fig. 2.1 Wild species of strawberry, which were crossed to give rise to the cultivated species. Left: *Fragaria virginiana*; right: *F. chiloensis* with one fruit of cultivated strawberry, *F. x ananassa*, included for contrast. (Courtesy Dr. R. S. Bringham, University of California, Davis.)

through the present U.S. Corn Belt during the nineteenth century, settlers brought with them the corn varieties of the eastern states, mostly flint types with long ears, straight rows, and big kernels, varieties that had probably originated somewhere in Guatemala. In the Midwest these varieties met the local types carried over from the agriculture of the Indians. The local varieties, which had moved northward from the central Mexican plateau since prehistoric times, had a high row number and conspicuous denting. The marriage of these two types apparently occurred in many places, and gave many successful selections—or gene recombinations.

Where asexual reproduction is possible by using cuttings, buds, bulbs, etc., interspecific hybridization permits the development of useful types, even if they are sterile. Such types are confined mostly to ornamental or horticultural crops.

Introgression. This method involves the transfer of a small amount of the germ plasm from one species to another.¹ It follows hybridization of two species and backcrossing of the recombination products over several generations to one parental species. This may result in the transfer of certain features of one species to another without impairing the latter's taxonomic integrity.

There is good evidence that corn has borrowed characteristics from its

wild relatives, teosinte and *Tripsacum*, through a system of introgression, and Mangelsdorf believes that it is doubtful if any variety of maize is free from these characteristics.¹³ Where teosinte grows abundantly in and around maize fields in Mexico and Central America, contamination of maize with teosinte characteristics has been obvious. Knobs on chromosomes of maize appear to have come from *Tripsacum*.

Sorghum in areas of Africa, where it was domesticated, appears to have absorbed some characteristics from the closely related wild weedy types, and vice versa.⁶ This means that in any particular area, the wild and domestic types, though distinct, will have some characteristics in common. As expected, obvious hybrids are frequently seen.

Autoploidy. Any increase in the number of sets of chromosomes in a species is termed autoploidy, or autopolyploidy. The basic number is termed a *genome*, and is symbolized by x . Sometimes n is used in place of x , but more often $2n$ is used to symbolize the chromosome number of the sporophytic or somatic tissue, and n the chromosome number of the microspores, megaspores, and gametophytic tissue. Thus, an alfalfa plant, an autotetraploid, may be symbolized as $2n = 4x = 32$. A diploid ($2x$) species has two genomes.



Fig. 2.2 Thompson Seedless grapes: normal diploid ($2n = 38$) below and tetraploid ($2n = 76$) above. (Courtesy Dr. H. P. Olmo, University of California, Davis.)

Autoploid plants have three or more similar genomes. Almost invariably, compared to the diploid, they have larger cells and cell nuclei, and frequently have larger plant parts. Where the larger plant part is edible or ornamental, autoploids have been of value. Examples would be grapes (Fig. 2.2), apples, pears, potatoes, and many garden flowers.

Almost without exception, autoploids are reduced in fertility, and the triploid often produces no seed. As a consequence, autoploidy has yielded very few crop plants that are grown for their seed. On the other hand, considering perennial fruit or ornamental crops, seedlessness may be an advantage. An example is the banana of commerce, a triploid.

Allopolyploidy. Allopolyploidy, or allopolyploidy, occurs when (1) two species with different genomes cross to produce a hybrid, and (2) the chromosome number is doubled. Usually the hybrid is sterile because its two genomes are so divergent that they fail to pair. Doubling of the chromosomes restores diploidy and usually restores fertility.

A large proportion of cultivated crops are allopolyploids, perhaps as many as 50 per cent. Prominent among these are wheat, cotton, tobacco, and many of the forage grasses.

Domesticated species of cotton (*Gossypium*) fall into three groups. One group, consisting of a number of diploid species, in the Old World has 13 pairs of chromosomes; these species have been given the genomic formula AA. A second diploid group, also with 13 pairs of smaller chromosomes, is a native of the New World; these species have the genomes DD. The third group, also found in the New World, has 26 pairs of chromosomes, with 13 pairs slightly larger than the other 13 pairs. It has been assumed that at some time in the past the Old World and New World diploid species crossed to give an F_1 with the genomic formula AD. Doubling of the chromosomes in the F_1 then occurred, giving an allotetraploid with 26 pairs (AADD). Careful studies of cotton hybrids indicate that the diploid species actually involved in the production of the allotetraploid were *Gossypium herbaceum*, a species of Africa and western and central Asia, and *G. raimondii*, a wild Peruvian species.

Selection

The products arising from the genetic forces described above are numerous. Sorting out the superior types is accomplished by both natural and human selection. Mangelsdorf concluded that selection by man was not very important in the development of corn until fairly recently.^{12,13} His conclusion was based on a study of the evolution of corn through 3,000 years, as shown by the remains discovered in Bat Cave in New Mexico. This period is believed to have extended from about 2500 B.C.

to 500 A.D. Corn remains show an increase in average size of ear and kernel, but the small primitive corn present at the beginning had not become extinct at the end (Fig. 2.3), as it surely would have been with rigorous selection in favor of large ears. Some 500 to 1,000 years later, however, when America was discovered, the American Indian was cultivating corn that approached present-day corn in size of ear and kernel.



Fig. 2.3 The largest maize cobs (one-half natural size) found in each of six successive strata in the Bat Cave material. Although there has been a progressive increase in maximum size from stratum to stratum, the type found in the first stratum has persisted throughout the series. (Courtesy of Professor Paul C. Mangelsdorf, Harvard University. From *J. Heredity* 40 (1949) 39-43.)

Selection by man is now much more important than natural selection, though natural selection has by no means been eliminated. Selection is the most difficult part of plant breeding. It is relatively easy to develop variability; it can be done by introducing new types, through hybridization within or between species, through mutagens, and by changing chromosome number and structure. The task requiring time, effort, and all the plant breeder's skill is the identification of the plant or group of plants with the most desirable combination of genes and chromosomes.

Genetic Drift

A phenomenon only recently recognized, which in part shapes the pattern of evolution, is genetic drift. As the term implies, it is a drift in gene frequencies associated with small numbers and random sampling. When populations of crop plants are cultivated as small populations, perhaps with a few plants chosen to supply seed stocks for the next year, there is a good chance that populations raised by different farmers may drift apart without natural or human selection being involved. It has been noted in Mexico that adjacent valleys with similar environments may have quite different varieties of corn.¹²

Résumé

It is humbling to the plant breeder to realize that the greatest accomplishments in plant breeding were made before the dawn of recorded history. At the same time it should be flattering to know that the accomplishments of those early primitive plant breeders were basic to the development of civilization.

Consciously or unconsciously, man directed the evolution of his crops along several routes, just as nature guided the development of natural species and varieties. In some cases, man exploited the spontaneous gene mutations occurring in the species of interest to him. In other cases he hybridized species, and selected among the recombination products of the genetic material. Finally, he took advantage of the different combinations of chromosome sets that nature offered to him. Like nature, and often aided by nature, he selected severely among his developing crops, molding them into something of greater use to him.

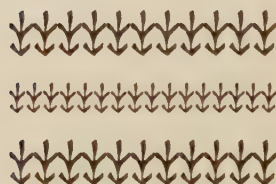
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3

PLANT INTRODUCTIONS



There is in fact an increasing and pressing need for introductions of new plant material to meet the requirements of an ever-increasing world population, both human and animal.

WHYTE, 1958

THE MOVEMENT of economic plants from place to place has been one of the most important features of the development of world agriculture. Originally this must have been quite haphazard and incidental to man's wandering, although his natural curiosity would have prompted him to collect seeds or plants and bring them home for trial. The distances that seed traveled were directly related to the span of the trade routes and the movement of invading armies. Perhaps the greatest landmark in the

history of plant introductions was the discovery of the western hemisphere. The exchanges of crops that followed this event profoundly affected the agriculture of both hemispheres.

Historical Background

Introductions during the colonial period. On his return voyage to the West Indies in 1493, Columbus brought seeds of all the crops common in Spain. Many of these were immediately successful, ultimately finding their way to the continent. Later, colonists to other areas repeated the process. The emphasis placed on introducing plants of all sorts is well illustrated by a list of those sent with the Endicott expedition in 1628, which included:

Wheate, Rye, barley, oates, a hhed of ech, in the eare, benes, pease, stones of all sorts of fruites, as peaches, plumes, filberts, cherries, peare, aple, quince kernells, pomegranats, Woad seed, Saffron heads, liquorice seed. . . *

Introductions during the post-colonial period. The settlement of the Great Plains area and the western and southwestern parts of the United States, often by immigrants, brought many more introductions. The Mennonites, who settled principally in Kansas during 1870 to 1880, brought with them the wheat that they had grown in Russia. This wheat, which became known as Turkey, established the hard red winter wheat area, one of the greatest wheat-producing regions of the world (Fig. 3.1).

Another example is provided by the introduction of alfalfa into the western United States. Introduced into South America by the Spanish explorers early in the sixteenth century, it soon became distributed over Chile and Peru. Many attempts to establish it along the east coast of the United States met with limited success. The first real success with alfalfa followed its introduction from Chile into California in 1851. In that state it produced remarkably good crops from the beginning. From California it gradually spread eastward.

The rather diverse experiences related above illustrate two points of interest to the plant breeder: (1) that there must be some similarity in soil and climate between the region of origin and a new area of production; and (2) that an introduced crop will have a greater chance of success if it has a considerable amount of adaptability, permitting it to fit into a wide variety of environments. Turkey wheat was adaptable because, considering the large number of introductions that had been made by the migrating settlers, it was a mixture of different types. Alfalfa was adaptable, partly because it was a mixture of different types and partly

* From *The Beginnings of Agriculture in America* by Lyman Carrier. Copyright 1923, McGraw-Hill Book Company. Used by permission.



Fig. 3.1 Monument commemorating the introduction of Turkey wheat to Newton, Kansas, where one of the first plantings of this variety was made in the United States. (Courtesy of the Newton Chamber of Commerce.)

because it was cross-pollinated. These points are given more detailed consideration in later chapters.

Organized plant introductions. Ryerson has described the development of an organized program of plant introduction in the United States.¹³ The first appropriation for agriculture by Congress, and the beginnings of the U.S. Dept. of Agriculture, occurred in 1839. Largely through the influence of Henry L. Ellsworth, commissioner of the Patent Office of the State Department, an appropriation of \$1,000 was obtained for the collection and introduction of seeds and plants and agricultural information. In his report of 1843, Ellsworth mentioned that 12,000 packets of

seeds were distributed, and recommended that the diplomatic corps be used to expand and systematize plant and seed collections in other countries.

Plant introduction in the United States was given new emphasis in 1862 by (1) the establishment of the landgrant colleges by the Morrill Act, and (2) the organization of the Department of Agriculture as a separate unit. With the landgrant colleges came the development of agricultural experiment stations in the separate states, and these provided a mechanism for determining the local adaptation of varieties introduced from other areas. Among the several functions of the new Department of Agriculture was the collection, testing, and distribution of seeds and plants. Plant materials were soon being exchanged nationally and internationally on a scale previously unknown. For the most part, however, these exchanges depended on personal arrangements among individuals.

Stability and direction to this activity came with the establishment within the U.S. Dept. of Agriculture of a separate organization to handle introductions. In 1896, N. E. Hansen was sent to Russia by the Secretary of Agriculture in search of cold-resistant fruits and cereals for the Great Plains area. Hansen proved such a highly successful collector that the "Section of Foreign Seed and Plant Introduction" had to be created in 1898 to handle the quantities of plant materials he collected. Mark Carleton, the first plant explorer of the new agency, was sent to Russia to collect wheat. Among the varieties he introduced were the ones that formed the basis of the durum wheat industry of the northern plains area.

Soybeans were introduced in large numbers from the Orient after the turn of the century by explorers from the Department of Agriculture, and selections from these provided the basis for the increase in area of this crop from 50,000 acres in 1907 to 4,000,000 acres in 1932; by 1965 the area had grown to approximately 34,500,000 acres. One recent introduction that has become successfully established is safflower. Work in Nebraska demonstrated both that the crop was adapted to the western part of the northern Great Plains, and that N-852, a variety introduced from the Sudan, would yield enough oil to make it attractive to the oil-seed processor. However, the successful establishment of the crop on a large acreage followed the introduction of safflower on a commercial scale to California in 1950.

Types of Introductions

Every country with an organized agriculture will bring in a wide variety of plant material each year. Specific needs of agriculture will dictate the

instructions to plant explorers in terms of areas to be visited and materials to be sought. These materials may be grouped as follows: (1) new crops; (2) new varieties of established crops; and (3) new characteristics for established crops.

New crops. The term "new crop" is used here to mean any crop brought into an area or a country for the first time. Several factors encourage an interest in such new crops. Most important of these is the market. In the United States there has been a market for 250 to 300 million pounds of castor oil for several years; this has stimulated research and developmental work on castor beans. In Canada the acreage of rape developed from almost none in 1950 to 629,000 in 1957 because large amounts of vegetable oil were imported each year. There is a market in the United States at present for (1) vegetable oils for specific industrial purposes, (2) a cheap source of fiber for paper manufacture, and (3) a cheap source of protein.²⁰ This has stimulated a search for and an evaluation of plants yielding such products from many parts of the world.

Another important factor stimulating interest in an introduced crop is vacant land. The increased interest in soybeans in the U.S. Corn Belt was influenced strongly by the replacement of mules and horses by tractors; this released several million acres formerly devoted to the growing of oats for feed. Acreage allotments for cotton in 1950 forced many farmers in California to turn to alternative crops, among them safflower and castor beans.

New varieties of established crops. Improved varieties of a vast array of horticultural and field crops are moving from countries with advanced breeding programs to other countries all around the world. Without question this has resulted in more and higher quality food for a vast number of people. Countries with an advanced agriculture rarely introduce a variety that is successful in the form that it is received. The reason is that their breeding programs have already produced well-adapted varieties. It is obvious, of course, that countries having similar climatic and soil conditions can sometimes exchange varieties successfully. The state of California has a long history of introducing successful wheat varieties from Australia, and recently has introduced wheat varieties resistant to stripe rust from Mexico.

New characteristics for established crops. Most often, introductions are made because of some characteristic they possess. It may be resistance to disease or insects, earliness, a color, or improved quality. Sometimes the search for such characteristics may extend to related wild species. In other words, the interest is in a gene or a group of genes, and not in the entire genotype. This has led to the development of what has been termed "gene

reservoirs" in many countries. The value of gene reservoirs is becoming more apparent as the genes are being lost in their areas of origin.

Centers of Diversity

Attempts to build up reservoirs of variability in our crop plants sooner or later focus attention on natural reservoirs of such variability. The more that is known about the geographic location, the range, and the pattern of the variability, the more intelligently can a plant explorer sample it.

The work of Russian plant explorers, and particularly that of Vavilov,¹⁷ during the early part of this century has shown that certain areas of the world are centers of much of the diversity in crop plants. Vavilov identified eight centers (Fig. 3.2), some of which were subdivided. These were: I, Chinese; II, Indian; IIa, Indo-Malayan; III, Central Asiatic; IV, Near-Eastern; V, Mediterranean; VI, Abyssinian; VII, South Mexican and Central American; VIII, South American (Peruvian-Ecuadorian-Bolivian); VIIIA, Chiloe; and VIIIB, Brazilian-Paraguayan.

These centers are separated by deserts, mountain ranges, or water. In other words, they have been centers for the development of independent agricultural civilizations, isolated from other civilizations. The total area of these centers makes up about one-fortieth of the land area of the world, if mountainous or desert areas within the centers are excluded. Of the crop plants that Vavilov associated with these centers, over 500 belong to the Old World, and approximately 100 to the New World. Within the Old World, southern Asia was the source of over 400 crop plants.

Some plants do not originate from these centers. Among these are: the date palm, with an unknown origin; sunflowers, from the Great Plains area of North America; the Macadamia nut, from Australia; and several forage species, many of them Eurasian in origin but north of Vavilov's centers.⁹

Often one species or one genus was identified with one center, but sometimes it would be found in two or more. Where present in more than one center, the types in each were usually distinctive. Vavilov recognized *primary centers*, where maximum diversity occurred, and where he felt that the crop had been first domesticated. *Secondary centers* developed from types that had migrated from a primary center. For example, the primary center for the origin of maize is in Mexico, but a secondary center for waxy types has developed in China.

Centers of origin. Vavilov considered these centers of diversity to be centers of origin. His feeling was that diversity was primarily a function



Fig. 3.2 Centers of origin of crop plants. (Adapted from N. I. Vavilov. *The Origin, Variation, Immunity and Breeding of Cultivated Plants*. Translated by K. Starr Chester. Copyright 1951. The Ronald Press Company, New York.)

of mutations and that mutations over a long period of time would accumulate, particularly if they were preserved by deliberate selection.

Centers of recombination. Harlan⁸ and Anderson¹ question the association of diversity with center of origin. Their observations would indicate that the diversity has characteristics of hybrid populations from crosses of diverse types. They feel that the centers are meeting places of these diverse types and that they actually may be recent in origin. Diversity, according to their view, is not so much a function of mutation as it is of recombination.

Migrations with civilizations. Darlington⁴ and Burkill² believe that shifts and developments of civilizations strongly influenced variability in crops. Shifts in civilizations brought migrations of crops, with changed selection pressures and opportunities for crosses with related types. As a consequence, variability was increased. Furthermore, as civilizations developed they improved their agriculture to the extent that they made planned introductions and practiced selection for particular types.

Features of centers of diversity. The pattern of variability in the centers of diversity is incompletely understood, and probably varies with each crop. Vavilov felt that the maximum amount of variability and the maximum concentration of dominant genes were in the center of the area of distribution and decreased toward the periphery, where recessive genes expressed themselves either as a result of strong selection pressure, isolation, or inbreeding. He noted that exceptional types were often found on the margins, as, for example, waxy corn and large-seeded hull-less oats in China. From the Himalayas to the Mediterranean he found a progressive increase in the size of fruits and seeds. Hartley and Williams point out that most of the successful meadow grasses of Europe, including species of *Poa*, *Festuca*, *Lolium* and *Dactylis*, were probably derived from woodlands and forest margins, not from steppes or prairie areas.⁹

Vavilov found that resistance to disease might be localized in certain areas, as resistance to Phylloxera and mildew in American grapes. Abyssinia was the only source of high levels of resistance to yellow dwarf of barley.¹⁴ A certain characteristic was sometimes common to several crops in one area; for example, earliness in introductions of wheat, barley, lentils, and fenugreek from Yemen.

Harlan noted microcenters of diversity, where the evolution of different types was proceeding at a more rapid rate than in other areas.⁷

Law of homologous series. Vavilov's exhaustive studies impressed upon him the existence of parallelisms in variability among related species, a phenomenon noted earlier by others. Vavilov called it the "law of homologous series in heritable variation." Among cotton species, in-

cluding Indian cotton (*Gossypium arboreum*), Asiatic cotton (*G. herbaceum*), and American cottons (*G. hirsutum* and *G. barbadense*), there were similarities in fiber color (white, brown, and green), degree of hairiness, color of seed, type of branching, form of leaves, color of stems, and opening of bolls. The importance of this to the plant explorer is that he can to some extent predict the existence of forms not yet described. For example, rye (*Secale cereale*) had been considered much less variable than wheat (*Triticum aestivum*), but a thorough study of the introductions from southern Asia showed close parallelisms in the variability of 28 characters, even though these two species belong to different genera.

Plant Introduction Procedures

Plant introduction has become such an important aspect of plant breeding endeavors that many nations have developed a staff devoted to this subject alone. The Food and Agriculture Organization of the United Nations acts as a clearinghouse for the plant explorations of the separate nations.¹⁹ Because of its scope it has been necessary to establish some system for its operations. The remarks below are based mostly on the system used in the United States.^{10,11}

Communication. Correspondence was responsible for many successful introductions in the past, and today a large volume of plant material is moving over the world as a result of written requests. It is not difficult to get in this way materials that are in botanic gardens, experimental stations, or commercial nurseries, but it is difficult to obtain a wide variety of types from local areas. Wild species are difficult to obtain also.

Organized explorations are usually sponsored by governmental agencies, but private organizations have also sent them out. Usually, objectives are rather specific; otherwise there may be needless repetition or scattering of effort. The purpose may be limited in scope, like that of an expedition sent to Abyssinia to look for wheat varieties carrying resistance to race 15B of stem rust. Where limited amounts of germ plasm of a crop are available, the plant explorer may search a wide area for as much variability as possible. In some cases a small area is explored in great detail for the variability present in all crops.

Where to search. The choice of the area to explore is often difficult to make. From what has been said to this point, an explorer would be wise to choose an area with a climate similar to his own, located in a center of diversity. As a general rule this would be true, particularly where the maximum amount of variability, rather than specific types, was desired. Vavilov noted that certain crops, such as wheat or barley, would usually succeed only when the climate in Russia corresponded with the climate in the country of origin. But he noted also that certain cosmopolitan plants would succeed in a variety of climates. Conspicuous

among these is the potato, which is tropical in origin. A list of these adaptable plants includes many ornamentals, also of tropical or subtropical origin, such as petunias, snapdragons, lobelia, nasturtium, asters, and stocks, which have been successful over a wide range of latitude and climate. As Vavilov says, "To speak with certainty of the suitability of a species or a variety to new conditions, it must be subjected to direct testing." *

What to collect. Most introductions are obtained as seeds, and these present no particular problems in shipment. Vegetative materials, except in the case of dried bulbs or corms, require special packing and rapid transport. Even greater care and speed is required in the shipment of pollen. Introductions of legumes have often been made without the indigenous nodule bacteria, so techniques have been developed for their introduction as well.¹⁹

Field No.	K 1434	Latin name	Carthamus tinctorius
Local name	KOUSHEH	P. I. No.	
Locality data	10 km southeast of Isfahan, Iran, and near the Zayandehrud River with chick peas		
Plant description	Plants about 30" high; red, yellow and orange flowers, mostly spineless. Two plants about 48" high, and not yet in bloom		
Photo			
Special notes	Wild? ☐ Cult? ☒		
Collector	Date July 13/58		
PLANT INTRODUCTION SECTION, HORTICULTURAL CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE			
UNITED STATES DEPARTMENT OF AGRICULTURE			
HC-1 (7-54)		16-70713-1 GPO	

Fig. 3.3 Plant introduction record obtained by the Plant Introduction Section of the U.S. Dept. of Agriculture. (The junior author made this collection.)

Records. The field records made by plant explorers of the U.S.D.A. are illustrated in Fig. 3.3. It is particularly important that the location of the collection be clearly indicated in case it is necessary to repeat the collection. When the introductions are received in the U.S. Dept. of Agriculture, each is given a plant introduction number (P.I. No.).

Quarantine. The importance of good quarantine cannot be over-

* N. I. Vavilov. *The Origin, Variation, Immunity and Breeding of Cultivated Plants*. Translated by K. Starr Chester. Copyright 1951. The Ronald Press Company, New York.

emphasized, since introductions in the past have been the source of many pathogens and insect pests from other countries, many of which came into the United States during a period of expanding agriculture in the nineteenth century.¹² In some instances, the damage to domestic crops has been spectacular, as in the case of the virtual extermination of the eastern chestnut by the blight fungus (*Endothia parasitica*). The list of diseases and insects not yet introduced to the United States is very long and justifies continued efforts to keep them out. Rooted plants of a woody nature are most likely to carry disease, since they have had ample time to accumulate various pathogens, and provide many sites for infection—bark, leaves, stems, and roots. Though more easily decontaminated, seeds can carry many diseases.

All samples introduced under the auspices of the U.S. Dept. of Agriculture are sent to the quarantine station in Washington termed the "Plant Inspection House" where they are fumigated and, if necessary, treated against disease. For the first year of growth in the United States, an introduction is usually isolated in a greenhouse or at one of the primary introduction stations—in both cases out of the area of commercial production. Primary introduction stations are located at Pullman, Washington; Ames, Iowa; Experiment, Georgia; and Glenn Dale, Maryland.

Special procedures are required for certain vegetative materials. Introduced budwood is grafted on stocks grown under isolation in a greenhouse at Glenn Dale, Maryland. Buds from this are propagated on other stock, also under isolation in another section of the greenhouse. Finally, another transfer is made to a third section. If it has remained free from diseases and insects in these transfers, the budwood may be distributed to plant breeders. Out-of-doors quarantine stations for vegetative materials are maintained at Glenn Dale, Maryland; Savannah, Georgia; Miami, Florida; and Chico, California.

Preservation of the Germ Plasm

The preservation of introduced materials is often a more formidable task than bringing them in. When stocks were small, individual plant breeders often did this. In this regard, botanic gardens have long made a notable contribution to agriculture by conserving living material. However, with many thousand introductions of some crops, it has been necessary for the U.S. Dept. of Agriculture to develop special agencies for this purpose. Each of the four plant introduction stations mentioned above have been assigned particular crops, and each must periodically grow out all introductions as frequently as necessary to maintain the

seed in a viable state. Plant breeders in various parts of the nation often cooperate in this program.

Cross-pollinated crops present a problem when large numbers of introductions must be grown at one time. The problem becomes even greater if the introductions are self-incompatible. Special techniques of bagging are required, with several plants of each introduction being involved.

A seed repository has been developed at Fort Collins, Colorado, where seed may be kept at optimum storage temperature and humidity for long life. Both introduced and domestic germ plasms are preserved there.

Description and Evaluation

When introductions are grown at the primary introduction stations, some important commercial variety is included in the nursery as a basis of comparison. The introductions are classified in as much detail as possible, and the descriptions are distributed to plant breeders who might be interested in them. If introductions are grown elsewhere, a similar classification is made and included with the original description which is kept at the primary plant introduction station. Where the number of introductions is very large, the information has been put on punch cards for computer analysis.¹⁸

Often entire collections are grown at a station to measure the resistance of each introduction to a particular pathogen. This was done at Davis, California, for the world collection of barley, to find resistance to net blotch (*Helminthosporium teres*).¹⁵ Of the 4,526 entries, 218 were promising enough for additional testing, and these were reduced to 75 in subsequent tests.

Classifications should attempt to systematize the variability and relate it to the areas where the introductions originated so that any area providing valuable introductions can be sampled again. A serious study of the pattern of variability in a crop may shed light on its course of evolution.

Evaluations abroad are useful when resistance is needed to some pathogen or insect that has not yet been imported. When a serious virus disease of rice threatened to invade the United States a large collection of rice germ plasm was tested for resistance in Puerto Rico where the disease had become well established.

These evaluations are straightforward, but they deal mostly with evaluations of qualitative characters. Much more difficult are the evaluations for genes that can contribute to quantitative characters such as yield, quality, and adaptation. This requires gene recombinations and evaluations under a variety of environments. The most promising ap-

proach seems to be some form of composite crossing of a large number of introductions.^{6,8,16} Actually such evaluations become a matter of plant breeding, in its widest sense. The procedures utilized will be discussed in later chapters.

Résumé

As crop husbandry improves in a nation, it is axiomatic that its genetic base will shrink. In self-pollinated crops this has been accomplished with pure-line varieties, and in cross-pollinated crops by hybrid varieties. In such a situation it is imperative that a system be established to preserve the present store of germ plasm. It is imperative also that the storehouse of germ plasm be augmented by supplies from other nations. Such a store of germ plasm insures future resources, since it protects against: (1) the disappearance of germ plasm, which is taking place at an increasing pace in many areas; (2) the occurrence of unforeseen calamities that may threaten a crop, such as a new insect or disease; and (3) the changing requirements of the marketplace, which may dictate changes in the architecture of a crop or its chemical constitution.

The task is so large and so important that it has required thorough organization to: (1) sample the germ plasm from all areas of the world; (2) prevent the introduction of pests; (3) catalog the germ plasm; (4) evaluate it; and (5) renew it for future use.

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4

MODE OF REPRODUCTION IN RELATION TO PLANT- BREEDING METHODS



This extraordinary diversity in the means for favouring or preventing cross- and self-fertilization in closely allied forms probably depends on the results of both processes being highly beneficial to the species, but directly opposed in many ways to one another and dependent on variable conditions.

DARWIN, 1877

SUCCESSFUL improvement of a crop through breeding depends on a thorough knowledge of the reproductive process. The techniques of handling breeding materials must take into account several features of reproduction: whether it is sexual, asexual, or a combination of the two;

the process in all its details. Sexual reproduction involves two phases or generations in the life of a plant; these are (1) the sporophytic generation and (2) the gametophytic generation.

The sporophyte in higher plants is conspicuous; it is the generation that begins with fertilization of the egg cell and which carries on through the seed, the seedling, and the fully developed plant to the production of flowers (Fig. 4.1). The flowers of the sporophyte produce two types of spores; the male spores produced in the anther are called microspores, and the female spores in the ovary are termed megaspores. The sporophytic generation is diploid ($2n$).

The microspores and megaspores are haploid (n). The microspore develops into a pollen grain in the anther, and the pollen grain into a pollen tube on the stigma and in the style. The pollen tube, when it reaches the embryo sac, is the fully developed male gametophyte. Note (Fig. 4.2) that the pollen tube never develops more than three nuclei:

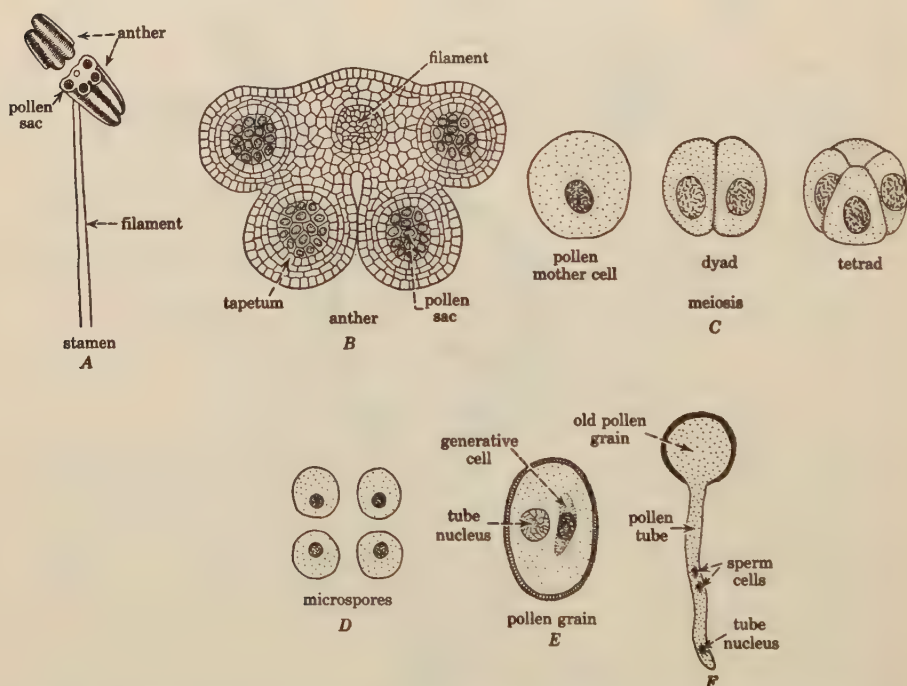


Fig. 4.2 Development of pollen from a pollen mother cell to the pollen tube. A, stamen; B, cross section of anther; C, development of a tetrad of cells from the pollen mother cell by meiosis; D, four microspores; E, pollen grain; F, germination of pollen grain into pollen tube. (By permission, from *Botany* by W. W. Robbins, T. E. Weier, and C. R. Stocking, John Wiley & Sons, Inc., 1964.)

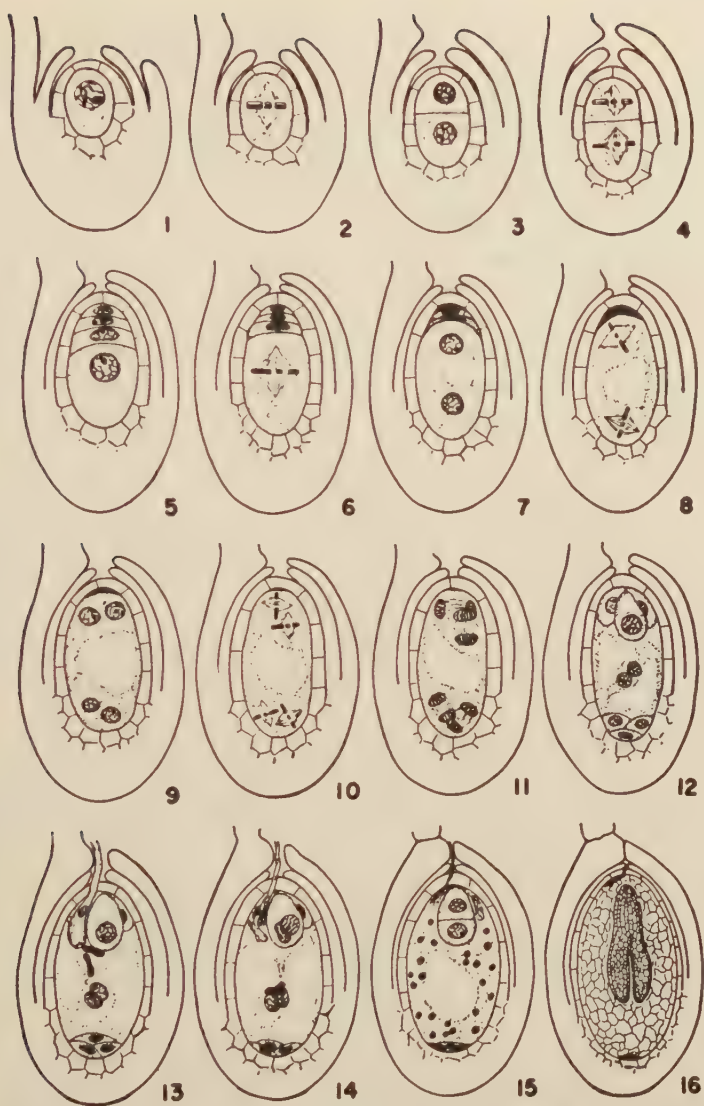


Fig. 4.3 Megasporogenesis, development of the female gametophyte, and early embryogeny in an angiosperm, of which maize is an example. (1-5) meiotic divisions leading to the formation of a linear tetrad of megaspores; (6-11) development of the embryo sac by three successive mitoses in the innermost megaspore; (12) egg and two synergids at the top, two polar nuclei in the center, and three antipodals at the bottom; (13) discharge of two sperm cells into the embryo sac from the penetrating pollen tube; (14) double fertilization, with one sperm cell fusing with the egg nucleus and the other with the fused polar nuclei, to give respectively a diploid zygote and a triploid endosperm nucleus; (15) two-celled embryo and the endosperm in a free nuclear state; (16) embryo with two cotyledons and a cellular endosperm. (From *Introduction to Cytology* by L. W. Sharp. Copyright 1934. McGraw-Hill Book Company. Used by permission.)

one, the tube nucleus, organizes the development of the pollen tube down the style; and the other two, the generative nuclei, travel down the pollen tube and enter the embryo sac where they fertilize both the egg nucleus and the endosperm mother cell (Fig. 4.3, No. 13 and 14). The generative nuclei are male gametes.

The male gametophyte is subject to many hazards, for the pollen grain often must be transferred from one plant to another by wind or insects, and the fragile pollen tube often has to grow several centimeters to reach the embryo sac. A very large number of pollen grains compensates for these handicaps. It is perhaps no surprise to realize that the male gametophyte is a very effective screen that eliminates male gametes which are defective in any way.

Only one out of four megaspores, usually the one nearest the micropyle, develops into a gametophyte (Fig. 4.3, No. 5) which is termed an embryo sac. Typically, there are three divisions of the megaspore nucleus, giving a total of eight nuclei in the embryo sac. One of these becomes the egg nucleus, and two fuse to become polar nuclei. The egg nucleus and polar nuclei organize around themselves cytoplasm and a membrane, which become an egg cell and endosperm mother cell, respectively. Both are called gametes.

Because the female gametophyte develops within the ovary, it is less subject to elimination by the hazards of the environment. For this reason defective gametes may be transmitted through the female gametophyte whereas they would be eliminated if present in the male gametophyte.

After fertilization, the egg cell develops into an embryo ($2n$), and the endosperm mother cell into the endosperm ($3n$). In some species the endosperm disintegrates soon after it begins development, so that the mature seed consists only of a seed coat and embryo.

Meiosis. Meiosis is the process in the sporophyte generation whereby microspores and megaspores are produced. It involves two nuclear and cell divisions, and is illustrated in Fig. 4.4. The significant feature of meiosis is that it provides an orderly procedure for the reduction of the chromosome number from the paired (diploid) condition to the single (haploid) condition. So important is this process in the sexual propagation of plants, that it is described here in some detail.

During prophase I of the first nuclear division, there is pairing (zygotene) and contraction (pachytene) of homologous chromosomes. Paired chromosomes are called *bivalents*. During diplotene the paired chromosomes separate, and it is apparent that each is now double and that there has been *crossing-over* of the pair of doubled chromosomes. Each unit of the doubled chromosome is called a *chromatid*, and where the

chromatids cross over from one chromosome to the other, the cross-shaped configuration is called a *chiasma* (plural *chiasmata*). At diakinesis the centromeres of the doubled chromosomes push farther apart, and the chromosomes contract further. This completes prophase I.

During metaphase I, chromosomes conglomerate on the metaphase plate, and the nuclear membrane disappears. At anaphase I, centromeres of bivalents migrate to separate poles, pulling the chiasmata off the ends of

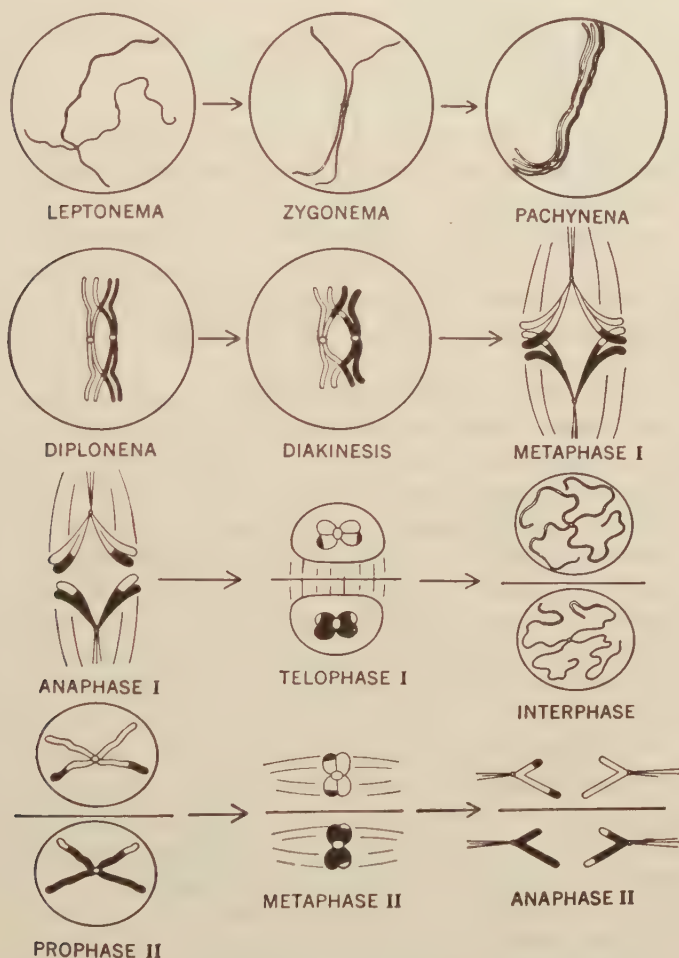


Fig. 4.4 Stages in meiosis I and II, showing only one pair of chromosomes. For the terms leptanema, zygonema, pachynema, and diplonema of the illustration, the terms leptotene, xygotene, pachytene, and diplotene, respectively, are used more often. (From M. M. Rhoades, *J. Heredity* 41 (1950) 59.)

the chromosomes. In anaphase movement, chromatids are usually obvious. At telophase I, a nuclear membrane is formed around the chromosomes at each pole, and the first nuclear division is complete. It is often called the *reductional division* because the chromosome number is reduced from the diploid to the haploid level.

The second nuclear division of meiosis resembles mitosis. The chromatids of each chromosome migrate to separate poles. This is called an *equational division*.

Meiosis is of particular importance to the plant breeder because it is a mechanism to provide variability. This is accomplished by: (1) reduction division; (2) independent segregation of each pair; and (3) crossovers in pachytene.

The role of reduction division in providing variability may be easily understood if it is assumed that there is only one chromosome pair in the cells of the sporophyte and that the chromosomes of the pair are different. Male and female gametes will be of two kinds; they will have one or the other of the two chromosomes. When fertilized, these gametes will give three different sporophytes, one having two chromosomes of one type, one with two chromosomes of the other type, and one with two different chromosomes.

If there are two pairs of chromosomes in meiosis, with the members of each pair different (symbolize these as A^1A^2 and B^1B^2), then independent assortment gives rise to four different gametes: A^1B^1 , A^1B^2 , A^2B^1 , and A^2B^2 . After fertilization these give rise to nine different sporophytes, though not in equal frequencies. With three chromosome pairs, again with the members of each pair different, there would be eight different gametes and 27 different sporophytes. If there were n different chromosome pairs, the number of different gametes would be 2^n , and the number of different sporophytes 3^n . It is clear that the independent segregation of chromosome pairs provides an important means of increasing variability.

Finally crossing-over in pachytene (illustrated in diplotene and diakinesis in Fig. 4.4) provides a means of subdividing chromosomes, thereby increasing the number of different gametes and sporophytes. It is obvious that the variability released by crossing-over will depend on its frequency and location. Crossing-over is less near the centromere.

Asexual Reproduction

Plants may reproduce asexually in a variety of ways without undergoing reduction of chromosome numbers (in meiosis) and restoration of the doubled number (in fertilization). These are grouped under *vegetative propagation* and *apomixis*.

Vegetative propagation. Some familiar means of asexual reproduction will include: rhizomes, or underground stems, as in Johnson grass, brome-grass, or hops; bulbs, as in onions or tulips; corms, as in the gladioli; tubers (a form of underground stem), as in the potato or Jerusalem artichoke; runners, or stolons, as in the strawberry; bulbils, as in onions or lilies; and suckers, either on the roots as in poplars, roses, and elms, or on stems as in the mints. Often, both vegetative and sexual reproduction are present, but sometimes a species or varieties of species will rely on the asexual form only.

Apomixis. Apomixis is a form of asexual reproduction in which seed is produced, but (1) the embryo develops without fusion of male and female gametes and (2) it develops from an unreduced cell ($2n$) of the ovule. There are many ways in which this can occur: A cell of the integuments or nucellus grows into an embryo, termed *adventitious embryony* if the haploid embryo sac is present, and *apospory* if it is not; or the megaspore mother cell develops directly or indirectly into an embryo sac either without meiosis or with meiosis modified to prevent reduction in the number of chromosomes, a process termed *diplospory*. The term *apomixis* has often been used as a synonym for asexual reproduction.^{6,7,8,13,14}

Parthenogenesis is the development of an egg cell into an embryo without fertilization. When an egg cell is unreduced, as in the situations described above, the embryos will all have the same genotype as the parent plant. Up to this point parthenogenesis is synonymous with apomixis. However, parthenogenesis has been extended to include those situations in the normally reduced embryo sac where the egg cell develops without fertilization to give rise to a haploid (n) plant. Such plants are usually sterile and often weak. Haploid progeny from a heterozygous plant will have different genotypes, and can be quite variable.

In plants reproducing apomictically, pollination and the development of the pollen tubes may be necessary for seed production. The pollen provides a stimulus only; its materials are not included in the developing embryo. The term for this phenomenon is *pseudogamy*. In some species the embryo develops apomictically, but fertilization is necessary for the endosperm to develop.

Identifying apomixis may be a problem. Development of seed in the absence of pollen, particularly if the seed give uniform progeny like the parent plant, is positive identification. If pseudogamy is involved, with pollination necessary for seed production, the uniform progeny from a single plant is suggestive of apomixis, particularly if the species is cross-pollinated. In both cross- and self-pollinated species, if carefully controlled crosses of diverse types of plants give progeny exactly like the female

parent, apomixis is almost surely involved. Histological studies, of course, will be necessary to identify the exact nature of apomictic development.

Facultative apomixis is the term given to the reproductive process in those plants or species that produce seed both apomictically and sexually. Kentucky bluegrass (*Poa pratensis*) provides an example of how complex the situation can be. Brittingham² found:

1. Apomictic ($2n$) types like the parent plant.
2. Sexually produced ($2n$) types from reduced gametes.
3. Haploid (n) types produced parthenogenically from a reduced gamete.
4. A triploid ($3n$) type from a combination of an unreduced female gamete ($2n$) and a reduced male gamete (n).

To the plant breeder the significant feature of vegetative reproduction and apomixis is that they perpetuate a type without change. If a particularly successful variety is found or developed, it will reproduce itself faithfully. In this respect asexual reproduction would be an advantage, particularly if the new variety could be propagated rapidly. On the other hand, from an evolutionary point of view, it is a dead end except for somatic mutations. Apomixis has been found in a number of species of forage crops, among them Dallisgrass (*Paspalum*) species,¹ *Calamagrostis purpurea*, and bluegrass (*Poa*) species. It is present in several other crop plants, among them *Citrus* and *Rubus*.

The formation of fruit is no guarantee that seeds have been formed, either with or without apomixis. Some varieties of horticultural crops normally yield fruit without seed, as in seedless grapes, seedless bananas, navel oranges, and pineapples. The ovules in such types develop without the stimulus of pollination and fertilization. This phenomenon, called *parthenocarpy*, may be induced in some plants, such as tomatoes and melons, by treating the stigmas with an extract from pollen or with a growth-promoting substance such as indoleacetic acid or naphthalene-acetic acid.

Self-pollinated Crops

The classification of plants as self-pollinated or cross-pollinated is not always easy. Species range through all degrees of cross-pollination, from none to complete. Plants classified as self-pollinated may have up to 50 per cent cross-pollination, as in cotton. The mechanisms for imposing self-pollination on plants are not very numerous. The most positive mechanism is *cleistogamy*, a situation in which the flowers never open. An example is provided by California oatgrass, the inflorescence of which

is borne between the culm and leaf sheath and never emerges. In certain of the annual fescues, such as *Festuca megalura*, the inflorescence emerges from the boot, but the florets do not open. In lettuce, wheat, and barley, as examples, the flowers open and the pollen is available for transport by wind or insects (they are *chasmogamous*), but the receptive stigma is pollinated before or just as the flowers open. In the tomato, pollination follows the opening of the flower, but the stamens form a cone in such a way that self-pollination is almost always ensured. In sorghums the stigma and anthers push out as the glumes open. The anthers dehisce as they are extruded, or shortly afterward.

In all cases where the flowers open, occasional cross-pollination is possible. Various factors may affect the level of self-pollination. The nature and amount of the insect population is an important factor, bees being particularly important in the crop species they visit. Proper air currents increase pollen movement in species that rely on wind for pollen dispersal. Some species, such as mountain brome (*Bromus carinatus*)⁹ and a species of needle grass (*Stipa cernua*), may be cleistogamous at one stage of development and cross-pollinated at another. In some crop plants, temperatures just below freezing may sterilize the pollen without affecting the ovules, thereby giving pollen of other plants an opportunity to effect fertilization. Even such a small change as higher than normal night temperatures arrested the normal development of anthers in annual bluegrass (*Poa annua*).¹⁰ Following controlled pollinations of florets subjected to high night temperatures, 96 per cent of the seeds were hybrids.

Typically, a self-pollinated population is a mixture of homozygous lines. Even if a cross occurs in the population, the resulting heterozygosity disappears rapidly. Complete self-pollination imposes two restrictions on a population: (1) It prohibits new gene combinations and the production of new and perhaps more successful genotypes; and (2) as a consequence of the first restriction, it confines mutations to a single line or to the progenies of the plants in which they occur. This does not mean that self-pollinated populations will be stable and unchanging; the proportion of different types present will change with the type and amount of selection pressure imposed by nature or by man. The variability, however, will be in terms of proportions, not in terms of the emergence of new types. Self-pollination quickly exposes recessive deleterious genes, permitting their immediate removal by nature or the plant breeder. Recessive mutants, favorable or otherwise, are quickly uncovered in a similar way.

Actually, very few species are completely self-pollinated. Even a very low level of outcrossing permits new gene combinations that, if they have a selection advantage, will increase rapidly in a population. Never-

theless, populations will be more inflexible than in cross-pollinated species.

There is good evidence that many of the self-pollinated domestic crops evolved from natural species that were cross-pollinated, in spite of systems of incompatibility favoring cross-pollination.¹⁴ Under cultivation, however, often with the seed of one or a few plants carefully chosen as the planting stock for the next year, types that were productive under conditions of some self-fertilization were apparently favored. In many cases man not only reduced the population but also selected for types to suit his purpose.

In a number of crops the amount of natural crossing is so low that no protective measures are required to prevent outcrossing. Important among such crops are barley, oats, wheat, common beans, cowpeas, peanuts, peas, soybeans, flax, tobacco, tomatoes, and some grasses and legumes (see Table 4.1). In these, natural crossing rarely exceeds 4 or 5 per cent, and is usually much lower. At the other extreme, some crops classified as self-

Table 4.1 Partial list of self-pollinated crops.

FIELD CROPS	
Annual sweet clover	Strawberry clover
Barley	Subterranean clover
Bur clover	Tobacco
Castor beans ^a	Velvet beans
Chick peas	Vetches
Common beans	
Cotton	
Cowpeas	
Flax	
Foxtail millet	
Lespedeza species	
Lima beans	
Mountain bromegrass	
Mung beans	
Oats	
Peanuts	
Peas	
Rice	
Slender wheatgrass	
Soft chess	
Sorghums	
Soybeans	

HORTICULTURAL CROPS	
Apricots	
Cucumbers ^a	
Egg plant	
Lettuce	
Muskmelons ^a	
Nectarine	
Okra	
Peaches	
Pepper	
Potato	
Pumpkin ^a	
Squash ^a	
Sweet peas	
Tomato	
Watermelons ^a	

^a These crops are highly cross-pollinated but are included here because they are not depressed by inbreeding.

pollinated require some protective measures under most conditions. Out-crossing is prevented in sorghum by bagging the heads, and in cotton by securing the petals with a rubber band or a fine copper wire before the flower opens.

Cross-pollinated Crops

Among natural species, cross-pollination is much more common than self-pollination. The common occurrence of cross-pollination indicates that it is a very successful breeding system. A partial list of cross-pollinated crops is given in Table 4.2.

The methods of ensuring cross-pollination vary in form and effectiveness. The most effective method separates the sexes in *dioecious* species, as in the date palm, hops, and hemp. Cross-pollination in such species is, of course, obligatory, and the breeding structure is the same as in animals.

Table 4.2 Partial list of cross-pollinated crops.

FIELD CROPS	
Alfalfa	Carrots
Alsike clover	Cauliflower
Bird's-foot trefoil	Celery
Crimson clover	Chard
Hemp ^a	Cherries
Hops ^a	Chestnut
Maize	Citrus
Orchard grass	Collard
Red clover	Date palm ^a
Rye	Fig
Rye grasses	Filbert
Smooth bromegrass	Grapes
Sweet clover	Kale
Tall fescue	Mangels
Timothy	Olive
White clover	Parsnips
	Pears
HORTICULTURAL CROPS	
Almond	Pecan
Apples	Plums
Asparagus ^a	Raspberries
Beets	Rutabaga
Blackberries	Spinach ^a
Broccoli	Strawberries
Brussels sprouts	Turnips
Cabbage	Walnuts

^a Dioecious crops.

The sexes may be separated on the same plant in *monoecious* species, which reduces but does not preclude self-pollination. In corn the male and female flowers are widely separated, whereas in the castor bean they are on different parts of the same raceme.

When the flowers include both sexes, often termed *hermaphroditic*, obligate cross-pollination has been achieved in various ways. Stamens may mature ahead of the pistils (protandry), or vice versa (protogyny); flower structure may be adapted to facilitate the transfer of pollen from plant to plant by insects and to prohibit self-pollination; or the pollen of a plant may not be functional on its own stigma or in its own style. The latter failure of pollen to function is termed self-incompatibility. Such incompatibility systems are so important to the plant breeder that the entire Chapter 15 is devoted to them. Here it is sufficient to know that they render a plant essentially self-sterile. A feature of cross-pollinated crops is wide pollen dispersal, chiefly by wind and insects.

When pollination is by wind, the flowers are usually inconspicuous, as in many of the forage grasses, cereal rye, and nuts. The pollen is light, dry, and easily blown, and usually produced in abundance. For example, one corn tassel produces about 25,000,000 pollen grains, or 25,000 for each kernel. This averages about 42,500 grains for every square inch of the field.¹¹ The stigmas are feathery, exposing a large surface area to the flying pollen. Wind-dispersed pollen may be moved long distances.

More species rely on insects to effect cross-pollination, such species being adapted in their structure to ensure most efficient results from insect activities. Often an insect and a flower may show a remarkable degree of coadaptation, with each appearing dependent on the other for its existence. The principal pollinating insects are bees, which visit flowers for both nectar and pollen. Such pollen is sticky and not easily blown by the wind, but readily adheres to the body of the insect, to be rubbed off on visits to other flowers.

In contrast to homozygosity in self-pollinated crops, heterozygosity is characteristic of the genetic structure of cross-pollinated crops. Such crops are continually exploring all possible combinations of genes. They are flexible, quickly developing the optimum combination of available genes for a particular situation.

A characteristic feature of most cross-pollinated crops is that inbreeding depresses their vigor and productivity. Cross-pollination, in contrast, restores and maintains vigor. These two facts have an important bearing on procedures in breeding this group of crops. In the few cases where inbreeding depression does not occur, the crop may be bred as if self-pollinated.

Establishing the amount of cross-pollination. For most crops the amount

of cross-pollination is known exactly, or at least closely enough to indicate whether they are largely self- or cross-pollinated and whether selfing will cause inbreeding depression. With crops of recent concern to the plant breeder, or with well-established crops moved to a new environment, it may be necessary to find the level of outcrossing through experiments.

Such experiments may require bags or cages to exclude foreign pollen or insects that carry pollen. The bags or cages must be carefully chosen to ensure that no pollen enters, particularly when the bag is manipulated. If no seed is set under this form of isolation, the crop may be assumed to be cross-pollinated. But the results must be judged with caution, for the environment of the cage or bag may itself be adverse to seed production. In addition, the production of seed indicates only that the crop can be self-pollinated; without the bag or cage it might actually be largely outcrossed. Complete isolation of the plant will test cross-pollination without the unnatural environment of the bag. If selfed seeds, either from bagged or isolated plants, yield progeny that sooner or later are depressed in vigor, the crop in question is normally cross-pollinated.

The level of outcrossing is often determined by growing two distinctly different varieties in alternating rows and counting the number of hybrid seed in each variety when sown the following year. Actually, this measures pollen movement from row to row and not down the row. For best results, alternate plants of the two varieties will give a more realistic measure of the amount of natural crossing if the varieties flower at the same time. Such studies are greatly facilitated if hybrid seeds or seedlings are readily distinguished from those of one or both parents.

Most experiments have indicated that varieties may differ considerably in natural crossing. This seems particularly true of crops with intermediate levels of cross-pollination; if highly outcrossed, particularly if self-incompatibility is involved, all varieties will be similar; and if highly self-pollinated, the range in varieties will be small. The effect of both

Table 4.3 Percentage of crossing in red leaf and virescent yellow cotton stocks per flowering period with a common green leaf stock as the pollen parent.⁵

FLOWERING PERIODS	RED LEAF		VIRESCENT YELLOW	
	<i>Seedlings</i>	<i>Per Cent</i>	<i>Seedlings</i>	<i>Per Cent</i>
	<i>Scored</i>		<i>Scored</i>	
Early Bloom	9,538	57.2	1,396	86.5
Middle Bloom	8,760	52.3	478	81.4
Late Bloom	6,473	32.7	306	56.4

variety and stage of development on outcrossing is apparent from a study on cotton.⁵ A virescent yellow stock showed considerably more outcrossing to a green type than a red-leaved stock (Table 4.3). Flowers produced early on both stocks had more outcrossing.

The classification of plants as self- or cross-pollinated in Tables 4.1 and 4.2 is not final; it may change with new information. The lists are only partial and almost devoid of ornamental crops. For information on the latter consult Crane and Lawrence³ and the U.S.D.A. *Yearbook of Agriculture* for 1937.¹⁵ It will be noted that the cucurbits are included among self-pollinated crops in Table 4.1. The reason is that breeding procedures for this group are essentially those of self-pollinated crops.

Résumé

In Table 4.4, self- and cross-pollinated crops are compared with respect to their genetic and mating characteristics, and the breeding tech-

Table 4.4 Characteristics of, and breeding techniques for, self- and cross-pollinated crops.

CHARACTERISTIC OR BREEDING TECHNIQUE	SELF-POLLINATED SPECIES	CROSS-POLLINATED SPECIES
<i>Characteristics of Natural Populations</i>		
Genotypes of Sporophyte	Homozygous	Heterozygous
Genotypes of Gametes of a Single Plant	All the same	All different
Progeny of a Single Plant	Homogeneous	Heterogeneous
Self-incompatibility	None	Frequent
Inbreeding Depression	None	Usual
<i>Breeding Technique</i>		
Introductions	Yes	Yes
Pure-Line Selections	Often	Only as parents for hybrid varieties
Mass Selection	Occasionally	Frequently
Pedigree Breeding	Yes	No
Bulk Populations	Yes	Yes
Backcross Breeding	Yes	Yes
Hybrid Varieties	Occasionally	Yes
Recurrent Selection	Occasionally	Yes
Synthetic Varieties	Occasionally	Yes

niques used in their improvement. Actually, this table outlines generally the content of this book.

It should be kept in mind that a self-pollinated crop is an assembly of homozygous plants and that a commercial variety of such a crop is

often one single genotype reproducing itself faithfully. The breeder's job is to find or develop the most desirable single genotype or combination of genotypes.

A cross-pollinated crop may be compared to a pool of genes that combine in various ways to form the genotypes of each generation. A single plant produces many different kinds of gametes, which combine mostly or always with the gametes of other plants. The breeder's objective is to develop the most desirable pool of genes. If hybrid varieties are being developed, lines must be found that will combine to produce vigorous hybrids.

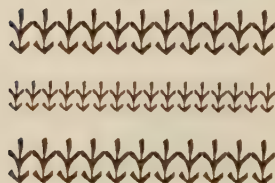
In the past, breeding techniques appropriate to self- and cross-pollinated crops were distinct. Now they tend to overlap, and this trend will continue in the future. For example, breeding programs are presently under way to develop hybrid wheat, the crop that has been the focus of breeding techniques for self-pollinated crops.

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5

VARIABILITY IN PLANTS



During the 50-year period under consideration there have been many improvements in experimental design, and new uses of statistical procedures are being constantly developed. These greatly aid in reaching sound conclusions and have played an important part in developing sound plant breeding techniques. . . . Statistical procedures should not, in the speaker's viewpoint, take the place of a knowledge of the biological nature of the organisms with which the experimental work is being performed.

HAYES, 1957

THE FIRST THING that impresses one on a visit to a plant-breeding nursery is the variability that is present. From row to row, or plot to plot,

there will be differences in such characters as height, rate of development, vigor, and uniformity. Where spaced plants are grown, each may have its unique characteristics. Then the visitor wonders, or asks the questions: "How does one catalog and summarize this variability?" "How does one make comparisons between the different types?" "How does one decide which row or plot is the best?"

Anyone who works with biological material must soon find ways of summarizing, expressing, and managing the variability; a plant breeder is no exception. The main business of plant breeding is to develop populations that will be evaluated or compared in various ways.

This chapter will introduce some of the statistics used in describing populations, and will discuss some of the simpler procedures that are used in comparing them. Statistical procedures for separating variability due to environment from that due to heredity are deferred to Chapter 8. Experimental designs appropriate for simple comparisons of varieties or selections are given in Chapter 30.

For the professional plant breeder, statistics and their application to biological material must necessarily be an important part of his training. Important as statistics are, however, it must be remembered that they are part of the tools of the trade, not the trade itself.

Scale of Variability

When the variability among plants may be easily assigned to a few classes, it is usually spoken of as *qualitative*. Such differences would be those relating to color, shape, texture, and the presence or absence of certain characters. Usually the classification will hold for any year, location, or environment.

The variability is termed *quantitative* when it grades gradually from one extreme to another. Plants, when being classified, cannot be assigned to discrete classes. Many characters of importance economically are quantitative in nature. Some of these are yield, period of development, height, and vigor. Often these characters are very sensitive to the environment.

It is obvious that some characters may not arbitrarily be designated qualitative or quantitative. Color, usually a qualitative character, may grade through many intermediate shades to another extreme and be better handled as a quantitative character. The same would be true of texture and shape. Earliness is usually quantitative, but plant populations may sometimes fall into two or three maturity classes, so that earliness is classified as a qualitative character. The same is true of height or branching. Vigor, adaptation, and yield, however, are rarely qualitative in nature. The criterion of difference between quantitative and qualitative

characters, then, is classification; if different types fall into discrete classes, the character is qualitative, and if not, it is quantitative.

Qualitative Variability

Plant breeders are much concerned with qualitative differences. So important are they that Chapter 6 is devoted entirely to a consideration of how such differences are exploited in the development of superior varieties. Such characters were the basis for much of the early development of genetics. The only statistics that will be used for qualitative characters are those involved in the *chi square* (χ^2) test.

Genetic ratios. The chi square test has been adapted to measure the significance of two types of differences of interest to the plant breeder. The first of these deals with comparisons of segregating populations to genetic ratios, and the second with comparisons of two populations variable in a qualitative way.

The simplest use of the chi square test in determining the fit of segregating populations to genetic ratios is illustrated by data of Briggs and Stanford¹ in Table 5.1. The question is: Does the F_2 population

Table 5.1 F_2 segregation in the cross, Goldfoil $\times$ Nepal 595, where linkage is involved.¹

	ACTUAL NUMBER	EXPECTED NUMBER			$\frac{(a - e)^2}{e}$	TOTAL	
CHARACTERS	<i>a</i>	<i>e</i>	<i>a - e</i>	$(a - e)^2$		χ^2	<i>P</i>
<i>3:1 Ratio Hooded to Awned</i>							
Hooded (<i>K</i> ₋)	562	577.5	-15.5	240.25	0.416	} 1.664	0.10 to 0.20
Awned (<i>kk</i>)	208	192.5	15.5	240.25	1.248		
<i>3:1 Ratio Mildew-Resistant to Mildew-Susceptible</i>							
Mildew-resistant (<i>MI</i> ₀ -)	588	577.5	10.5	110.25	0.191	} 0.764	0.20 to 0.50
Mildew-susceptible (<i>ml</i> ₀ <i>ml</i> ₀)	182	192.5	-10.5	110.25	0.573		
<i>9:3:3:1 Ratio</i>							
Hooded Resistant	387	433.1	-46.1	2125.21	4.907	} 68.695	Less than 0.001
Hooded Susceptible	175	144.4	30.6	963.36	6.484		
Awned Resistant	201	144.4	56.6	3203.56	22.185		
Awned Susceptible	7	48.1	-41.1	1689.21	35.119		
<i>Assuming a Crossover of 18.77% between <i>k</i> and <i>MI</i>₀</i>							
Hooded Resistant	387	391.8	-4.8	23.04	0.059	} 1.942	0.20 to 0.50
Hooded Susceptible	175	185.7	-10.7	114.49	0.616		
Awned Resistant	201	185.7	15.3	234.09	1.261		
Awned Susceptible	7	6.8	0.2	0.004	0.006		

with 562 hooded and 208 awned plants fit a 3:1 ratio? The formula for the chi square is

$$\chi^2 = \frac{(a - e)^2}{e} \tag{5.1}$$

where a = the actual value, and e = the expected value, calculated from the genetic ratio. The two chi squares add up to 1.664. Using the chi square table (Appendix, Table A.3), and 1 degree of freedom (one less than the number of classes), the probability, P , is found to be between 0.1 and 0.2. This indicates that a deviation as great or greater than the actual values would be expected to occur by chance alone between 10 and 20 per cent of the time. By a similar analysis the value of P for a comparison of the segregation of mildew-resistant and mildew-susceptible plants to a 3:1 ratio is between 0.2 and 0.5. Where the probability is less than 0.05, the difference between the actual segregation and the expected values from the genetic ratio is considered significant; in other words, there is a poor fit. On this basis the segregations for both the hoods versus awns and mildew resistance versus susceptibility show a good fit to a 3:1 ratio.

Since these two segregations fit a 3:1 ratio, it is expected that the combined data will fit a 9:3:3:1 ratio. Chi squares are calculated, added, and the probability determined (for 3 degrees of freedom) to be less than 0.001. This indicates a very poor fit of the actual and expected values. This happens because parental types are too abundant, and the recombination classes are too few. In other words, the genes for hoods and mildew susceptibility (and awns and mildew resistance) are linked.

Assuming a crossover value of 18.77 per cent between the genes for expression of hoods or awns and mildew infection, the actual and expected values are in close agreement. It should be noted that the degrees of freedom are 2 instead of 3; 1 degree of freedom is lost because the crossover value is calculated from the observed data.

Contingency tables. Chi square tests will measure differences between two populations that are classified in a qualitative way. We shall assume

Table 5.2 Contingency table comparing two selections for survival in the presence of wilt.

	DEAD	ALIVE	TOTAL
Selection A	16	68	84
Selection B	25	25	50
Total	41	93	134

in a test of two selections, A and B, for resistance to wilt that 16 out of 84 plants of A and 25 out of 50 plants of B die. They may be compared by putting them in contingency tables such as Table 5.2.

If these selections have the same resistance to wilt, they should have the same proportion of dead plants. In other words, the expected number of dead plants in each would be in the same proportion as the totals; A would be expected to have $(41/134) \times 84 = 25.7$, and B would have $(41/134) \times 50 = 15.3$. The expected number of living plants are obtained by subtraction, namely, 58.3 for A and 34.7 for B. Four chi squares are calculated from actual and expected numbers and, when added, give a value of 14.137. Degrees of freedom are 1, the product of one less than the number of selections times one less than the number of classes. The value of P in the example would be less than 0.001. If A and B are similar in resistance, a difference as great or greater than that of the example would be expected by chance alone in less than 0.1 per cent of similar trials. Since the limit of P for significance is 0.05 (or 5 per cent), these populations may be judged as not the same but significantly different. The contingency table could have been extended to include more selections and more classes; for example, four selections and three classes (dead, partly wilted, and healthy). This would require that 12 chi squares be calculated and added. Degrees of freedom, using the same procedure as above, would be $3 \times 2 = 6$.

Quantitative Variability

The greatest contribution of statistics in plant breeding has been in the measurements and comparisons of quantitative characters. An understanding of the inheritance of quantitative characters has been and will be greatly facilitated by statistical procedures. Statistics will be developed here for:

1. A single quantitative character of a single variety.
2. Comparisons of two varieties for one quantitative character.
3. Associations of quantitative characters.

Parameters. Parameters are characteristics of populations. Examples of such characteristics are the mean, the variance, and the standard deviation. It is apparent that populations apply to one quantitative character such as the height of a new variety, or days to mature of a promising new hybrid. Implicit in the idea of a population is an infinite number of plants with the quantitative character under study. Because populations (measurements of a single character) are infinite in number, parameters are never precisely obtained, but are estimated. The estimates are *statistics*.

Statistics. Statistics are obtained from *samples*. Samples are taken at random so that they represent as closely as possible the entire population being considered. This seems straightforward, but samples are often non-representative and sometimes difficult to obtain. But measurements, no matter how numerous or how carefully they are made, are no better than the sample on which they are based. It is very easy to be selective in taking a sample, often without being aware of it.

Statistics for a Single Population

The single population to be considered here is seed length in a pure-line selection of Pinto beans. As a pure line, all plants will have the same genotype. The selection was grown from a single planting in a uniform area. In other words, heritable variability was reduced to zero, and environmental variability was reduced as much as possible. Statistics were determined from a sample of 100 seeds.

Frequency distribution. Where measurements are numerous, it facilitates calculations if they are grouped into *classes*, with each class having a *range* and a *center*. Usually the range of each class is the same. For the example (Table 5.3) there are ten seed-length classes, each with a range of 5 mm. An examination of the data indicates that most of the measurements fall about the middle of the total range, with progressively less at the extremes.

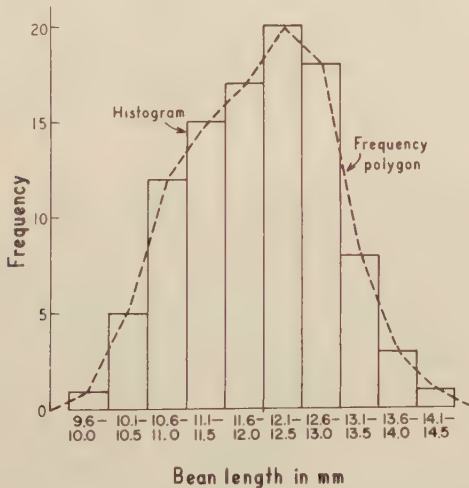


Fig. 5.1 Frequency polygon and histogram prepared from data on length of bean seeds in Table 5.3.

Table 5.3 Frequency table and statistics obtained from the lengths of 100 seeds of Pinto beans.^a

CLASS		FREQUENCY		DEVIATION FROM MEAN			MACHINE CALCULATION		
<i>Center</i>									
<i>Range</i>	<i>x</i>	<i>f</i>	<i>fx</i>	<i>x</i> − <i>x̄</i>	(<i>x</i> − <i>x̄</i>) ²	<i>f</i> (<i>x</i> − <i>x̄</i>) ²	<i>x</i>	<i>x</i> ²	<i>fx</i> ²
9.6-10.0	9.8	1	9.8	−2.2	4.84	4.84	9.8	96.04	96.04
10.1-10.5	10.3	5	51.5	−1.7	2.89	14.45	51.5	106.09	530.45
10.6-11.0	10.8	12	129.6	−1.2	1.44	17.28	129.6	116.64	1,399.68
11.1-11.5	11.3	15	169.5	−0.7	0.49	7.35	169.5	127.69	1,915.35
11.6-12.0	11.8	17	200.6	−0.2	0.04	0.68	200.6	139.24	2,367.08
12.1-12.5	12.3	20	246.0	0.3	0.09	1.80	246.0	151.29	3,025.80
12.6-13.0	12.8	18	230.4	0.8	0.64	11.52	230.4	163.84	2,949.12
13.1-13.5	13.3	8	106.4	1.3	1.69	13.52	106.4	176.89	1,415.12
13.6-14.0	13.8	3	41.4	1.8	3.24	9.72	41.4	190.44	571.32
14.1-14.5	14.3	1	14.3	2.3	5.29	5.29	14.3	204.49	204.49
Total		100	1199.5			86.45	1199.5	1472.65	14,474.45

^a With deviations from mean and by machine calculation. All measurements in millimeters.

The mean:

$$\bar{x} = \frac{\sum fx}{n} = \frac{1199.5}{100} = 11.995, \text{ or } 12.0$$

The standard deviation, s_x :

$$\text{Using deviations} = \sqrt{\frac{\sum f(x - \bar{x})^2}{n - 1}} = \sqrt{\frac{86.45}{99}} = 0.93$$

$$\begin{aligned} \text{Using actual values} &= \sqrt{\frac{\sum fx^2 - (\sum fx)^2/n}{n - 1}} = \sqrt{\frac{14474.45 - 1199.5^2/100}{99}} \\ &= 0.93 \end{aligned}$$

Graphic presentation of data. Often data are easier to understand if they are given in graphic form. Two methods are used. In the frequency histogram (Fig. 5.1), columns are used, with the height of each column representing frequency, and the width the class range. On the other hand, in a frequency polygon (Fig. 5.1), dots above the class centers represent frequencies, and the dots are joined by straight lines.

The normal curve. It is apparent that the frequency polygon obtained from the measurements of bean seeds takes on the appearance of a bell in outline. This is true of measurements of quantitative differences in a great host of characters in the biological world. If class ranges are reduced and the number of measurements increased, the shape becomes a smooth curve. Such a curve (Fig. 5.2) is called a *normal curve*. A normal

curve is defined by its mean and standard deviation, two measurements to be considered later. It is apparent that the normal curve of Fig. 5.2 and the frequency polygon of Fig. 5.1 are not exactly the same shape. Of great importance, however, is that one approximates the other so closely that the statistics calculated from the sample will approximate those of the normal curve. By obtaining the mean and standard deviation of the sample, one can say a great deal about the population from which it was drawn.

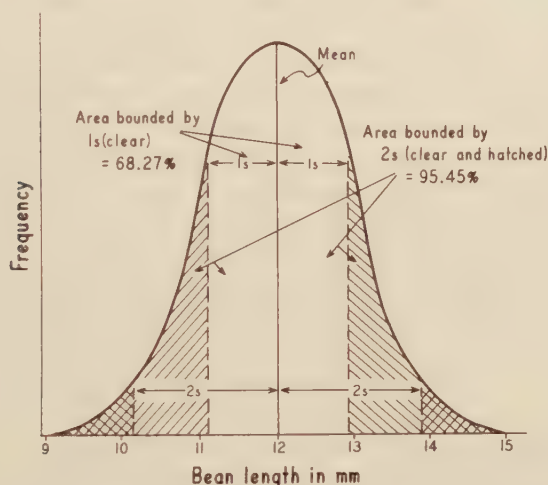


Fig. 5.2 Normal curve prepared from the statistics of the bean sample of Figure 5.1.

Measures of central tendency. There are three measures of central tendency: the mean, the mode, and the median. Only the mean can be handled statistically.

The arithmetic *mean* of the sample is an extremely useful statistic. If the individual measurement (in the example, bean length in millimeters) is represented by x , the mean, $\bar{x}$, is obtained by summing all the values of x and dividing by the number of measurements. This is represented in formula form by

$$\bar{x} = \frac{\sum x}{n} \quad (5.2)$$

where Σ = sum of; and n = number of measurements. In the example, mean bean length is 12.0 mm. Where frequency tables are used, the mean,

$\bar{x}$, is given by the formula $\Sigma f x / n$, where f = frequency of classes and x = class centers (see Table 5.3). It should be remembered that this is the mean of the sample. It will be close to the mean of the population of bean lengths, but probably is slightly different.

The *mode* is the class of greatest frequency. In the example (Fig. 5.1) it has a class center of 12.3 mm, which is very close to the mean of 12.0 mm.

The *median* is the midpoint in the range, which is 12.0 mm in the example.

Measurements of variability. The variability in bean length cannot be ascribed to heredity because a pure-line variety was used. It must, then, be due largely to the variability in the environment. Such environmental variability would include differences in soil and microclimate, effects of insects or diseases, and the position of the developing bean seed on the plant or in the pod. In any sample some variability will be due to chance, which is expressed as the extreme unlikelihood of drawing two samples that have exactly the same variability. Three measures of variability will be considered: the standard deviation, the standard error of a mean, and variance.

The *standard deviation*, s_x , of a population may be estimated from a sample by the following formulas:

$$s = \sqrt{\frac{\Sigma (x - \bar{x})^2}{n - 1}} \quad (5.3)$$

For frequency distributions,

$$s_x = \sqrt{\frac{\Sigma f(x - \bar{x})^2}{n - 1}} \quad (5.4)$$

and for machine calculations,

$$s_x = \sqrt{\frac{\Sigma x^2 - (\Sigma x)^2 / n}{n - 1}} \quad (5.5)$$

What does this mean? It means that all the deviations from the mean are squared, summed, and divided by a number that is one less than the number of measurements. Then the square root is taken of the quotient. With the data assembled in a frequency distribution, as in Table 5.3, formula 5.4 is more convenient (f = frequencies of classes and x = class centers). It is more convenient with a calculator to use formula 5.5. In formula 5.5 the term $(\Sigma x)^2 / n$ is often termed the *correction factor*. All formulas give the same standard deviation.

The merit of the standard deviation is that it will tell rather precisely the range of variability of the population. It will say that in the range

of the mean $\pm$ the standard deviation ($\bar{x} \pm s_x$, or 12.0 ± 0.93 mm in the example), 68.27 per cent, or $\frac{2}{3}$, of all measurements will be included. It will further say that in the range of the mean $\pm$ twice the standard deviation ($\bar{x} \pm 2s_x$, or 12.0 ± 1.86 mm in the example), 95.45 per cent, or $\frac{19}{20}$, of all measurements will be found. In other words, 68.27 per cent will fall in the range 11.07 to 12.93 mm and 95 per cent in the range 10.14 to 13.86 mm.

If a second sample of 100 seeds is drawn from the same lot of beans, it is very likely that its mean and standard deviation will differ slightly from those of the first sample. These differences are due to chance. Such means have a normal distribution, and the standard deviation of the mean is termed the *standard error of the mean*, $s_{\bar{x}}$. The standard error of the mean may be determined from the standard deviation of the first sample or from the original measurements of the first sample by the following formula:

$$s_{\bar{x}} = \frac{s}{\sqrt{n}} \quad (5.6)$$

Applied to the example, $s_{\bar{x}}$ is $0.93/\sqrt{100} = 0.09$ mm. This means that in $\frac{2}{3}$ of the samples of 100 seeds drawn from the population, the mean will be in the range of 12.0 ± 0.09 mm, and in $\frac{19}{20}$ of the samples in the range of 12.0 ± 0.18 mm. Means of variety yields, heights, seed weight, and other quantitative characters are usually expressed as $\bar{x} \pm s_{\bar{x}}$ without explanation.

Variance. Variance, V , is the standard deviation squared, s^2 , and is calculated in the same manner as the standard deviation (formulas 5.3, 5.4, and 5.5) except that the square root sign is omitted. For example, formula 5.3 becomes

$$V = \frac{\sum (x - \bar{x})^2}{n - 1} \quad (5.7)$$

Variance is very frequently used in describing biological materials. Variance analyses are considered in Chapter 30.

Coefficient of variability. The coefficient of variability, CV , expresses the standard deviation in percentage of the mean as follows:

$$CV = \frac{s_x}{\bar{x}} \times 100 \quad (5.8)$$

For the bean population, $CV = (0.93/12.0) \times 100 = 7.75\%$.

Coefficient of variability is useful in comparing the variability of items with quite different means or measurements. The variability in heights of maize and wheat in adjacent plots might be compared, or the variability in wheat yield on the same piece of ground in different years.

Comparison of Two Populations for Quantitative Characters

Plant breeders often make comparisons between a new variety and the one it will replace. Comparisons are invariably made for yield, but may also be made for height, seed dimensions, fruit diameter, and so on. Statistics provides a method for such comparisons.

Unpaired quantitative characters. Considering the example of bean seeds, it may be assumed that, in response to a market demand for a longer seed, a new variety has been developed which has a mean seed length of 12.50 ± 0.11 mm, as determined from a sample of 100 seeds. The difference from the previous variety, with a mean of 12.00 ± 0.09 , is 0.5 mm. Is this difference significant? Significance is determined by first calculating a *standard error of a difference*, $s_{\bar{d}}$. The formula for its calculation is

$$s_{\bar{d}} = \sqrt{s_{\bar{x}}^2 + s_{\bar{y}}^2} \quad (5.9)$$

where $s_{\bar{x}}$ and $s_{\bar{y}}$ are standard errors of the means of the two varieties under comparison. For the example, $s_{\bar{d}} = \sqrt{(0.09)^2 + (0.11)^2} = \sqrt{0.0202} = 0.14$ mm.

The t test is used to determine the significance of the difference. The t value is obtained by

$$t = \frac{\bar{d}}{s_{\bar{d}}} \quad (5.10)$$

where $\bar{d}$ is the mean difference between the means. For the example, $t = 0.5/0.14 = 3.57$.

The significance of t is determined from a t table (Appendix Table A-1). Degrees of freedom in the comparison are 198 (99 for each variety). If the two varieties have the same seed length, a t value of 1.97 or greater will occur by chance with a probability of 0.05, and of 2.60 or greater with a probability of 0.01. Since the t value in the example is much greater than 2.60, the chances of the varieties having the same seed length are very low. They are significantly different.

Paired quantitative characters. It will have occurred to the reader that the bean seeds of the varieties being compared may have been collected from two different environments, one more favorable to one variety and thus increasing its seed length. Because of these possible effects of environment, comparisons are paired as much as possible. A real comparison of the yields of two barley varieties is given in Table 5.4. Vaughn was a new variety being considered as a replacement for Atlas. Yield comparisons were obtained over several years, with both varieties always in the same test. The mean yields of Vaughn and Atlas are respectively 90.3 and

79.5 bu per acre, with the difference $\bar{d} = 10.8$ bu per acre. Using formula 5.5 the standard deviation (s_x) and standard error of the mean ($s_{\bar{x}}$) are calculated for Vaughn, and the corresponding values (s_y and $s_{\bar{y}}$) for Atlas. Using formula 5.9 for unpaired comparisons, $s_{\bar{d}}$ is calculated, $(\sqrt{6.12^2 + 6.02^2})$, to be 8.58 bu per acre, and the t value ($10.8/8.58$ from formula 5.10) is 1.26. For a significant difference if there are 24 degrees of freedom, t should be 2.06. In other words, the differences between these varieties would be judged to be nonsignificant, if unpaired comparisons are made.

The paired comparison is obtained by considering differences (d) in the yields of the varieties as variates, and by computing a standard error ($s_{\bar{d}}$) for these differences. Note that the differences fluctuate considerably and

Table 5.4 Comparisons of yields^a of Vaughn and Atlas barley grown over a period of years at Davis, California.

YEAR	VAUGHN		ATLAS		DIFFERENCE		$x \cdot y$
	x	x^2	y	y^2	d	d^2	
1925	90.7	8,226.49	49.2	2,420.64	41.5	1,722.25	4,462.44
1926	93.2	8,686.24	78.8	6,209.44	14.4	207.36	7,344.16
1927	86.2	7,430.44	81.2	6,593.44	5.0	25.00	6,999.44
1928	81.5	6,642.25	58.8	3,457.44	22.7	515.29	4,792.20
1929	63.3	4,006.89	62.7	3,931.29	0.6	0.36	3,968.91
1930	112.3	12,611.29	103.8	10,774.44	8.5	72.25	11,656.74
1931	54.4	2,959.36	50.2	2,520.04	4.2	17.64	2,730.88
1932	129.8	16,848.04	124.2	15,425.64	5.6	31.36	16,121.16
1933	87.5	7,656.25	92.2	8,500.84	-4.7	22.09	8,067.50
1934	85.0	7,225.00	78.4	6,146.56	6.6	43.56	6,664.00
1935	121.5	14,742.25	91.1	8,299.21	30.4	924.16	11,068.65
1936	101.5	10,302.25	92.9	8,630.41	8.6	73.96	9,429.35
1937	66.9	4,475.61	69.8	4,872.04	-2.9	8.41	4,669.62
Totals	1,173.8	111,832.36	1,033.3	87,781.43	140.5	3,663.69	97,975.05
Means	90.3		79.5		10.8		

^a Yields in bushels per acre.

$$s_x = \sqrt{\frac{111,832.36 - (1,173.8)^2/13}{12}} = 22.07$$
$$s_{\bar{x}} = \frac{22.07}{\sqrt{13}} = 6.12$$

$$s_y = \sqrt{\frac{87,781.43 - (1,033.3)^2/13}{12}} = 21.70$$
$$s_{\bar{y}} = \frac{21.70}{\sqrt{13}} = 6.02$$

$$s_d = \sqrt{\frac{3,663.69 - (140.5)^2/13}{12}} = 13.37$$
$$s_{\bar{d}} = \frac{13.37}{\sqrt{13}} = 3.71$$

$$r_{xy} = \frac{97,975.05 - (1,173.8 \times 1,033.3)/13}{\sqrt{111,832.36 - (1173.8)^2/13} \times \sqrt{87,781.43 - (1033.3)^2/13}} = 0.814$$

that they usually favor Vaughn. But, most important, note that the varieties tend to have the same response to the environments of the different years. This method of comparison screens out similar responses of the varieties to these changing environments. The formula for the calculation of $s_{\bar{d}}$ is

$$s_{\bar{d}} = \sqrt{\frac{\sum d^2 - (\sum d)^2/n}{n(n-1)}} \quad (5.11)$$

For Vaughn and Atlas this is 3.71 (Table 5.4).

The t value ($10.8/3.71$ from formula 5.10) is 2.9. For 12 degrees of freedom (one less than the number of comparisons), the t table gives values of 2.18 and 3.06, respectively, for P values of 0.05 and 0.01. The odds are greater than 19:1, but less than 99:1, that these varieties differ in mean yield at Davis, California.

It is clearly seen that the paired test is much more sensitive than the unpaired test, where comparisons are made over a range of environments. Its use by plant breeders is limited because it permits comparisons of only two varieties. Where several varieties are involved, some form of variance analysis (Chapter 30) will give control of the environment.

Measures of Association

The degree of association between two variables may be measured by correlation or regression. Correlation is used where the variables are not dependent upon one another. For example, the yields of Atlas and Vaughn barley (Table 5.4) over a 13-year period were correlated; both tended to go up in good years and down in poor, without one variety influencing the other. Regression is used when one variable is dependent on another. For example, yield of wheat will be dependent upon average seasonal rainfall; obviously, seasonal rainfall is not dependent on yield.

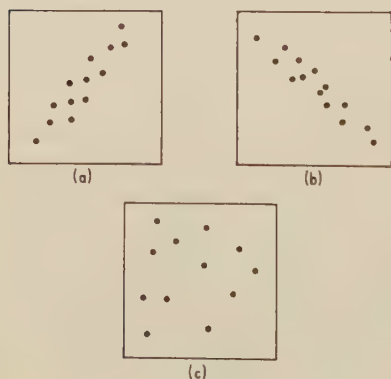


Fig. 5.3 Dot diagrams showing (a) a positive correlation, (b) a negative correlation, and (c) no correlation.

Correlation. Correlations may be positive or negative. The yields of Vaughn and Atlas barley provide an example of positive correlation; both varieties respond in the same manner to growth stimuli. Another example is that of length and width of a sample of bean seed, where both dimensions respond in the same direction to factors of the environment.

Correlations are measured by a correlation coefficient, r , which will vary from 0 to 1.0 for a positive correlation and from 0 to -1.0 for a negative correlation. The larger the coefficient, the stronger is the correlation.

A scatter or dot diagram (Fig. 5.3) will indicate the presence and nature of the correlation. Each dot represents a single comparison, with the two axes representing the two variables. The orientation of the dots in Fig. 5.3a indicates positive correlation; those in 5.3b, negative correlation; and

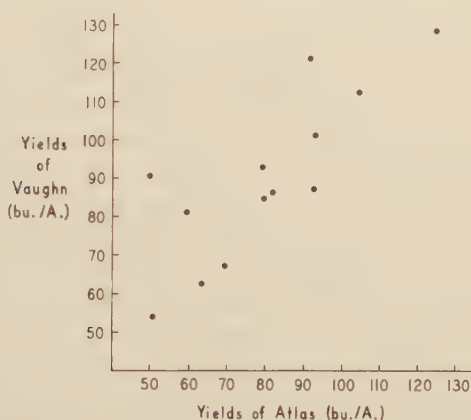


Fig. 5.4 Dot diagram showing relationship of yields of the barley varieties Vaughn and Atlas in different years. Data from Table 5.4.

those in 5.3c, no correlation. Figure 5.4 is a dot diagram showing the positive correlation of the yields of Vaughn and Atlas barley (Table 5.4).

Correlation is calculated by

$$r_{xy} = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sqrt{\sum(x - \bar{x})^2 \times \sum(y - \bar{y})^2}} \quad (5.12)$$

or, for machine calculation,

$$r_{xy} = \frac{\sum xy - n\bar{x}\bar{y}}{\sqrt{[\sum x^2 - (\sum x)^2/n][\sum y^2 - (\sum y)^2/n]}} \quad (5.13)$$

The computations using formula 5.13 for the correlation of the yields of Vaughn and Atlas are given in Table 5.4. The coefficient r_{xy} is 0.814. Its significance is measured by a t test, where

$$t = r\sqrt{(n-2)/(1-r^2)} \quad (5.14)$$

For the barley varieties this becomes $0.814\sqrt{11/(1-0.814^2)} = 4.65$. In Appendix Table A-1 the value of t at the 5 per cent level of significance for 12 degrees of freedom is 2.18. Since the t value for the barley comparison is much greater than this, it may be assumed that there is more than a random association between their yields.

A perfect correlation would be extremely rare in biological material, though values above -0.90 and 0.90 are not uncommon. It is difficult to give a clear interpretation of different values of the correlation coefficient, but values above -0.5 or 0.5 are considered to indicate a close relationship; those between -0.3 and -0.5 (or 0.3 and 0.5), moderately close; and those below -0.3 or 0.3 , little or no relationship. It is sometimes stated that the quantitative relationship between the two variables is given by the square of the correlation coefficient, if complete interdependence is given by 1. In the yields of the barley varieties, with a correlation coefficient of 0.814, this would mean that 0.66 (or 66 per cent) of the variability in one variety is associated with variability in the other. This means also that an increase in the correlation coefficient from 0.5 to 0.6 will increase the association from 0.25 to 0.36 (or 0.11), whereas an increase from 0.8 to 0.9 will increase the association from 0.64 to 0.81 (or 0.17). In other words, differences in the size of the correlation at higher values for r have more meaning than similar differences for low values.

Regression. Regression is used frequently by plant breeders because many characteristics of plants may be dependent on other characteristics. For example, corn yields frequently are higher with later maturity, height may be increased by higher temperatures, and disease ratings may be higher with higher humidities.

The relationship between a dependent and independent variable is given by

$$y = \bar{y} + b(x - \bar{x}) \quad \text{or} \quad \bar{y} - b\bar{x} + bx \quad (5.15)$$

where y is the unknown dependent variable, x is a known value of the independent variable, and b is the regression coefficient of y on x . If b is known, $(\bar{y} - b\bar{x})$ becomes a constant, expressed as a ; then the formula becomes

$$y = a + bx \quad (5.16)$$

The regression coefficient is obtained as follows:

$$b_{yx} = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sum(x - \bar{x})^2} \quad (5.17)$$

or, for machine calculation,

$$b_{yx} = \frac{\sum xy - (\sum x \sum y)/n}{\sum x^2 - (\sum x)^2/n} \quad (5.18)$$

Knowing r_{xy} , the value of b_{yx} may be obtained by

$$b_{yx} = r_{xy} \frac{s_y}{s_x} \quad (5.19)$$

The relationship between oil content (y) and hull content (x) in F_2 safflower plants from a cross of varieties N-1 and N-8 is given in Table 5.5

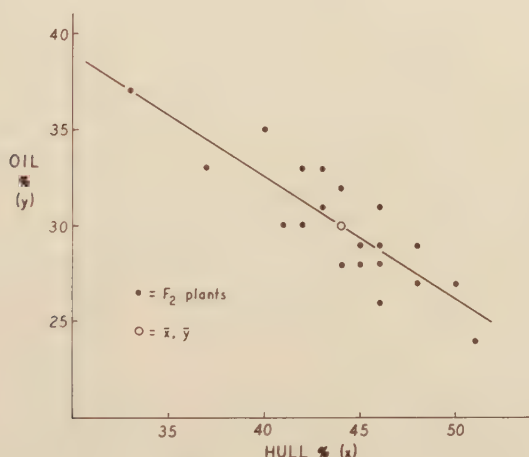


Fig. 5.5 Regression of oil content on hull content in F_2 safflower plants. Data from Table 5.5.

and Fig. 5.5, showing only 20 of the 372 plants studied by Claassen et al.² The oil and hull contents of N-1, N-8, and the F_1 were

	Oil Content, %	Hull, %
N-1	28.0	45.0
N-8	34.0	38.0
F_1	35.9	34.0

Statistics for the F_2 data are given in table 5.5.

Using formula 5.16, and assuming values of x to be 50 per cent and 30 per cent, the corresponding values of y are 26.1 per cent and 39.0 per

Table 5.5 Hull and oil percentage of safflower seed from F₂ plants of a cross between N-1 and N-8.²

PLANT NO.	HULL IN PER CENT, <i>x</i>			OIL IN PER CENT, <i>y</i>			$(x - \bar{x})(y - \bar{y})$
	<i>x</i>	$x - \bar{x}$	$(x - \bar{x})^2$	<i>y</i>	$y - \bar{y}$	$(y - \bar{y})^2$	
1	46	2	4	26	-4	16	- 8
2	44	0	0	28	-2	4	0
3	42	- 2	4	33	3	9	- 6
4	51	7	49	24	-6	36	- 42
5	48	4	16	27	-3	9	- 12
6	43	- 1	1	33	3	9	- 3
7	37	- 7	49	33	3	9	- 21
8	46	2	4	28	-2	4	- 4
9	46	2	4	31	1	1	2
10	33	-11	121	37	7	49	- 77
11	45	1	1	29	-1	1	- 1
12	50	6	36	27	-3	9	- 18
13	48	4	16	29	-1	1	- 4
14	43	- 1	1	31	1	1	- 1
15	46	2	4	29	-1	1	- 2
16	42	- 2	4	30	0	0	0
17	44	0	0	32	2	4	0
18	45	1	1	28	-2	4	- 2
19	40	- 4	16	35	5	25	- 20
20	41	- 3	9	30	0	0	0
Total	—	0	340	—	0	192	-219
Mean	44			30			

$\bar{x}$ (formula 5.2) = 44.0% $\bar{y}$ (formula 5.2) = 30.0% r_{xy} (formula 5.12) = -0.86
 s_x (formula 5.3) = 4.13% s_y (formula 5.3) = 3.18% b_{yx} (formula 5.17) = -0.644
 $s_{\bar{x}}$ (formula 5.6) = 0.95% $s_{\bar{y}}$ (formula 5.6) = 0.71% a (formula $\bar{y} - b\bar{x}$) = 58.34

cent. Joining the points with these coordinates in Fig. 5.5 gives the regression line of *y* on *x*, or oil content on hull content. It will be noted that it passes through a point with mean values for *x* and *y*.

Résumé

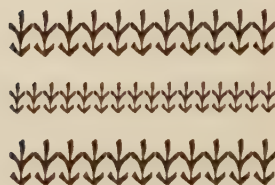
The important lesson to be learned from this chapter is that statistics provide powerful tools to help answer important questions relating to plant breeding materials. However, these questions are elementary. For more difficult questions relating to effects of genes in quantitative inheritance, fates of genes and genotypes in mixed population, and predicted performances, statistics are even more indispensable.

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6

GENES AND QUALITATIVE CHARACTERS



It is easy to see that the occurrence of a large mutation would provide an effective stimulus for human selection, whereas the existence of a range of variation in the same character due to minor genes might not. In this way, the establishment of disease resistance, awnlessness in cereals, some forms of pest resistance, besides such quality characters as grain colour and texture, has been brought about in large measure by selection of major genes.¹³

HUTCHINSON, 1965

ON THE REDISCOVERY of Mendel's work, in 1900, many felt that his laws of inheritance would apply only to conspicuous differences—the qual-

itative differences. They felt that other laws would explain the inheritance of differences of degree, where hybrid populations showed a continuous array of variability instead of falling into discrete classes. Research over the past 50 years has shown that these "blending" characters are in fact determined by genes obeying Mendelian laws, with the distinctive feature that the effect of the individual gene is small. These quantitative characters are discussed in the next chapter.

In most crop plants, one or a few genes may profoundly influence their utilization and economic value. This is perhaps not too surprising when it is realized that primitive man seized quickly upon obviously aberrant types and tested their value. Nor are all such genes discovered. Genetic literature continues to report their occurrence, some of them showing economic promise when they appear, others needing modification by other genes.

From the bewildering array of these genes, some have been selected because of their importance, and some because they illustrated particular actions or interactions of genes. Examples are given more or less in the order of plant development from a seedling to maturity. Genes involved in disease and insect resistance will be considered in later chapters.

As a plant breeder plans the architecture of his varieties, he often wonders what the ideal dimensions should be. What size of seed contributes most to high yield, what color of flower, what type of leaf? These questions have prompted research in recent years to get accurate answers, at least for a number of qualitative characters. This chapter concludes with some methods used in such research.

Inheritance of Crop Characters of Economic Importance

Chlorophyll appears to be very sensitive to influence by genes, perhaps because of the complex nature of the photosynthetic apparatus. Chlorophyll mutations are classified into three general types, based on color. These are albino, virescent, and luteus. Albinos are white in color, and because they have no chlorophyll and usually no chloroplasts, they die in the seedling stage. Virescent types may be almost white in early stages, but turn to various shades of green as they develop. They may eventually be quite normal in appearance, or they may die. Luteus types are yellow in color, and may die in the seedling stage or may complete development if some chlorophyll is present. In corn there are over 100 loci with genes that affect chlorophyll development. Most frequently, genes affecting chlorophyll are recessive.

Genes for *reduced height* have been popular in agronomic crops because the plants are easier to harvest mechanically. A dwarf-internode gene, *di*, has been incorporated into castor bean varieties.²³ Internodes

have been one-quarter to one-half the length of normal internodes, under similar genotypic backgrounds and environments (Fig. 6.1). Besides being easier to harvest, dwarf-internode types have been much more resistant

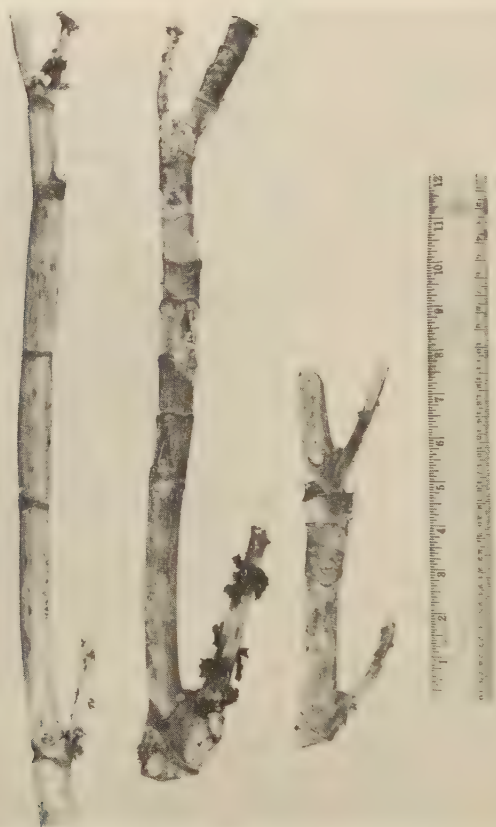


Fig. 6.1 Left, castor bean parent with normal stems having long internodes (genotype *DiDi*); center, short-internode parent (genotype *didi*), with eight instead of four internodes between racemes; and right, a segregate from the cross of the two parents with four short internodes. The segregate combines the short internodes of one parent with the reduced number of internodes in the other. (Courtesy L. H. Zimmerman, U.S.D.A.)

to being whipped about by wind. As a result, they have shattered less seed and have not lodged during storms.

Genes for reduced height have had a dramatic effect upon the success

of sorghum as a seed crop, primarily because shorter types have been easier to harvest mechanically. Recessive genes for short stems have been found at four loci, and their effects are shown in Table 6.1. Varieties

Table 6.1 Effect of genotype on the height of sorghum.¹⁹

VARIETY	GENOTYPE ^a	HEIGHT TO FLAG LEAF, cm	
		Range	Average
Recessive for One Gene			
Durra, Sumac, Shallu, Spur, Feterita, } Tall White Sooner milo, Standard } Yellow milo }	$Dw_1Dw_2Dw_3dw_4$	120-174	150.5
Standard broomcorn	$Dw_1Dw_2dw_3Dw_4$		207.0
Recessive for Two Genes			
Texas Blackhull kafir	$Dw_1Dw_2dw_3dw_4$		100.0
Bonita, Early Hegari, Hegari	$Dw_1dw_2Dw_3dw_4$	82-126	104.3
Dwarf White Sooner milo, Dwarf } Yellow milo }	$dw_1Dw_2Dw_3dw_4$	94-106	100.0
Acme broomcorn	$Dw_1dw_2dw_3Dw_4$		112.0
Japanese Dwarf broomcorn	$dw_1Dw_2dw_3Dw_4$		92.0
Recessive for Three Genes			
Martin, Plainsman	$dw_1Dw_2dw_3dw_4$	52-61	56.5
Double Dwarf White Sooner milo, } Double Dwarf Yellow milo }	$dw_1dw_2Dw_3dw_4$	53-60	56.5

^a Genes in homozygous condition.

with the same genotype varied considerably in height, owing to the effect of *modifying genes*. For example, where varieties were late, the height was increased because lateness is associated with high internode number. On the average, these recessive genes for shortness are cumulative in their effects. If genes had been recessive at all loci, height would have been further reduced about 10 cm.

One of the dwarfing genes in sorghum, dw_3 , has been avoided by sorghum breeders because it is *mutable*. In about one in 1,200 gametes, dw_3 mutates to Dw_3 . A variety with the genotype dw_3dw_3 will have 10 to 15 tall plants per acre, giving the field a ragged appearance. If not removed, there will be still more tall plants the next year.

Stem strength is determined usually by genes with small effects. One recent example of the effect of a single gene is provided by safflower.⁵ The basic effect of the recessive gene, *th*, for thin hull was to reduce the amount of secondary wall thickening in sclerenchyma cells. Since the

hull of the seed is mostly sclerenchyma cells, the hull of *thth* genotypes became very thin on drying (Fig. 6.2). Thinner-walled sclerenchyma cells resulted in weaker stems and branches. Also, because thin-walled sclerenchyma cells failed to open the anthers, practically no pollen was released, and the plants were structurally male-sterile. This is an example of *pleiotropy*, in which different characters are influenced by the same gene.

Fasciation of stems in peas was studied by Mendel and shown to be due to a single recessive gene, subsequently termed *fa*. Fasciation is the fusion, wholly or in part, of branches with the stem. Complete fusion gives a grotesque-appearing plant, often with a coarse, straplike, twisted stem. Flowers and fruits may rise directly from the stem. Studies of F_1 , F_2 , and test-cross materials from crosses of normal and fasciated types by Marx and Hagedorn¹⁷ showed that the *penetrance* of the gene *fa* was incomplete and variable from cross to cross. Penetrance in this instance is the proportion of plants of the genotype *fafa* that showed fasciation; the balance were normal in appearance. *Expressivity* was variable also, with fasciation varying from types in which it was almost imperceptible to those in which the plants were quite abnormal. By selection, presumably for the appropriate modifying genes, completely penetrant lines could be developed, with expressivity remarkably uniform.

Maturity is frequently controlled by genes with small effects. Perhaps the best example illustrating the effect of major genes is provided by sorghum. These genes, along with genes affecting height, have profoundly influenced the distribution and success of sorghum in the United States. Maturity genes are found at three loci, and their effects on earliness are given in Table 6.2.

An interesting feature of these alleles is that they manifest their effects

Table 6.2 Sorghum genotypes and their effects on number of days to anthesis.¹⁸

NO.	GENOTYPE	NUMBER OF DAYS	
		TO ANTHESIS	MATURITY
1	<i>Ma</i> _— <i>Ma</i> ₂ — <i>Ma</i> ₃ —	92-106	Ultralate
2	<i>Ma</i> _— <i>Ma</i> ₂ — <i>ma</i> ₃ <i>ma</i> ₃	92-106	Ultralate
3	<i>MaMa</i> <i>ma</i> ₂ <i>ma</i> ₂ <i>Ma</i> ₃ —	76-88	Late
4	<i>Mama</i> <i>ma</i> ₂ <i>ma</i> ₂ <i>Ma</i> ₃ —	90-98	Late heterozygous
5	<i>MaMa</i> <i>ma</i> ₂ <i>ma</i> ₂ <i>ma</i> ₃ <i>ma</i> ₃	64-74	Intermediate
6	<i>Mama</i> <i>ma</i> ₂ <i>ma</i> ₂ <i>ma</i> ₃ <i>ma</i> ₃	76-88	Intermediate heterozygous
7	<i>mama</i> <i>Ma</i> ₂ — <i>ma</i> ₃ <i>ma</i> ₃	46-60	Early
8	<i>mama</i> <i>ma</i> ₂ <i>ma</i> ₂ <i>Ma</i> ₃ —	46-60	Early
9	<i>mama</i> <i>ma</i> ₂ <i>ma</i> ₂ <i>ma</i> ₃ <i>ma</i> ₃	46-60	Early

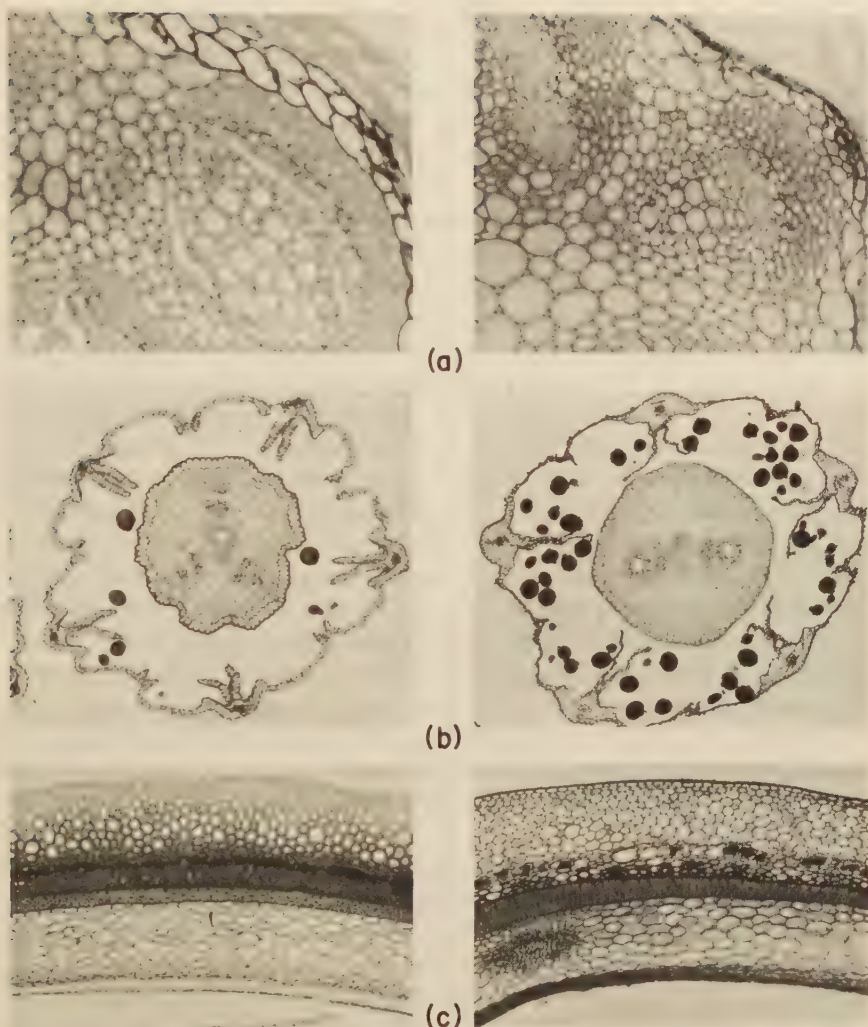


Fig. 6.2 Pleiotropic effect of *th* gene in safflower. The *thth* genotype has weak stems, fails to release pollen, and has thin hulls on the seed. The primary effect of the *th* gene, when homozygous, is to reduce secondary thickening of fibrous cells, which is shown above in the figures on the right. (a) a cross section of stems with a bundle of fibrous cells to the right of vascular tissue ($\times 160$). (b) cross section of anther tube and style after style elongation, showing most of pollen remaining in *thth* genotype, a result of the inner walls of the anther failing to swing open during style elongation ($\times 60$). Bottom: hulls (pericarp above and seed coat below) 20 days after fertilization, showing thin walls of pericarp of *thth* genotype ($\times 72$). The thin walls of the pericarp collapse on drying. (Courtesy Dr. W. W. Ebert, Sonoma State College, Rohnert Park, California.)

only under day lengths longer than 14 hr. With a 10-hr day, all genotypes are early. Considering one locus, the allele for earliness is recessive to the allele for lateness. The recessive gene *ma*, when homozygous, is *epistatic* to the dominant genes *Ma*₂ and *Ma*₃. This simply means that the effects of *Ma*₂ and *Ma*₃ are not expressed in the presence of *mama*, or that the plant is early. It was expected that the heterozygous genotype *Mama* (No. 4) would be intermediate between *MaMa* (No. 3) and *mama* (No. 8). Instead, it was later than the late parent. This would appear to be due to an interaction between *Ma* and *ma*, termed *intra-allelic interaction*. It is apparent from these data that four homozygous phenotypes are possible: ultralate, late, intermediate, and early. The sorghum breeder may pick the combination of genes that best fits his latitude, altitude, and rainfall pattern. As Quinby and Karper say it, they are "adaptation genes."¹⁸

Flower color and shape have long been favorite subjects for genetic studies, particularly in ornamental crops. In flax, flower characteristics may be affected by genes at eight loci.⁴ Some of the alleles affect anther or seed color also, or both, and are thus pleiotropic in their action.

Pollen characteristics or behavior may be under genetic control. Loege-ring and Sears found a gene *Ki* in wheat which killed all pollen grains with the genotype *ki* in a heterozygous plant (*Kiki*).¹⁵ Such pollen grains (50 per cent of the total) degenerated shortly after the second division of meiosis, and at the time of anthesis were almost empty. Megaspores of heterozygous plants were unaffected. The pollen-killer gene gave rise to distorted genetic ratios where it was closely linked to other genes, and actually was found because of its effect on the inheritance of resistance to stem rust.

Nectarless, a condition in muskmelon flowers, was found to be due to a single recessive gene *n*.³ The genotype *nn*, which had smaller than normal nectaries, produced little or no nectar and was unattractive to insects. Without the insect visits, pollinations were reduced and yields were low.

Seed color is frequently included in the description of market types of classes of farm crops. Mostly, these seed colors have a rather simple inheritance, though several genes may be involved. One example from corn is given below. The list could have been extended to include beans, wheat, oats, rye, barley, peas, sorghum, rice and several other crops.

Kernel color in corn may be in the pericarp, which is maternal tissue, or in the endosperm.⁶ If in the endosperm, it may be in the aleurone, which is the outer layer of cells, or throughout the endosperm. Endosperm colors are determined by the genotype of the endosperm and not by the genotype of the maternal tissue. This means that *F*₂ segregations are obtained from the kernels on the ears of *F*₁ plants. The genes *A*₁, *A*₂,

A_3 , C , and R must be present for red or purple color in the aleurone; these are called *basic color genes*. If Pr is present, the color is purple; if pr , it is red. The basic color factors are *complementary* in their effects. If crosses are made between varieties recessive for a different locus and both colorless ($CCrr \times ccRR$), the F_2 kernels will segregate nine colored ($C_R_$) and seven colorless (C_rr , $ccR_$, and $ccrr$).

If a dominant inhibitor gene I is present, it will erase the effects of all these color genes. If two varieties have all color genes present, but one has the genotype II (colorless) and the other ii (colored), the F_2 will segregate in a ratio of three colorless ($I_$) to one colored (ii). However, if the genotypes are $CCII$ (colorless) and $ccii$ (colorless), and all other basic color factors are present, the F_2 will segregate in a ratio of 13 colorless (9 $C_I_$, 3 $ccI_$, and 1 $ccii$) to 3 colored (C_ii).

In corn and in other crops where the endosperm or embryo characteristics may be affected by the genotype of the pollen, the phenomenon is called *xenia*. The term is usually reserved, however, not for the segregations discussed above, but for situations where two varieties distinct in their endosperm characteristics are crossed and the characteristics of the endosperm of the male parent are visible to a greater or lesser degree in the kernels of the female. This is a problem in the production of sweet corn, where stray pollen of field corn will be manifested as starchy instead of sugary kernels.

Chemical composition of crop products is often under the control of one or a few genes. Corn provides several genes effecting important changes in the nature of the *carbohydrates* in the endosperm. Some of the more important of these are:

1. fl , for floury endosperm, giving kernels consisting almost entirely of soft starch. Flour corns are used by American Indians for home use.

2. su , for sugary endosperm, with kernels so high in sugar that they are wrinkled and translucent when dry. All sweet corns have the genotype $susu$.

3. Y , for yellow endosperm, which is high in provitamin A, and characteristic of most varieties grown for feeds. y gives white endosperm, which is preferred in the manufacture of breakfast foods.

4. wx , for waxy endosperm, consisting mostly of amylopectin, and useful in the manufacture of adhesives.

5. ae , for endosperm high in amylose, used in the manufacture of plastics.

Oil quality is determined largely by fatty acid composition, and is mostly influenced by genes with small effects. However, in a few cases

one or two genes may completely change the characteristics of an oil. Three allelic genes, *Ol*, *ol*¹ and *ol*, determine in large part the proportions of linoleic and oleic acid in safflower oil.¹⁴ Their effects are listed in Table 6.3.

Table 6.3 Effects of alleles at one locus on fatty acid composition and iodine value of safflower.¹⁴

GENOTYPE	LINOLEIC ACID, %	OLEIC ACID, %	IODINE VALUE
<i>OlOl</i>	75-80	10-15	137-145
<i>ol¹ol¹</i>	42-54	35-50	115-125
<i>olol</i>	12-30	64-83	85-100

Commercial varieties have the genotype *OlOl*, and give an oil useful both in paints and margarines, whereas at the other extreme, *olol* yields an oil very similar to olive oil chemically and useful as a cooking oil. Genes at two loci determine the presence and amount of erucic acid in the seed of rape.¹⁰

Toxic substances have been removed from crop plants by breeding, and in the process it has been found that the presence of some of them is determined by a few genes. Coumarin is an example of one of these. This bitter organic compound is present in most species of sweet clover (*Melilotus*). Besides making the forage unpalatable, the anticoagulant dicumarol is evolved from coumarin if sweet clover hay and silage are allowed to mold under certain conditions. Blood of animals eating large amounts of such feed fails to coagulate, and they may die from internal bleeding. The presence or absence of coumarin in *M. alba* was determined by the alleles *Cu* and *cu*, with the genotype *cucu* deficient in coumarin.⁸ A second gene *b* changed the free coumarin to the bound form.

Resistance of plants to pesticides is often the basis for their successful use. In barley a single recessive gene gave resistance to DDT.¹¹ Susceptible plants became chlorotic when sprayed with DDT in seedling stages, which usually resulted in the early death of plants, and surviving plants were unproductive. In Chapter 18 it will be seen how these alleles may be used to facilitate the production of hybrid varieties of barley.

Even *symbiotic relationships* may be under relatively simple genetic control. In soybeans, a recessive gene *no* prevented nodulation by *Rhizobium*.²² Sister strains with genotypes *NoNo* and *nono* gave similar yields and chemical composition, when nitrogen was supplied. In the absence of nitrogen, and with *Rhizobium* present, the genotype *nono* had a yellow

leaf color, gave about half the yield of *NoNo*, and produced seed with reduced protein content.

Measurement of the Value of Genes
or Certain Morphological Characteristics

The value of the genes that have been discussed so far is rather obvious. Some genes, however, are difficult to evaluate, even though their effects on the morphology or development of the plant are clearly evident. Such genes would be those affecting flower color or type, plant height, branching characteristics, or earliness. In particular, plant breeders are interested in the associations of these characters with yield. Two procedures have been used to evaluate morphological characters, both involving the use of isogenic lines. *Isogenic lines* have the same genic constitution except for alleles at one or a few loci.

The first of these requires the addition of the character to a variety by backcrossing; the details of the process are given in a later chapter. If sufficient backcrosses are made, usually in the range of eight to ten, a character with a simple genetic basis may be transferred to another variety. In this way two versions of the variety are available, one with the character and one without. The advantage of this method is that it permits the evaluation of the character in a variety that is in use and adapted to a particular environment. If the new character does improve yield, the plant breeder has it incorporated in a variety accepted by farmers and the trade and fit for immediate release. The disadvantage of the method is that it tests the gene in only one genetic background. In

Table 6.4 Comparison over a period of three years of awned and awnless versions of Baart and Onas wheat in tests grown at 18 stations in western United States and Canada.²¹

CHARACTER AND AWN EXPRESSION	BAART VERSUS AWNLESS BAART ^a	AWNED ONAS VERSUS ONAS ^b
Mean yield (bu/acre)		
Awnless	39.4	47.8
Awned (difference)	4.1	1.8
Kernel weight (g/1,000 kernels)		
Awnless	41.5	36.8
Awned (difference)	1.6	2.8
Test weight (lb/bu)		
Awnless	60.1	58.2
Awned (difference)	1.1	0.9

^a Onas × Baart, backcrossed ten times to Baart.
^b Baart × Onas, backcrossed nine times to Onas.

other varieties, with different genotypes, the character might have different effects.

Using this method, Suneson and his associates obtained a measure of the value of awns in wheat.²¹ Starting with two different varieties Onas (without awns) and Baart (with awns), awns were transferred from Baart to Onas, and the awnless condition passed from Onas to Baart. At the end of the backcrossing program there were awned and awnless versions of these two varieties. The awned version of each variety (Table 6.4) exceeded the awnless in yield by about 7 per cent and had higher test weight and kernel weight. When these data became available, awned Onas, called Onas 53, was released for commercial production.

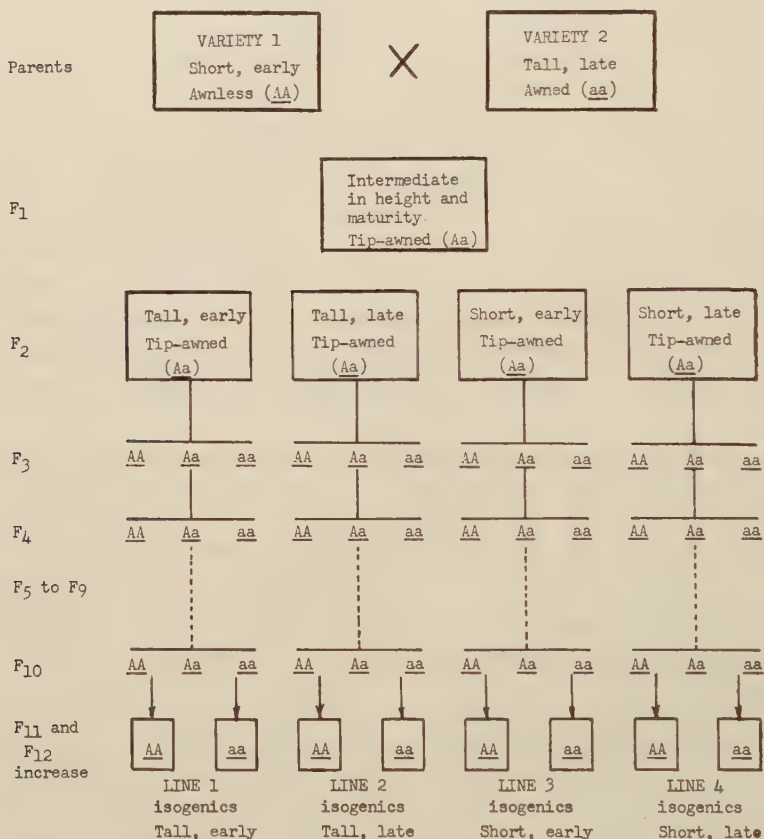


Fig. 6.3 Development of four different pairs of isogenic lines, one member of the pair awnless (AA), and the other awned (aa). It is assumed that the heterozygote may be identified because it has short tip-awns.

The second method, particularly adapted to self-pollinated species, involves the production of a series of isogenic lines from a cross of varieties with and without the character under study.¹ In the F₂, selections are made from different agronomic types, enough to represent the range of variability. All selections, however, should be heterozygous for the genes governing the character under study. For several generations these selections are propagated from single plants heterozygous for this character. This is illustrated in Fig. 6.3, where four different agronomic types are selected in F₂, each heterozygous for the locus determining the presence of awns. Since it is a self-pollinated species, with each line propagated from a single plant, the genes at all other loci will rapidly become homozygous (see Chapter 10 for details of the effect of selfing on homozygosity over several generations). This means, in this example, that at the end of ten years, four homozygous pure lines will be available, each in two versions, one with awns and one without. During the eleventh and twelfth generations, the four paired lines are increased to provide seed for testing purposes.

By using several isogenic lines the morphological character under study may be compared against a variety of genetic backgrounds. Thus, conclusions from the study will have wider application. One disadvantage, of course, is that the derivative types probably will not be usable commercially, since no particular effort is made to develop lines that are closely adapted to the environment or high in quality. The method is

Table 6.5 Comparison of ten awned and awnless isogenic lines of wheat from the cross Kanred X Clarkan^a grown at Denton, Texas.²

RESULTS REPORTED	1947	1948	1950	1952	AVERAGE
Yields (bu/acre)					
Awnless	24.8	31.6	15.6	27.4	24.9
Awned (difference)	2.2 ^b	1.2	2.1	−0.5	1.1
Kernel weight (g/1,000 kernels)					
Awnless	28.0	31.1	26.9	28.7	28.7
Awned (difference)	1.6 ^b	2.6	3.1	1.2	2.1
Kernels per head					
Awnless	16.6	18.7	17.0	22.9	18.8
Awned (difference)	0.5	− 0.7	− 0.4	− 0.4	− 0.2
Weather	Some stress for moisture	Favorable moisture	Very dry spring	Adequate moisture	

^a Selections made ten generations from the cross, and awned and awnless types increased.
^b The difference is highly significant (at the 1 per cent point).

not adapted for use in cross-pollinated crops because the selfing to produce homozygosity would bring inbreeding depression with it. Atkins and Norris,² in a study of the effects of awn on yield and other characters in wheat, obtained results similar to those of Suneson et al.²¹ described in Table 6.4. Awns did increase yields, particularly in dry years (Table 6.5).

Both types of isogenic lines differing in awn development raise the same questions about the basis for the higher yields of awned types. Are the higher yields due to some pleiotropic effect of the gene or genes for awns, or are they due to closely linked genes? In the case of awns, neither pleiotropy nor linkage is involved. Instead, the increase in yield appears to be due to a beneficial effect of the awn itself.⁹ Awns provide photosynthates that are readily available to the developing seed. As expected, the increased seed weight in awned types is primarily starch. The advantage that the awn provides is expressed to the greatest degree under conditions of moisture stress.

In breeding a semismooth-awned version of Atlas barley, it was found that the semismooth derivatives in the tenth backcross outyielded both rough-awned Atlas and rough-awned sibs. A careful study indicated that genes linked to the gene for semismooth awns were responsible for the higher yields.⁷ The linkage was close, as was expected from persistence of the association through ten backcross generations.

Résumé

It is sometimes stated that plant breeders have exhausted the possibilities of improvements of crop plants by using genes with major effects. The evidence, however, of many of the examples presented here contradicts this. It should be remembered, too, that only a few examples have been presented in this chapter, and no reference has been made to genes being exploited in the production of hybrid varieties and genes involved in resistance to diseases and insects.

As Hutchinson has pointed out, a major gene often has several genes modifying its expression, as is the case for the genes reducing the stem length of sorghum. Frequently, genes with major effects are unusable until they are incorporated into the proper genotypic background. In castor beans, for example, the dwarf internode gene, *di*, was not particularly successful when substituted for its *Di* allele in commercial varieties by backcrossing. Its best success was realized when attention was given to the development of a background genotype particularly suited to it. In safflower it appears that the characters stem strength, pericarp thickness and pollen release, all affected by the *th* gene, may be independently modified by genes with small effects. The gist of this is

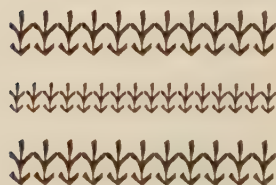
that plant breeding has been, is, and will be a matter of managing genes with effects over a wide range.

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7

QUANTITATIVE INHERITANCE



Polygenes are the genes of fine adjustment, clothing, as it were, the indispensable skeleton of major genes, and moulding the whole into the fine shape demanded by natural selection. They are the genes of smooth adaptive change and appear to be at the root of speciation. They are also the genes with which the plant and animal breeder is generally concerned in his endeavors to produce improved forms.

MATHER, 1949 *

THE IMPORTANCE of quantitative characters has encouraged research into the nature of their inheritance, since this will lead to more intelligent approaches to breeding programs for their improvement. Rapid

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advances have been made recently in developing theory and procedures for the inheritance of quantitative characters, and progress will continue in the future. It is a field of study requiring considerable training in mathematics and its application to biological material.

The purpose here is to give something of the history of the idea of applying Mendel's laws to the inheritance of quantitative characters, and to introduce some of the concepts of gene action that are involved. The student who is interested in details should consult such texts as those by Falconer,³ Mather,^{7,8} and Smith.¹¹

Terminology of Gene Action

Studies of the effects of genes on quantitative characters frequently refer to the type of gene action, without specifying the number of genes

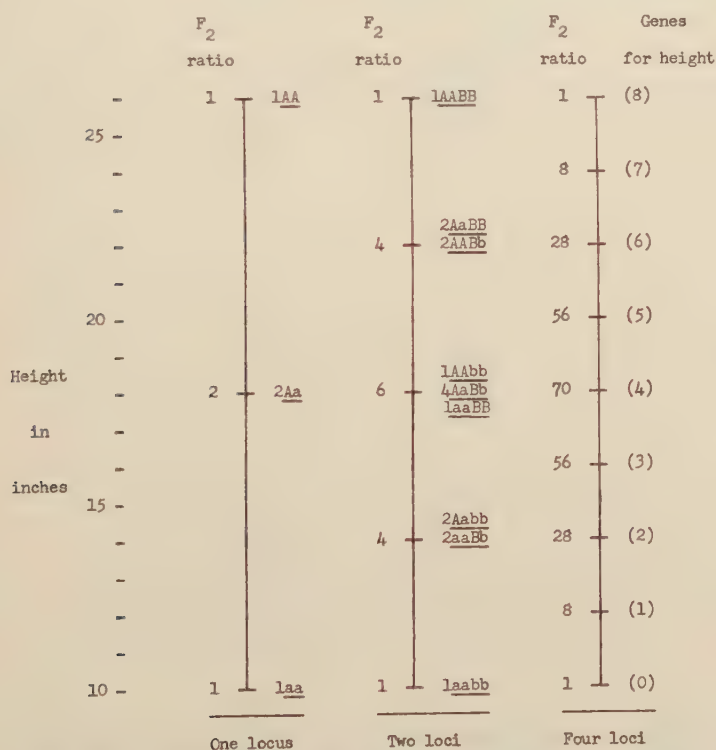


Fig. 7.1 Additive gene action in F_2 , assuming parents differing at one, two and four loci. Alleles A and B are assumed to contribute equally to height. For the four-loci model the number of genes, all equal in their effect, contributing to height are given in parenthesis.

involved. The type of gene action is not different from that for qualitative characters except that the effect of any one gene is small and the effects may be strongly influenced by the environment. Actually, the distinction between qualitative and quantitative effects of genes is one of degree; gene effects do not always fall neatly in one class or the other. The examples given below are assumed to apply to height.

Additive gene action. The word "additive" applies to relationships of genes at the same locus. Applied to two alleles, A and a , affecting height, the increase of AA over aa is twice that of Aa (see Fig. 7.1). If two loci are involved, with one parent $AABB$ and the other $aabb$, the F_1 , $AaBb$, will be intermediate. The F_2 will segregate as shown in Fig. 7.1, with five phenotypic classes. If there were four loci segregating, there would be nine phenotypic classes, assuming that the effects of the alleles for height at each locus would be the same.

As the number of loci increases, and assuming that the extremes in height do not change, the height classes are closer together. The frequencies in all cases are symmetrical, with the most frequent class being intermediate between the parents. Actually, with some environmental effects the height classes will not be discrete, but will overlap. This is illustrated in Fig. 7.2, where the distribution of each height class in the two-loci

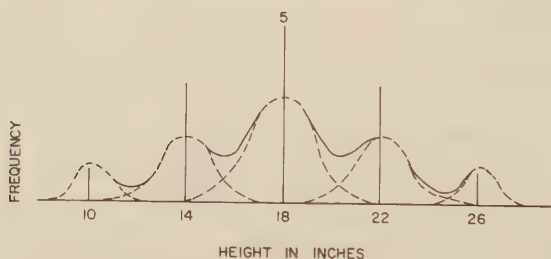


Fig. 7.2 Distribution of F_2 plants in height classes for two-loci model of Fig. 7.1, assuming some variation due to environment. The dotted lines represent the distribution of the separate height classes, if they could be studied separately, and the solid line the distribution actually obtained.

model of Fig. 7.1 is shown as a dotted line. If the overlapping areas were added together, the distribution would be represented by the solid line. Such a distribution, if found in a study of height, would clearly indicate the operation of two genes with additive effects. But if there were more

classes, and the environmental effects were greater, the distribution would take on the shape of the normal curve.

In Figs. 7.1 and 7.2 it has been assumed that the effects of *A* and *B* are the same. However, the effects of alleles at different loci may be quite different; that is, the effect of *A* may be quite different from the effect of *B*. Note that with additive gene action, the phenotype faithfully reflects the genotype, assuming no environmental effects. This means that selection for a particular genotype is accurate within the limits imposed by environmental effects.

Dominant gene action. Dominance applies to the relationships of alleles at the same locus. Complete dominance assumes, in our example of height, that *Aa* and *AA* are the same height. With two loci, and the dom-

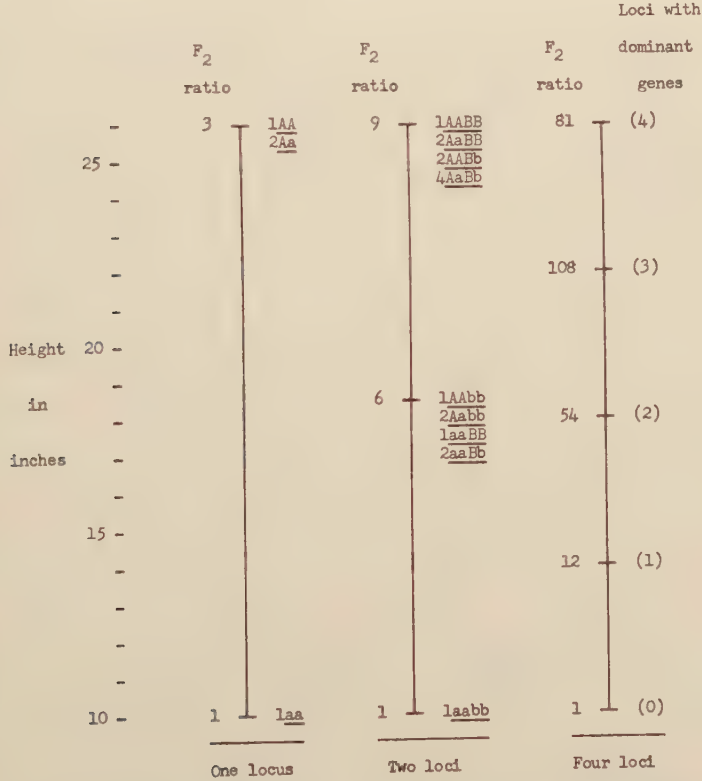


Fig. 7.3 Dominant gene action in F₂, assuming parents differ at one, two and four loci. Alleles *A* and *B* are equal in effect, and dominant to *a* and *b*, respectively. For the four-loci model the number of loci with dominant alleles present are given in parentheses.

inant alleles at each locus similar in effect, the distribution of the genotypes in F_2 is shown in Fig. 7.3. With four loci involved, there are five classes. In other words, the number of classes is one more than the number of loci, assuming, of course, that the alleles at each locus are similar in effect.

Note that the distribution is skewed, with the mode in the direction of dominance. This is quite obvious in situations where a few loci are in-

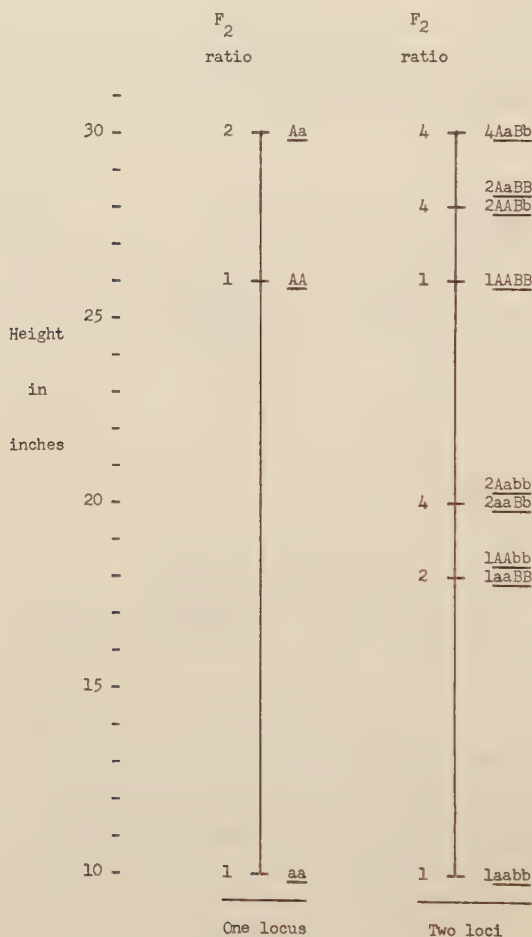


Fig. 7.4 Overdominant gene action in F_2 , assuming parents differing at one and two loci. Alleles A and B are equal in effect and both interact in a similar manner with a and b respectively.

volved. In actual fact, however, with a large number of loci segregating and strong environmental effects, the distribution again resembles a normal curve.

Note that the effect of an additional *A* in going from the genotype *aa* to *Aa* is quite different from that experienced when going from *Aa* to *AA*. Selection for a particular genotype is not so effective as with additive gene action because it is not possible to distinguish between *Aa* and *AA* by their appearance.

Overdominance. If overdominance were present in the example of height (Fig. 7.4) the genotype *AA* would be 26 in. high, *aa* would be 10 in., and *Aa* would be more than *AA*, perhaps 30 in. In other words, there

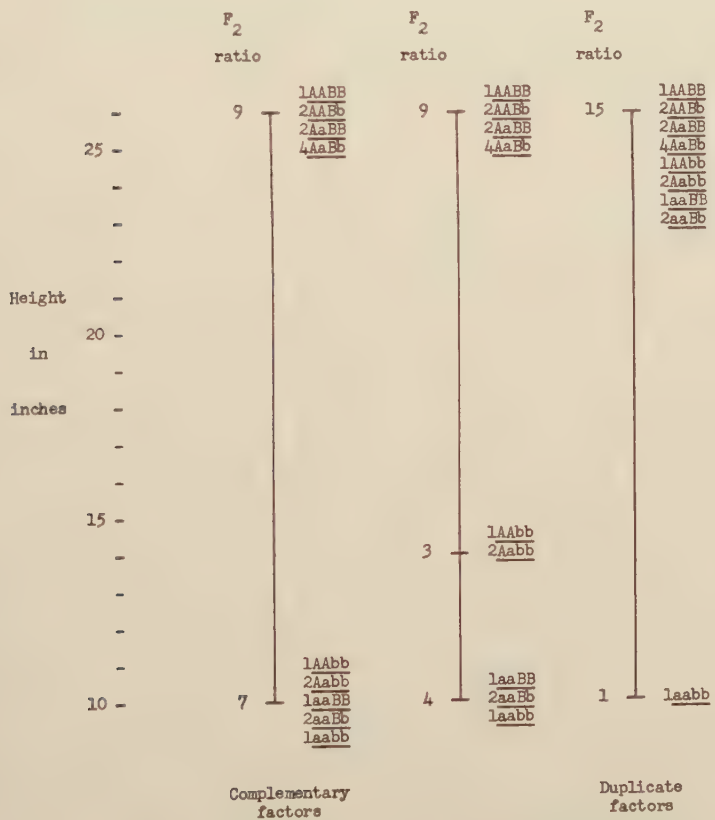


Fig. 7.5 Some examples of epistatic gene action involving two loci. Segregations are a reflection of interactions between genes at separate loci.

is some interaction between A and a to increase height. Note that it is impossible to "fix" the higher segregates in terms of developing types homozygous for the highest class.

Gene relationships at a single locus frequently occupy some point intermediate between additive, dominant, or overdominant gene effects. There is evidence to show that in one environment their expression may take one form, and another form in another environment.

Epistasis. Originally, and usually with qualitative characters, epistasis is the term used for the situation where a gene covers up the effect of another gene at another locus. In quantitative inheritance the term has been extended to include all situations where genes interact at two or more loci. A few of these situations are pictured in Fig. 7.5. Note the interactions involved. In 9:7 and 9:3:4 ratios, dominant genes at two loci interact to increase height, whereas in a 15:1 ratio, recessive genes at two loci interact to reduce height. Other dihybrid ratios involving interactions between loci are 13:3 and 12:3:1.

Modifying Genes

A large number of genes have no known effect except to intensify or diminish the expression of a major gene. The classical experiment showing the effects of modifying factors was carried out on the hoods of rats by Castle and Philips.¹ By continued selection they could modify the size of the hood over a wide range. The size of the pattern was inherited, but the effects of the individual genes were obviously very small.

The effect of modifying genes on heading date was observed in segregating populations derived from a cross between Ramona and White Federation varieties of wheat. The cross was made by the senior author while breeding a stem rust-resistant strain of Ramona that retained Ramona characteristics, its earliness in particular. Heading date was used as a measure of earliness. The usual difference in heading date between the two varieties is about seven days, and no difficulty was encountered in classifying the plants in the F_2 into a 3:1 ratio of early and late plants, and those of the first backcross of the F_1 to White Federation into a 1:1 ratio, the early and late classes being similar to the parents. None of the early plants, however, was as early as Ramona, and none appeared in any numbers until the fifth backcross generation to Ramona. It appeared that heading date in the hybrid was governed by one major gene and a number of modifiers.

The effect of modifiers is of concern to plant breeders working with most crops. Modifiers will be most certainly involved in producing small but important differences in such flower characteristics as different shades of color, slight changes in shape, and subtle variations in fragrance.

Likewise in fruits, modifiers will change appearance, aroma, and taste.

As more and more major genes are concentrated in the commercial varieties of any area, the modifying genes become increasingly important. Frankel⁴ emphasized that viewpoint in discussing the problems of breeding for yield. He believed that wheat breeding in New Zealand had reached the point where further progress was unlikely through adjustment of qualitative characters, but would require the management of genes with small quantitative effects. He further pointed out that the effects may be so small that they will be recognized only with difficulty and an uncertainty so great that breeding efforts and genetic studies along such lines may be of doubtful value.

Multiple Factors

The concept that multiple factors determine the inheritance of quantitative characters was introduced about ten years after the rediscovery of Mendel's laws. First to advance the concept was Nilsson-Ehle,⁹ from studies of kernel color in wheat and oats. He found that wheat kernel color, as between white and various shades of red, was determined by genes at three loci. The allelic genes at these loci are R_1 and r_1 , R_2 and r_2 , and R_3 and r_3 . The only genotype giving white kernels is $r_1r_1r_2r_2r_3r_3$, and all the rest give red. To the extent that classifications are based on red or white kernels, this would be called a qualitative character. Ratios of 3:1, 15:1 and 63:1 red to white kernels were observed in F_2 populations, depending on the genotype of the parents. For example, a cross of $R_1R_1R_2R_2r_3r_3 \times r_1r_1r_2r_2r_3r_3$ gave a ratio in F_2 of 15:1 redkerneled and whitekerneled plants, respectively.

The quantitative aspect of this study was involved in the intensity of the red color. It was found that the genes R_1 , R_2 , and R_3 increased the depth of red color as their numbers increased from one to six. This meant that in the cross of $R_1R_1R_2R_2r_3r_3 \times r_1r_1r_2r_2r_3r_3$, the following types were found in F_2 (r_3r_3 is omitted, since it is common to both parents):

1 $R_1R_1R_2R_2$	1 very dark red
2 $R_1R_1R_2r_2$ and 2 $R_1r_1R_2R_2$	4 dark red
4 $R_1r_1R_2r_2$, 1 $R_1R_1r_2r_2$, 1 $r_1r_1R_2R_2$	6 medium red
2 $R_1r_1r_2r_2$, 2 $r_1r_1R_2r_2$	4 light red
1 $r_1r_1r_2r_2$	1 white

It is apparent that the gene action is additive, tending toward slight dominance of R_1 , R_2 and R_3 over r_1 , r_2 , and r_3 , respectively.

These studies demonstrated the genetic basis of *transgressive segregation*. Transgressive segregation occurs when crosses of two parents give F_2 populations exceeding those of both or one of the parents in the character under study. When wheat varieties of the genetic constitution

$R_1R_1r_2r_2$ and $r_1r_1R_2R_2$ (both medium red) were crossed, the F_2 population was the same as that given in the preceding paragraph. In other words, two parents with medium-red kernels yielded F_2 ranging from very dark red to white.

East was active in research on the inheritance of quantitative characters at an early date, though perhaps his best paper² dealt with the inheritance of length of corolla in a cross between varieties of a wild species of tobacco (*Nicotiana longiflora*). He found corolla length to be particularly valuable for such studies because it was little affected by environment. His data, typical of many studies of quantitative characters, are given in Table 7.1 and Fig. 7.6. His postulates, which are still valid, were as follows:

1. Crosses between varieties or races that are uniformly homozygous, either through inbreeding or self-fertilization, should give an F_1 population of uniformity comparable to that of the parents.

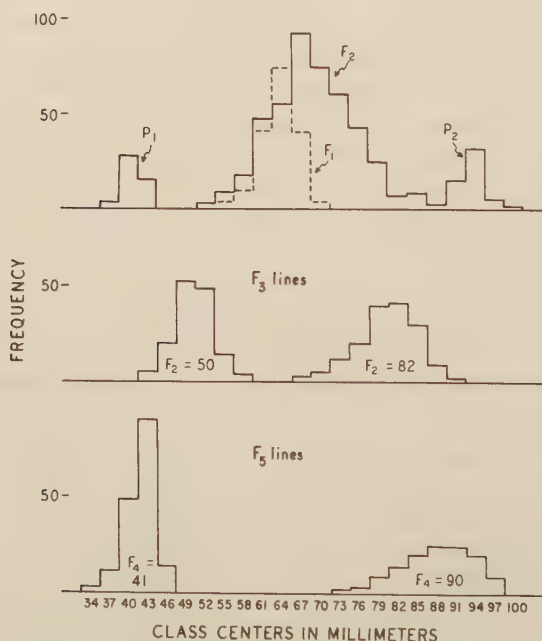


Fig. 7.6 Frequency distributions for corolla length of parents, F_1 , F_2 , F_3 lines, and F_5 lines from a cross of two varieties (P_1 and P_2) of *Nicotiana longiflora*. For F_3 and F_5 lines the corolla length of the parent plant is indicated.²

Table 7.1 Frequency distributions for corolla length of two varieties (P_1 and P_2) of *Nicotiana longiflora* and different hybrid generations from a cross between them.²

GENERATION AND YEAR GROWN	CLASS CENTERS, mm																				STATISTICS ^a						
	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100	n	$\bar{x}$	s_x	CV
P ₁ 1911																								125	40.5	1.75	4.33
P ₁ 1912	1	4	28	16																				49	40.6	2.00	4.92
P ₂ 1911																								88	93.2	2.29	2.46
P ₂ 1912																								57	93.4	2.23	2.39
F ₁ 1911																								173	63.5	2.92	4.60
F ₂ (1) 1912																								211	67.5	5.91	8.75
F ₂ (2) 1912																								233	69.8	6.79	9.73
F ₂ Total																								444	68.8	5.91	8.59
F ₃ 1913																								147	50.2	3.17	6.31
F ₃ 1913																								162	80.2	4.76	5.93
F ₄ 1914																								189	46.2	1.87	4.04
F ₄ 1914																								195	82.3	3.30	4.01
F ₅ 1915																								161	42.0	2.30	5.50
F ₅ 1915																								125	87.9	5.52	6.28

^a For computation of statistics see Chapter 5: n = number of plants; $\bar{x}$ = mean; s_x = standard deviation; and CV = coefficient of variability.

2. The variability of the F_2 population from such crosses should be much greater than that of the F_1 population.

3. When enough F_2 individuals are available, the grandparental types should be recovered.

4. Individuals from various points on the frequency curve of an F_2 population should give F_3 and later populations that differ markedly in their means.

An examination of the data of Table 7.1 reveals that the requirements proposed by East have been met for the most part. The F_1 was little more variable than the more variable parent. With both the parents and the F_1 , single genotypes were involved, the parents being homozygous and the F_1 highly heterozygous.

The variability was much greater in the F_2 than the F_1 , as expected, because it consisted of a number of genotypes, each of which would give a characteristic corolla length.

The grandparental types were not recovered, although the longest corolla length overlapped the lower limits of the one parent. Had a large enough population been grown, the grandparental types would have been recovered.

Populations carried into F_4 and F_5 generations were markedly different in their means, indicating that they possessed different genotypes. These represent segregation products that would occur only if the parents differed in the genes governing corolla length.

The concept of multiple factors visualized each gene, or at least each locus, as operating independently in its effect on quantitative characters. Genes were considered to be additive, and somewhat similar in their effects.

In any case, East's data may be analyzed on these assumptions. If one gene pair were involved, the extreme genotypes would be AA and aa , each making up $\frac{1}{4}$ of the F_2 population. With two gene pairs involved, each parental type would make up $(\frac{1}{4})^2$ of the population, and with n gene pairs, $(\frac{1}{4})^n$. East found that only one of the 444 F_2 plants approached the corolla length of the short parent. With four gene pairs, $(\frac{1}{4})^4$ or one out of 256 plants would be expected, and with five gene pairs, one out of 1024. The assumption of five gene pairs appears more reasonable, particularly since the actual population of 444 plants did not give any quite so short as the short parent. On this assumption the short parent would have the genotype $aabbccdde$ and the long parent $AABBCCDDEE$, making ten genes responsible for the difference between them of 52.7 mm (93.2 — 40.5). Each gene from the long parent could be assumed to contribute 5.3 mm to the length of the corolla tube. On this basis the F_1 ,

AaBbCcDdEe, would have a length of 67.0 mm [$40.5 + (5 \times 5.3)$], which is rather close to the actual value of 63.5 mm. With the parents differing by five gene pairs, there would be 11 phenotypic classes in the F_2 . Computations of F_2 genotypes will be rather involved (there would be 3^5 , or 243, genotypes), but will be facilitated if it is kept in mind that all gene pairs are similar. In this way one can use the expansion of the binomial ($a + b$) to the tenth power (this expansion is used because ten genes appear in each genotype). The expansion becomes

$$\begin{aligned} &a^{10} + 10 a^9b + 45 a^8b^2 + 120 a^7b^3 + 210 a^6b^4 + 252 a^5b^5 + 210 a^4b^6 \\ &\quad + 120 a^3b^7 + 45 a^2b^8 + 10 ab^9 + b^{10} \end{aligned}$$

The prefixes represent the number of individuals expected in each size class. The powers of *a* represent the numbers of genes from the short parent, and those of *b* the number of genes from the long parent. Hence, a^{10} would represent the short parent, b^{10} the long parent, and a^9b the genotypes *Aabbccdde*, *aaBbccdde*, *aabbCcdee*, *aabbccDdee*, and *aabbccddEe*. The expected and actual values are compared in Table 7.2.

Table 7.2 The probability of the occurrence of individuals in the various corolla length classes postulated on the basis of five segregating allelic pairs.^a

CLASS CENTERS, mm	THEORETICAL FREQUENCIES, NUMBER	ACTUAL FREQUENCIES, NUMBER	EXPECTED FREQUENCY FOR 444 INDIVIDUALS, NUMBER
40	1	0	0.4
46	10	0	4.3
52	45	7.5	19.5
58	120	46.0	52.0
64	210	125.0	91.1
70	252	151.5	109.3
76	210	85.5	91.1
82	120	23.5	52.0
88	45	5.0	19.5
94	10	0	4.3
100	1	0	0.4
Total	1024	444	443.9

^a Compared with combined F_2 data reported in Table 7.1 and illustrated in Fig. 7.6.

The rather close fit suggests that in fact five genes determined the differences between the two varieties in corolla length.

Geometric Gene Action

When the F_1 and the mean of the F_2 are intermediate between the parents, as in the corolla length of tobacco and in many other studies, dominance is usually not involved. In some studies, however, the F_1 and the F_2 means are not intermediate between the two parents, and the F_2 distribution is skewed. An example is provided by fruit weights of tomato, which are shown in Table 7.3.⁶ When the parents differed greatly in

Table 7.3 Mean fruit weight in grams of different tomato crosses.⁶

LARGER PARENT	SMALLER PARENT	WEIGHT OF PARENTS		F ₁	F ₂	GEOM. MEAN ^a	ARITH. MEAN ^b
		<i>Larger</i>	<i>Smaller</i>				
Parents differing greatly in size							
902 Selection	Red Currant	56.2	1.1	7.2	7.3	7.6	28.6
Honor Bright	Red Currant	150.0	1.1	9.4	6.9	12.3	75.5
Albino	Red Currant	312.0	1.1	12.6	12.3	17.5	156.5
Parents differing slightly in size							
Peach	Yellow Pear	42.6	12.4	23.1	26.2	23.0	27.5
Dwarf Aristocrat	Grape Cluster	112.4	55.8	73.3	81.1	79.0	84.1
Albino	Honor Bright	312.0	150.0	160.0	151.3	217.0	231.0

^a Geometric mean = $\sqrt{a \times b}$.

^b Arithmetic mean = $\frac{1}{2}(a + b)$.

size, the F_1 was closer to the geometric mean of the parents in most instances. In other words, fruit size of the F_1 was much closer to the small parent. The F_2 distribution was strongly skewed toward small size (positively), and its mean also was close to the geometric mean (Fig. 7.7). It was assumed that the effect of the action of the genes was cumulative; that is, they operated like compound interest, with each additional gene increasing size by a certain percentage.

Skewness in this example may be explained on other bases. It may be due to dominance of genes for small fruit size (note the skewness in the distribution in Fig. 7.3, showing dominant gene action), or it may be due to some physiological limit such that the products of genes for small size have profound effects in the upper size range, but have very little effect as the size gets very small.

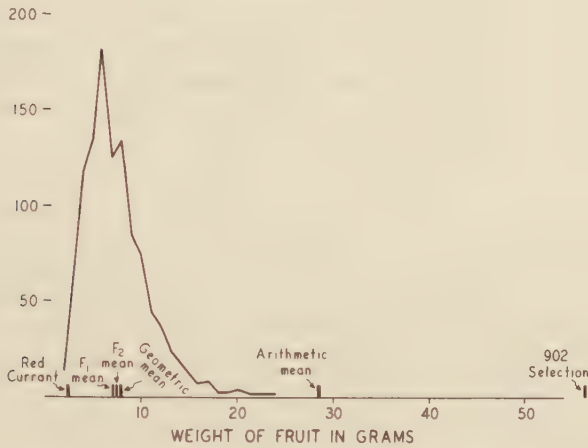


Fig. 7.7 Means and F_2 distribution of fruit weight in a cross of the tomato varieties 902 Selection and Red Currant. This is a positively skewed distribution. Adapted from MacArthur and Butler.⁶

Analysis by Partitioning

In studying the inheritance of quantitative characters in tomato, Powers introduced the partitioning method of genetic analysis. Partitioning means that separate components of development and yield are studied in segregating populations to determine the number and nature of the genes affecting each component. In a cross of Porter and Ponderosa tomato varieties, Powers et al.¹⁰ established the gene pairs determining several characters as follows:

Percentage of flowers that set fruit	$F_1f_1F_2f_2F_3f_3F_4f_4$
Period from seeding to first bloom	$B_1b_1B_2b_2B_3b_3$
Period from first bloom to first fruit set	$S_1s_1S_2s_2S_3s_3$
Period from first fruit set to first fruit ripe	$R_1r_1R_2r_2$
Number of locules	$Lc_1lc_1Lc_2lc_2Lc_3lc_3$
Weight per locule	$W_1w_1W_2w_2W_3w_3$

Some of the genes of the Ff and Ss series were considered to be identical, for the percentage of flowers that set fruit has an effect on the period from first bloom to first fruit set. Hence, it was assumed that 15 gene pairs, and not the 18 named above, were involved in the yield of ripe fruits.

The interrelationships of the genes affecting the component characters were studied to determine (1) whether the same genes were involved

(that is, whether pleiotropy was present), (2) whether linkage existed, and (3) whether the genes differentiating separate component characters interacted together. Finally, the interactions of genes with the environment were determined. The results indicated that the following were involved: linkage, pleiotropy, and complex genotype-environment interactions.

Polygenes

The term "polygenes" was introduced to genetics by Mather to refer to genes with small effects.⁷ In contrast to the multiple factor concept, in which each gene is considered to operate more or less independently, Mather believed that each polygene is dependent on, and interacts with, other genes present. Lerner⁵ summarized the main phenomena assumed to be characteristic of polygenic inheritance as follows:

1. Segregation occurs at an indefinitely large number of loci affecting the character.
2. The effects of allelic substitution at each locus are trivial compared to the total amount of observed variation in the character and may sometimes be comparable to the effects of isoallelic differences (i.e., they are detectable only under special circumstances).
3. The phenotypic effects of substitution at different loci are interchangeable, in the sense that identical phenotypes may be produced by a large variety of genotypes, even in the absence of dominance, epistasis, and environmental differences.
4. The phenotypic expression of polygenic characters is subject to considerable modification by differences in the intangible environment to which the members of a given population are exposed.
5. Most populations carry great reserves of heritable variability with respect to polygenically inherited traits; that is, they are genetically heterogeneous.
6. Polygenes tend to occur in balanced systems, the polygenic block being of particular significance in this connection.
7. Contrary to many interpretations of polygenic inheritance (including that in the original formulation of the concept), polygenes have pleiotropic effects and can act both as modifiers or suppressors of other genes and systems or as determinants of variation of traits for which no major gene differences are demonstrable.

Describing polygenic inheritance, Lerner also said, "This is an extremely intricate subject."⁵ With this warning in mind the summary quoted above may be discussed further.

Because segregation occurs at so many loci, or because the effect of the

individual gene is so small, it is impossible to describe the segregating population in terms of individual genes or to speak in terms of genotypes. Instead of discrete classes, the population has a continuous distribution, which is described according to means, variances, and the departure of the curve of the distribution from the normal.

With each gene having a small effect, the substitution of one allele for another will probably go undetected. This would be similar to comparisons of *isoalleles*; that is, alleles that are similar in effect on a character, at least under most circumstances.

A very important feature of polygenes is that different combinations will give the same phenotypic expression, so that a uniform open-pollinated variety may actually consist of many genotypes. A hybrid population that has been rigorously selected with a particular phenotype in mind—it may be a certain maturity date, a certain height, a certain seed size—may consist of rather different genotypes.

Environment will often modify the expression of polygenes, but its role is difficult to assess because of the difficulty or impossibility of measuring it for each plant. Environment includes both the several aspects of weather and the features of the soil in intimate contact with the plant. In selecting for character expressions determined by polygenes, it is important to have an environment similar to that where the crop is grown commercially.

The association of polygenes is important. Polygenes in a certain block on a short section of the chromosome often cooperate well and have a beneficial effect on some character. If their combination is particularly significant for the survival of a natural population, such genes may become extremely closely linked through some barrier to crossing-over, or the entire population may become homozygous for the block in question.

Sophisticated, and often complicated, methods of analysis have been, and are being, developed for polygenic systems that determine the inheritance of quantitative characters. These methods will indicate the contribution to the variance of a segregating population by: genes with additive effects; genes with dominant effects; interaction between genes, or epistatic effects; the effect of environment; and the interaction of genes and environment.

Résumé

The concept of the polygene has been useful in understanding the inheritance of quantitative characters. This concept recognizes that the polygene is subject to the same laws of inheritance as a gene with qualitative effects. Though individually polygenes exert very little effect on the phenotype, their total effect may be measured in terms of very im-

portant differences, even differences that characterize species. Individually, they are impossible to follow or to control.

Because they govern the inheritance of important economic characters, geneticists and plant breeders are developing analytical methods that will permit a clearer understanding of their collective operation. With understanding will come control.

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8

ROLE OF THE ENVIRONMENT IN PLANT BREEDING



In very general terms, it seems that an organism may adapt to climate either by evolving a genetic system which produces a stable phenotype regardless of the prevailing environment, or by assembling a set of genes which develop phenotypes adjusted to the environment encountered.¹⁵

LANGRIDGE, 1963

HISTORICALLY, plant breeding has moved toward increased control of the environment. This began when plant breeding materials were grown in a nursery, where weeds and plant pests were kept under control. Later, greenhouses were used during the winter so that two, and sometimes three, generations could be grown in one year. With the need for disease-

resistant varieties, breeding material was grown in environments that were optimum for differentiating between resistant and susceptible plants. Now plant breeders are making increased use of controlled environments to evaluate breeding materials for tolerance to heat, cold, drought, salt concentrations, nutrient deficiencies and excesses, and smog. Separate components of plant performance are being considered in relation to separate components of the environment.

Although the trend toward precise control of the environment will continue, most plant-breeding programs will have to be conducted in the field. For this reason it is important to have clearly in mind the procedures that are used to control and measure the effects of the environment. Plant breeders should never overlook the fact that the field nursery is their laboratory and that it merits the same concern and interest that the chemist gives the experiments on his laboratory bench.

Four aspects of the environment will be considered here: (1) control and evaluation of the environment in the nursery, (2) separation and measurement of heritable and environmental components of variation, (3) adaptation of varieties to an environment, and (4) Lysenko's theory of environmental adaptation of the genotype.

Control and Evaluation of the Environment of a Breeding Nursery

Often a plant breeder has little choice in the location of a nursery. Where a choice is possible, the nursery should be both uniform and representative of the area where the crop under improvement is grown.

The variability of a new nursery area may be measured rather accurately if a single crop is grown over the entire area. The variability in this crop can then be mapped, even to the extent of getting yield measurements from plots over the entire area. This may be done for more than one year. In succeeding years, yield tests will be located in areas where crop development has been uniform.

Soil variability will be reduced in several ways. Wherever possible, the nursery each year should follow a different crop which has been grown uniformly over the entire area. Where nurseries follow nurseries of the same or another crop, there is always a great deal of variability resulting from different treatments or planting rates of the previous year. In arid regions a year of summer fallow is a desirable substitute for another crop. Fertilizers and soil amendments should be added in amounts sufficient to permit optimum development of the crop.

In large part the weather cannot be controlled in the nursery. However, different temperatures can be provided by different slopes. For example, growers and breeders of certain fruit crops in some areas use northern slopes because temperatures are lower and development begins later

in the spring. In most areas, provision should be made for irrigation in case of severe drought, so that the data of an entire year will not be invalidated and valuable breeding materials will not be lost. Drainage will be necessary in areas of excessive rain. Breeders of forage crops have sometimes used artificial light in the nursery to extend day lengths. Sprinklers have been employed to increase relative humidities favorable for the development of specific diseases.

More often than not, the environment of breeding materials will be different to some extent from the environment of commercial production. Wider than normal spacings may be necessary for field crops, to permit single-plant evaluations. With fruit trees, closer than normal spacings may be used to conserve space. As early as possible in the breeding program, selections should be grown under conditions as similar as possible to those of commercial production.

However, a plant breeder should look beyond established commercial procedures. The search should be continuous for new combinations of genotypes and production techniques that will increase yield. This was emphasized by Borlaug in the development of varieties for Mexico,⁴ who did not develop varieties adapted to prevailing production practices. Instead, he bred wheat varieties for optimum conditions of the environment, and demonstrated that the proper combination of environment and variety could increase yields several-fold.

The milo variety Ryer 15 did not reach its full potential in California until production practices were changed. When it first became available, it was used primarily because it was early. Under prevailing production practices, with rows spaced 30 or more inches apart and plants 4 to 6 in. apart in the rows, Ryer 15 was rated as unproductive. It was unproductive because it would produce very few tillers. When row spacings were reduced to 20 in. and plants were spaced 1 to 2 in. apart, it was comparable in yield to other varieties.

Measurements of differences in the environment in a nursery may be obtained by using one or more *check varieties*, which are grown under the same conditions as the breeding material. Since the virtues and shortcomings of the check varieties are known, it is possible to use them as reference points when making selections.

Experimental designs will provide for control and measurement of environmental effects. Where superior genotypes are compared under several environments (different years, soil types, dates of seeding, and so on), the proper experimental design will permit measurements of the degree to which the genotypes vary in a similar manner with different environments, and the degree to which they do not. The use of simpler experimental designs used by plant breeders is discussed in Chapter 30.

Heritability

It is obvious that differences due to the environment will obscure differences due to inheritance. The greater the proportion of the total variability that is due to environment, the more difficult will it be to select for inherited differences. On the other hand, if environmental variability is small in relation to genetic differences, selection will be efficient because the characteristics of a selection will be transmitted to its progeny. The inherited portion of the variability has been termed heritability. Heritability is the degree to which the characteristics of a plant are repeated in its progeny.

The heritability of qualitative characters is usually high because breeding behavior can be predicted. In the example of isogenic lines of wheat (Chapter 6), where it was assumed that the genotypes *aa*, *Aa*, and *AA* were awned, tip awned, and awnless, respectively, the genotype of a plant could be readily identified from the appearance of the awn. The characteristics of the awns of the progeny of plants could be predicted also.

Heritability, h , of quantitative characters is expressed in terms of variance as follows:

$$h = \frac{V_h}{V_h + V_e}$$

where V_h = variance due to genotype and V_e = variance due to the environment.

Heritability in this instance is a measure of the contribution of the genotype to the total variation. It includes variability due to all types of gene expressions, including additive, dominant, overdominant, and epistatic effects.

Often heritability is now considered to include only the additive genetic variance, symbolized by V_g . If the variances due to dominance and epistasis are represented by V_d and V_i , respectively, heritability becomes:

$$\frac{V_g}{V_g + V_d + V_i + V_e}$$

The many ways of calculating heritability²² include parent-offspring regressions, variance components from an analysis of variance, and use of the variance of genetically uniform populations as a measure of non-heritable environmental variability.

Using one version of the latter method, the heritability of corolla length in East's tobacco experiment may be calculated (Table 7.1). The variability of the F_2 will be a consequence of a combination of genotypic and environmental effects. The standard deviation (s) of the F_2 is 5.91, so that V or s^2 will be 34.9, which is $V_h + V_e$. From Table 7.1, the vari-

ances of the parents P_1 and P_2 , both pure lines, in 1912 may be averaged and taken as a measure of the environmental variance. Respectively these are 4.0 and 4.9 (2.0^2 and 2.23^2), and the average is 4.4. V_h is obtained by subtraction ($34.9 - 4.4$), and is 30.5. Heritability h is

$$\frac{V_h}{V_h + V_e} = \frac{30.5}{34.9} = 0.874$$

Heritability may be expressed as a fraction, as in the preceding equation, or as the corresponding percentage, which in this example would be 87.4 per cent. Heritabilities of this level are considered to be very high, and are rare in studies of quantitative characters. With heritability at this level, a plant breeder would expect the progeny of single plant selections to repeat closely the performances or characteristics of the plants from which they came. Actually, this is what East found.⁹ He made nine F_2 selections and grew progenies from them; results for corolla length (in mm) are given in Table 8.1.

Table 8.1 Comparison in tobacco of corolla lengths of F_2 plants and their F_3 progeny.⁹

LENGTH OF F_2 SELECTION, mm	LENGTH OF F_3 PROGENY, mm	
	Range	Average
72	61-79	73.1
46	43-64	53.5
50	43-58	50.2
60	43-64	56.3
77	49-85	73.0
81	61-91	76.3
80	61-85	74.0
50	46-58	53.0
82	67-91	80.2

The maximum departure of the F_3 average from the F_2 corolla length was 7.3 mm, and most of the F_3 averages were quite close to the dimensions of F_2 parent plants.

Some estimates¹⁹ of percentage heritability in corn are given in Table 8.2. As in most studies, yield has a low heritability. The direct components of yield (in this case ear number, length, and diameter) have a low heritability also. As shown by the data in Table 8.2, heritability will vary, depending on the method used in its computation. It will also vary with

Table 8.2 Heritability of different characters of corn.¹⁹

CHARACTER	METHOD OF ESTIMATION		
	<i>Components of Variance</i>	<i>Female-Offspring Regressions</i>	<i>Male-Offspring Regressions</i>
Plant Height	70.1	42.6	59.7
Ear Height	55.4	41.0	46.8
Husk Extension	49.5	71.8	61.7
Husk Score	35.9	47.7	44.8
Ear Number	23.6	15.9	24.3
Ear Length	17.3	16.2	13.4
Ear Diameter	14.1	22.6	14.1
Yield	20.1	9.5	15.5

the year and the location. A series of studies, however, will establish a general average for separate characters. These averages will indicate the level of confidence that may be used in making selections for the characters under consideration.

Adaptation of Varieties to an Environment

A superior variety is the result of a combination of genes that cooperate well with the environment. Since the environment fluctuates in time and space, it becomes difficult to decide the most desirable relationship between a variety and its environment. Should a variety be developed that is closely tuned to one environment at the expense of good performance in other environments? This relationship has been termed *specific adaptation*. Or should a variety have *general adaptation*, which means that it has the ability to do well over a wide range of fluctuations in the environment, but is probably not outstanding in any one? Actually, the plant breeder is not limited to these two extremes; it is possible to work toward some middle position.

Often in improving a crop for an area, the first varieties will have general adaptation and will perform above average for many years and in different locations. Later, other varieties will appear which will be recommended for specific situations such as early dates of seeding, sandy soils, and heavy grazing. Often a state or a province is divided into zones, with varieties listed in order of excellence for each zone.

Buffering. A variety is buffered in relation to the environment when it is not too sensitive to changes or adversities; it is able to continue or complete development over a wide range of differences in temperature, humidity, and other components of the environment. Part of this buffer-

ing is related to development of each individual and part of it to variation between individuals.

Homeostasis is a term proposed by Cannon for the ability of the human body to develop and maintain itself in a steady state in spite of exposure to a wide range of environments.⁵ The term has been widely applied in biology, but has been qualified as *developmental* homeostasis, as contrasted to *genetic* homeostasis. Allard and Bradshaw prefer the term *individual buffering* to developmental homeostasis.² Homeostasis or buffering will be considered here in relation to plants.

Developmental homeostasis applies to the buffering of a single genotype or a group of plants with a single genotype. Where homeostasis prevails, the plant has the ability to develop normally in spite of adverse conditions of the environment. Homeostasis will be expressed by reduced variability when a group of plants with a single genotype is grown under different environments. Examples of varieties that exhibit homeostasis are: successful pure-line cereal varieties that have occupied millions of acres; clonally propagated varieties of horticultural crops that have been grown in many countries of the world; and popular single-cross corn hybrids.

There is good evidence that single-cross hybrid corn varieties owe their developmental homeostasis to their heterozygosity.¹ When such hybrids with different levels of heterozygosity were compared at different locations and in different years in South Dakota (Table 8.3), variability de-

Table 8.3 Coefficients of variability for groups of single-cross corn hybrids with different levels of heterozygosity.¹

TRAIT	LEVEL OF HETEROZYGOSITY ^a					
	0.031	0.077	0.139	0.262	0.508	1.00 ^b
Plant Weight ^c	26.2	24.6	21.8	24.2	19.4	15.7
Ear Weight ^d	37.2	36.0	30.8	34.8	20.6	16.3

^a Level of heterozygosity = $1 - F$, where F is the coefficient of inbreeding.
^b Single crosses of inbreds of diverse origin, and assumed to be at the maximum level of heterozygosity.
^c Average for four station-years.
^d Average for three station-years.

creased with increasing levels of heterozygosity. There is also evidence that heterozygotes in self-pollinated crops have better buffering than homozygotes, which is indicated by the high yields of both homozygotes

and heterozygotes in good years and higher yields of heterozygotes in poor years.^{2,3,15}

Superior varieties of cross-pollinated crops appear to be buffered as a result of both developmental and genetic homeostasis.¹⁶ Genetic homeostasis was a term advanced by Lerner to identify the situation in which a population can perform in a steady state because of differences in the genotypes. As applied to cross-pollinated crops, genetic homeostasis will be due to (1) the ability of different heterozygous genotypes to develop similar phenotypes, (2) the ability of some genotypes in one environment to compensate for others not well adapted, and (3) different genotypes having different spatial requirements in the three-dimensional environment of the crop. One of the best examples of populations exhibiting genetic homeostasis is provided by double-cross varieties of corn. Double-cross varieties, which consist of several different heterozygous genotypes, have been less variable in different environments than have single-cross varieties, which consist of only one heterozygous genotype.^{14,21}

Allard and Bradshaw have summarized the evidence which shows that mixtures in self-pollinated crops are also buffered.² Instead of genetic homeostasis they prefer the term *populational buffering*.

It will be apparent, however, that buffering based on genetic homeostasis will be vulnerable to natural selection. In one environment there will be developed one assembly of genotypes; in another environment, another assembly. This is discussed in more detail in Chapter 16.

Environmental Modifications of the Genotype

When the idea of evolution first became acceptable, the earliest concepts assumed that the characteristics displayed by an organism in a particular environment were to some extent inherited and that several generations in the same environment would fix the acquired characteristics. Lamarck was the leading advocate of this point of view during the first part of the nineteenth century, and Lamarckism became the name for this concept. Even though Darwin accepted the idea, he believed that natural selection was a more potent evolutionary force. Mendel's discoveries, and research in genetics since the rediscovery of his laws at the beginning of the twentieth century, pushed Lamarckism into disrepute.

Genetic research has shown that mutagens will alter the structure of genes and chromosomes, but it is clear that the changes are random and hence usually not in the direction of better adaptation to the environment. It is also well established that environment is a powerful agent of natural selection. But the evidence has been overwhelming against the

environment molding in any direct way the materials of inheritance so that they produce organisms better adapted to the environment.

It was surprising, then, to learn in 1948 that neo-Lamarckism and neo-Darwinism had become the officially accepted viewpoints of geneticists and plant breeders in Russia, and that Mendelism and Morganism had been rejected as pseudoscientific. It was largely the personality and efforts of one man, Trofim D. Lysenko, that persuaded or pushed Russian scientists and political leaders to the nineteenth century point of view. This "new" concept of genetics was called Michurinism, after I. V. Michurin, a famous Russian plant breeder who lived from 1855 to 1935. Michurin made notable contributions to Russian agriculture by developing new varieties of fruit trees. Without formal training in genetics, he had developed his own ideas about inheritance.

Lysenko's published writings on heredity and variability are confusing and not supported by convincing evidence. His views of interest here are summarized below:^{13,17,23}

1. Environmentally induced changes in heredity are adaptive; the organism acquires characteristics induced by the environment.
2. The conservation of the plant (its tendency to reproduce itself) may be shattered by adverse environmental conditions, by hybridization, and by grafting. This makes the plant more sensitive to factors that induce a changed developmental cycle.

Research was cited by Michurinists to support their points of view. In practically all cases their data could be explained on the basis of established genetic laws. Almost invariably they have committed three errors: They have not been careful in their selection of stocks; they have not used adequate controls; and they have refused to submit their data to statistical tests.

For example, it was claimed that spring wheats have been changed to winter types by exposing them to the environment of a winter wheat. This was said to shatter the conservation of these varieties and to make them more sensitive to factors in the new environment, leading to a more appropriate developmental cycle. In some cases only a period of a few generations was required for the transformation. All the evidence suggests that the spring varieties were mixtures in the beginning, with the distinct possibility that natural selection preserved the types tending toward or having the winter habit. Transgressive segregation following natural crossing is also a possibility.

Much was made of grafting as a means to transform varieties. It was

pointed out that the scion would to some extent take on the characteristics of the stock, and vice versa. This was not new; it had been common knowledge among horticulturists for many years. The rootstock may modify the following characteristics of the scion: vigor in apples, cherries, and citrus; fruit set in persimmons, plums, and grapes; winter hardiness in plums; and fruit quality in pears and citrus.¹⁰ Similarly, the scion may influence the rootstock, particularly with respect to vigor and duration of growth during the season. The basis for the response is not clearly known in all cases, but it would appear to be due in part to water and nutrient supply from the rootstock, carbohydrate, and hormone production of the scion, and from interactions between the scion and rootstock at the point of union. There is no evidence that these environmentally induced changes are inherited.

Michurinists, however, claim that the changes are inherited, and term the process "vegetative hybridization." This method of changing heredity was considered to be intermediate between changes in environment and sexual hybridization.

In potatoes, Howard found that the short-day wild species, *Solanum demissum*, from Mexico normally produced very low or no yields of tubers under long-day conditions.¹² But if scions of varieties of cultivated potato, *S. tuberosum*, which is tolerant of long days, were grafted onto the wild species, the yield of the latter was much larger—an increase from a range of 7 to 10 grams per pot to a range of 130 to 142 grams. It was also found that a second year of grafting might increase the yields still more. Tubers from grafted plants produced plants the next (second) year, which emerged and flowered earlier, but did not yield more than tubers from ungrafted plants. All effects had disappeared in the following (third) year.

Several grafting experiments in the tomato have given no evidence of inherited changes being induced as a result of effects of the stock upon the scion, or vice versa.¹⁸ In some cases, as expected, there were effects of the stock on the scion. An interesting example of the response of the scion to the rootstock is provided by reciprocal grafts of tobacco and tomato.⁷ On a tomato rootstock, tobacco did not produce nicotine because the roots are the center of nicotine synthesis in the tobacco plant. For the same reason, tomatoes grown on tobacco rootstocks contained nicotine. Some potato varieties that normally will not flower may be induced to do so by grafting their scions onto tomato stocks.⁶

In a few instances, variants have been obtained from grafts that combine the characteristics of both rootstock and scion. These have been shown to be *chimeras*, which in various ways combine cells of both rootstock and scion (Fig. 8.1). Of most interest are the *periclinal chimeras*

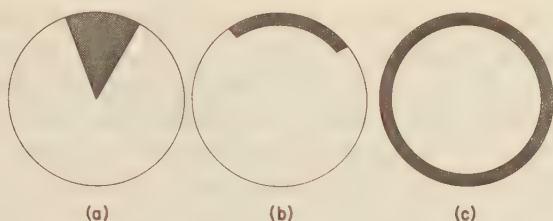


Figure 8.1 Different types of chimeras that may be obtained from graft hybrids: a, sectorial; b, mericlinal; and c, periclinal.

because they do not immediately betray their bipartite nature, since they actually look like a vegetative hybrid combining nuclear elements of both rootstock and scion. Studies of periclinal chimeras have shown clearly that the growing point consists of three different layers.^{8,20} It was shown in *Datura* (Fig. 8.2) that the outer layer of cells developed into the epidermis, the second layer developed into tissues below the epidermis and included the sporogenous tissue producing pollen and embryo sacs, and the third layer developed into the internal tissues.

Periclinal chimeras occur frequently in species propagated vegetatively, and some are of economic value. Thornless forms of the youngberry and of other species of *Rubus* may have only the epidermis of thornless tissue;

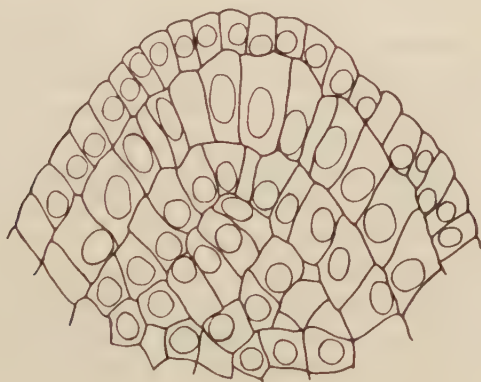


Figure 8.2 Periclinal chimeras in *Datura stramonium*, indicating the three distinct layers of the growing point. The outer layer is diploid ($2n$), the second layer octoploid ($8x$), and the third layer tetraploid ($4x$). (Satina, S., A. F. Blakeslee, and A. G. Avery, *Amer. J. Botany*, 27 (1940), 895.)

the balance of the stem tissue may be similar to the normally spined types. In vegetative propagation, periclinal chimeras may give rise to two different types. A stem cutting will usually develop its shoot from a bud having all layers of the growing point, but roots rise adventitiously, usually from the third layer. Thus, in the case of the periclinal thornless *Rubus* varieties, the stem cutting will have a periclinal thornless shoot system and a root system entirely of tissue of the thorny type. Further propagation of this type from stem cuttings would again give plants with chimeral shoots and nonchimeral roots, but root cuttings would give only spiny nonchimeral types.

Tubers of potato varieties that are periclinal chimeras may also give rise to two types, which are quite obvious if the genotypes for skin color of the epidermis and lower layers are different.¹² Plants rising from eyes will be periclinal chimeras, but plants developing from adventitious buds will produce the skin-color characteristic of the lower layers of the tuber. Adventitious buds in the tuber may be stimulated to develop by removing the eyes. In many instances sports of vegetatively propagated plants involve periclinal chimeras. Their appearance and behavior explain some of the observations made by Michurinists, which they attribute to vegetative hybridization.

Periclinal chimeras reproduce sexually from the subepidermal layer. This means that their progenies exhibit the characteristics of this layer and not those of the epidermal layer.

Lysenko's theories have been considered here in some detail because they have been accepted by a large number of plant breeders in some countries. In part they were accepted because they seemed reasonable and superficially fitted the facts. There was nothing wrong in Lysenko's challenge to classical genetics and breeding procedures established thereon: the error was in closing the door to alternative explanations for the data that he obtained and the observations he made. The preservation of, and advances in, a science will always be made in an environment of free inquiry. Fortunately, Lysenko no longer holds his position of the only authority on plant breeding in Russia, and there is room now for other viewpoints in genetics and plant breeding.

Résumé

The evidence is clear that a particular environment cannot directly change a genotype to perform better in that environment; the environment does not adapt plants. But the environment plays a powerful role in plant breeding. It writes in large part not only the specifications of a new variety but also the specifications of diseases, insects, and weeds that will plague it.

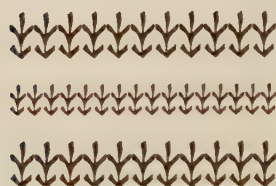
The environment is important, but also complex. For this reason it is desirable that plant breeders achieve greater control of separate components of the environment. With better understanding of the relationships among components of the environment and components of yield and quality, plant breeding will become more a science and less an art.

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9

SELECTION IN SELF- POLLINATED CROPS



It is now easily understood that the action of selection cannot go beyond the known limits—it must stop when the purification, or practically speaking, the isolation of the most strongly divergent line is complete.

JOHANNSEN, 1903 *

SELECTION is a feature of all plant breeding. Indeed, one of the ingredients of successful plant breeding, no matter what method is used, is the ability to recognize superior types in a limited or vast array of variability. The purpose of this chapter is to trace briefly the role of selection in improving some self-pollinated crops and to point out re-

* From James A. Peters, ed., *Classic Papers in Genetics*, © 1959. Reprinted by permission of Prentice-Hall, Inc., Englewood Cliffs, N. J.

strictions imposed on selection by genetic laws. The focus is on the function of selection in exploiting variability occurring naturally in a crop. It should be realized, however, that the role of, and restrictions on, selection in self-pollinated crops will be the same where the variability has been developed by hybridization or mutagenic agents.

Toward the end of the eighteenth century several plant breeders had demonstrated that selection would lead to worthwhile improvements. During the nineteenth century several varieties of self-pollinated cereal crops were developed by selection and proved superior to the varieties from which the selections were obtained.

As the nineteenth century closed, it was apparent from the observations of these plant breeders that the breeding behavior of self-pollinated crops had been realized in part. They noted that some selections bred true, and that these selections were often better than the population from which they came. There was no agreement on the value of continued single-plant selection.

The Pure-Line Theory

It remained for W. Johannsen,⁵ a Danish biologist, to explain the genetic basis of pure lines. In announcing his "pure-line theory" shortly after the rediscovery of Mendel's publication, he not only provided the plant breeder with a sound scientific basis for selection in self-pollinated crops but also made one of the most significant contributions to the science of genetics.

Johannsen's experiments dealt with selection for seed weight in beans, a highly self-fertilized crop. He chose a common variety named Princess, which had seeds of many different sizes. When he started the experiment, he was not sure whether the variability was due to inherited differences or environmental effects.

In a planting of this variety he chose 19 plants and determined the mean seed weight of each. These means varied over a wide range. To show that he did in fact have 19 distinct lines, each with a characteristic seed weight, he selected seeds of different weights from each line. From these seeds, termed mother seeds, he grew plants and determined their seed weight. The significant observation which he made was that all mother beans from a line gave plants with similar bean weights. For example, in line 2, mother beans of 40, 50, 60, and 70 cg gave progenies with mean seed weights of 57.2, 54.9, 56.5 and 55.5 cg, respectively.

Johannsen studied the variability within a pure line in still greater detail. In each line he selected in both a plus and minus direction for six years. In other words, for each line he developed two sublines, one being carried forward from the progeny of the smallest beans, the other

from the largest. After six years the mean yields of sublines of lines 1 and 19 were as follows:

	<i>From smallest beans</i>	<i>From largest beans</i>
Line 1	69.07	67.66
Line 19	37.36	36.95

Continuous selection had not changed the mean seed weight of a pure line! Variability of selected lines was due entirely to environmental influences.

He defined a pure line as the progeny of a single self-fertilized homozygous individual, the definition that is still in use today. From these and other experiments Johannsen concluded that the original variety, Princess, consisted of a mixture of pure lines.

Johannsen clearly saw that there were definite limits to the improvements of self-pollinated plants by selection. This is obvious today, but it was a disturbing new concept in the first decade of this century when many students of evolution accepted the idea that natural selection would improve populations almost without limits.

Land Variety

Princess would serve as an example of a land variety of bean. A land variety has three principal attributes: It is endemic to an area, with its origins sometimes going back several hundred years; it is a mixture of types; and it is well adapted to the environment. In a self-pollinated crop the ingredients of the mixture will be mostly homozygous.

The mixture may be conspicuous, as in the case of a land variety of wheat that has heads awned and awnless; glumes that are red, white, and black; and kernels that are white and various shades of red. Or the differences between plants may be minute and may affect quantitative characters such as height, time to mature, and seed size. Variability in a land variety of field peas is illustrated in Fig. 9.1.

Because they are usually very old and because they have been thoroughly tested by their environment, land varieties are well adapted. As mixtures, they are buffered against the different hazards of separate seasons. If one type fails to yield well in a particular year, another will compensate for it. Some constituents of the mixture may resist the attack of some races of a disease; other constituents, other races.

Most land varieties are unacceptable to a highly developed agriculture, for obvious reasons. Often their variability makes them difficult to grow and harvest mechanically. Uniformity is necessary also in the marketplace.



Figure 9.1 Variability in seeds (each group of three from a single plant) of peas in one field near Erzurum, Turkey, in 1964. An example of a land variety.

Level of Heterozygosity

Ideally, a self-pollinated crop is considered to be a mixture of homozygous plants, each of which will breed true. In actual fact, however, most self-pollinated crops will have some outcrossing, the amount varying with the crop and the environment (see Chapter 4). Even though outcrossing in each generation will raise the level of heterozygosity, this tendency is offset by the disappearance in a self-pollinated population of one-half of the heterozygosity in each segregating generation (see Chapter 10). This means that the level of heterozygosity does not rise much above the level of outcrossing in one generation.

However, there is evidence that the level of heterozygosity will be higher than that expected from outcrossing alone. It would appear that heterozygosity imparts some advantage to a self-pollinating plant and permits it to contribute more seed to the next generation.

Mass Selection

The obvious first step in the improvement of a self-pollinated variety is the elimination of undesirable types. This may be achieved by simply

roguing them as the variety grows in the field. Another approach is to tag desirable types as they grow, and to harvest only these at maturity. Either way, the results are similar; the best plants in the variety are identified and bulked.

A refinement of mass selection is to harvest the best plants separately and to grow them out as pure lines for comparison. Only those pure lines that are superior and similar are bulked as an improvement of an established variety. It might even be a new variety if it is obviously different from the original.

In many instances mass selection is the first step in the improvement of land varieties where they are still in use. By this method the characteristics that have made the land variety successful will be maintained, and its obvious shortcomings will be eliminated.

Similarly, in the introduction of a new crop, the initial improvement may be achieved by mass selection. When Claassen¹ identified an introduction of safflower from the Sudan as obviously superior to other introductions, he released this introduction as N-852, *after* he had rogued obvious offtypes.

Modern plant breeding employs mass selection to preserve the characteristics of established varieties. Usually, this involves the harvest of about 200 typical plants or heads of the variety, the number being large enough to preserve the identity and the original variability of the variety. These are grown in rows, and those that are not typical will be destroyed, preferably before they flower. The remaining crop is harvested in bulk. This process is repeated as often as necessary to preserve the characteristics of the variety.

Pure-Line Selection

The improvement of a variety by mass selection could be quickly followed by pure-line selection, as was practiced by Johannsen in selecting beans.⁵ Again this is a particularly effective method of improving a land variety, both in its area of development and in some new area into which it is introduced.

The development of Atlas barley is used as an illustration because the senior author had occasion to observe much of its development. It was obtained from Coast, and was undoubtedly a variety from North Africa that had been introduced into California by early Spanish settlers, probably in the latter part of the eighteenth century. It was a successful, well adapted, and widely grown variety over much of California. In 1917, Dr. H. V. Harlan and his associates made several hundred head selections of Coast from commercial fields. Seeds from each of these were grown in single rows the following year. There were many minor differences

among selections, most of them not great enough to have caught the eye if they had been grown as a mixed population. Several selections were saved for further evaluation. One of these was tested for several years and released in 1924 as the variety Atlas.¹⁰ Compared to Coast, it was four to seven days earlier and stiffer strawed.

The safflower variety N-852 was listed as an example of mass selection. In this variety, single-plant selections were made and one of them was increased and released in Nebraska as the variety N-10.² N-10 was more uniform than N-852, higher in yield, and higher in seed-oil content.

Sources of Variability in Pure Lines

Gene mutations. The discussion to this point would imply that pure lines remain homozygous and "pure" indefinitely, as long as self-pollination occurs. Convincing evidence has been obtained to indicate that this is not the case. East,³ in his long experience with *Nicotiana* (tobacco) species and crosses between them, observed types that developed parthenogenically from unfertilized haploid egg cells. A few were small and sterile, and were presumed to be haploids (monoploids). Others were diploid as a result of chromosome doubling at an early division of the haploid embryo. The striking feature of these latter types was the uniformity of their selfed progeny; they were more uniform than any inbred that East had observed. After three or four years of continued self-fertilization, the uniformity was no different from other selfed populations. This was attributed to frequent mutations with small effects on quantitative characters.

It remained for Stadler to obtain positive evidence of the frequencies of particular gene mutations.⁸ He selected as favorable material for study those genes governing endosperm characters in corn. An examination of the seed of a single ear established the genotype of several hundred individuals. Stadler's results (Table 9.1) show that the natural frequency of mutation may vary widely for different genes. Later studies⁹ showed that the mutation rate for the *R* locus differed with different alleles, and for the same allele differed in different genetic stocks because of the effects of modifying genes. These data are in general agreement with the mutation rate found in a number of other organisms. Usual mutation rates are in the order of one per 100,000 to one per million gametes, although higher and lower frequencies are recorded.

Selfed progenies of maize developed from doubled monoploids were studied from S_3 through S_6 (S_1 in this example is the doubled progeny of a monoploid) to determine the frequency of mutations affecting quantitative traits such as plant height, ear length, leaf width, date of silking, and yield.⁷ Mutations occurred in each trait at an average rate

Table 9.1 Frequency of spontaneous gene mutations in maize.⁸

GENE	NUMBER OF GAMETES TESTED	MUTATIONS OBSERVED	MUTATION RATE PER MILLION GAMETES
<i>R</i> (color factor)	554,786	273	492
<i>I</i> (color inhibitor)	265,391	28	106
<i>Pr</i> (purple)	647,102	7	11
<i>Su</i> (sugary)	1,678,736	4	2
<i>Y</i> (yellow)	1,745,280	4	2
<i>Sh</i> (shrunken)	2,469,285	3	1
<i>Wx</i> (waxy)	1,503,744	0	0

of 4.5 mutations per 100 gametes tested. A similar experiment on long-time inbred lines gave a mutation rate of 2.8 mutations per trait per 100 gametes.⁶ As Sprague et al.⁷ state, "It is a well-established fact that changes in phenotype do occur, since long-time inbred lines, when exchanged and maintained by different workers over a period of time and under different environments, become noticeably distinct." Jensen has reviewed the evidence on offtypes in small grains.⁴

Even very low general mutation rates are high enough to account for the great variability in cultivated crops when it is considered how long most of them have been grown under domestication. Despite the low rates and the fact that nearly all mutations are expected to be unfavorable, the possibility of new favorable mutations should not be ignored.

Hybridizations and recombinations. It was pointed out earlier that some cross-pollination occurs in normally self-pollinated crops. This provides a mechanism for the recombination of characters existing among individuals in the population. Hybridization may also occur occasionally with members of other populations, bringing in characters not previously present. Recombinations may be superior to any existing in the population.

Résumé

In improving self-pollinated crop plants, selection plays its greatest role among materials that are mixed, as in land varieties. For such crops it is the obvious first stage in their improvement, with mass selection preceding single plant selection.

In established crops, selection has long ago exploited most of the variability that is present, and plant breeders have turned to other techniques of improvement. Mass selection is used as a technique in the

propagation of new and old varieties, to preserve their variability and identity.

In a sense, selection exploits nature's store of variability. We turn now to directed efforts to increase and manage variability in self-pollinated plants.

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Table 10.1 Relationships between the number of heterozygous loci in F_1 plants and the number of genotypes and phenotypes in F_2 .

NUMBER OF HETEROZYGOUS LOCI	DIFFERENT GAMETES IN F_1^a	DIFFERENT GENOTYPES IN F_2	TOTAL GENOTYPES IN F_2
1	2	3	4
2	4	9	16
3	8	27	64
4	16	81	256
10	1,024	59,049	1,084,576
21	2,097,152	10,460,353,203	4,398,046,511,104
n	2^n	3^n	4^n

^a Also gives (1) the number of different phenotypes in F_2 , assuming complete dominance; (2) the number of homozygous genotypes in F_2 ; and (3) the number of genotypes in backcross F_1 .

ably a minimum number in most varietal crosses. To grow enough F_2 plants (Table 10.1) to supply all possible genotypes, about 50 million acres would be required. Of the 2,097,152 homozygous genotypes, all but those of the two parents represent potential new varieties. These numbers give a realistic idea of hybrid populations and illustrate the importance of efficient techniques in screening these populations.

Number of alleles at each locus. In a cross between two pure-line varieties or inbred lines, as described above, each locus will have a maximum of two different alleles, one from each variety. By appropriate crosses of a number of pure-line varieties, and certainly in crosses of different plants from two cross-pollinated varieties, it is possible to have some loci represented by a number of different alleles. The number of genotypes increases greatly as more loci are represented by more than two different alleles. For example, with only two alleles at each of three loci, 27 genotypes are possible, but four different alleles at these loci make 1,000 different genotypes possible. For the progeny of a single F_1 diploid plant, the maximum number of different alleles for any one locus is two. The situation in polyploids, where more than two alleles may be present in a single plant, will be considered later.

Linkage. In dealing with the recombination products of a large number of genes, it is almost certain that some will be linked. Linkage affects the frequency of gene combinations, favoring those of the parents at the expense of the recombinations. The magnitude of the effect will depend on the degree of linkage (Table 10.2). Linkage does not affect the rate at which homozygosity results from inbreeding.

Table 10.2 Effect of linkage upon the proportion of AB/AB genotypes expected in F_2 from the double heterozygote, AB/ab or Ab/aB .

RECOMBINATION OR CROSSOVER VALUE	PER CENT OF AB/AB (OR ab/ab) INDIVIDUALS IN F_2 IF THE F_1 IS:	
	AB/ab (Coupling)	Ab/aB (Repulsion)
0.50 (independence)	6.25	6.25
0.25	14.06	1.56
0.10	20.25	0.25
0.02	24.01	0.01
0.01	24.50	0.0025
p	$\frac{1}{4}(1 - p)^2$	$\frac{1}{4}p^2$

The effect of linkage may be illustrated by considering the Turkey (T) gene and the Rio (R) gene for resistance to bunt in wheat. These have been found to be linked, with approximately 15 per cent crossing over, and occur singly in Turkey 3005 and Rio varieties of wheat, respectively. They occur together in Turkey 10016. If Turkey 10016 were used as a source of resistance in a cross to a susceptible variety ($ttrr$), 81.9 per cent of the F_2 plants would be resistant because of the presence of one or the other or of both genes. Of the resistant plants, 83.1 per cent would have both resistant genes. Furthermore, 22 per cent of these would be homozygous for both resistant genes.

Had these genes been independent, 93.75 per cent of the plants would have been resistant in F_2 , but only 60 per cent would have both genes for resistance and only 6.25 per cent would be homozygous for both genes.

If the resistant genes had come from two parents, as Turkey 3005 ($TTrr$) and Rio ($ttRR$), the recombination would follow another pattern. Approximately 99.5 per cent of the F_2 plants would be resistant, with almost half having both genes, but only 0.6 per cent would be homozygous for both genes for resistance. For easy comparison, these three situations are shown in Table 10.3.

In another situation a desirable and undesirable gene may be linked. There is, for example, a tight linkage between the genes governing stem rust resistance, derived from *Triticum timopheevi*, and late maturity. This created an unhappy situation because plant breeders were interested in early maturity as well as rust resistance. After growing very large populations, resistant strains with adequate early maturity were recovered. Once the crossover was obtained, the plant breeders were in a very favorable position, since earliness was then linked to resistance.

It is important to realize, when two pure-line varieties are crossed,

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It is important to realize, when two pure-line varieties are crossed,

Table 10.3 Proportion of the various genotypes in F_2 where two genes for resistance are considered: (1) assuming linkage with 15 per cent of crossing-over when these enter the cross in both the coupling and repulsion phase, and (2) assuming no linkage.

GENOTYPE	PHENOTYPE	COUPLING RT/rt , %	REPULSION Rt/rT , %	NO LINKAGE $RrTt$, %
$TTRR$	Resistant	18.06	0.56	6.25
$TtRR$	Resistant	6.37	6.37	12.50
$TTRr$	Resistant	6.37	6.37	12.50
$TtRr$	Resistant	37.25	37.25	25.00
$TTrr$	Resistant	0.56	18.06	6.25
$Ttrr$	Resistant	6.37	6.37	12.50
$ttRR$	Resistant	0.56	18.06	6.25
$ttRr$	Resistant	6.37	6.37	12.50
$ttrr$	Susceptible	18.06	0.56	6.25

that the maximum number of different gene combinations is achieved in F_2 ; actually the combinations occur on the F_1 plant at the time of fertilization. Accordingly, F_2 populations should be as large as possible, consistent with the objectives of the program and the availability of land and labor. Large populations in later generations will do little to increase the number of different gene combinations. The reason for this is that most of the plants will be homozygous, when breaks in linkage will produce no new combinations of genes.

There is some evidence that the linkage of certain genes may be favored in evolution. The effectiveness of a gene or a number of genes is apparently magnified in the presence of other genes, and natural selection will favor changes in the structure of the chromosome complement that bring together the genes that cooperate successfully.^{7,8} Assuming the genes that cooperate well together are on the same chromosome, inversions will affect their spatial relationships and the degree of their cooperation. Inversions bringing them closer together would be favored by natural selection, since they would be inherited as a unit; crossovers would be rare between them. Because selection favors these changes in linkage relationships in natural populations, plant breeders may profitably explore the possibilities of changes in gene arrangements for more efficient crop production.

Structural differences in chromosomes. If two varieties differ in the structure of (or arrangement of the genes on) their chromosomes, gene recombinations may be affected. Reciprocal translocations result in new linkages of genes, but they do not place a barrier to crossing-over except

in the immediate area of the translocation break in the chromosomes involved. The barrier to crossing-over may extend from the translocation point to the centromere in species with an excess of alternate segregation of the chromosomes at meiosis.²

In plants heterozygous for large inversions, crossing-over in the inverted segment usually results in deficiencies, duplications, fragments, or dicentric chromosomes, and gametes possessing them are mostly nonfunctional. In other words, the genes in the inversion are inherited as a unit. In the inversion homozygote, of course, crossing-over will occur within the inverted section, but when two varieties differing by an inversion are crossed, the genes of the inverted section are inherited as a block.

Generations After F_2

The distinctive feature of hybrid populations of self-pollinated plants is their behavior after F_2 . In the F_3 and succeeding generations there is a decrease in heterozygosity and an increase in homozygosity. As a consequence, the hybrid population becomes a mixture of homozygous genotypes.

Gene frequencies. It is important to remember that the gene frequencies of an F_1 population will remain the same in all succeeding generations of the hybrid population, even though the frequencies of the genotypes will change. Stable gene frequencies will not prevail, of course, if there is selection against a particular genotype, if some genotypes are less productive than others, or if the mutation rate of a gene is high. The plant breeder will, of course, manage populations to increase the frequencies of genes favorable to the objectives he has in mind.

Homozygosis. Mendel was aware of the effect of self-pollination, or inbreeding, and showed that its effect on a heterozygous locus Aa was to reduce heterozygosity by 50 per cent in each generation. The progeny of Aa would be $1AA$, $1aa$, and $2Aa$; that is, one half would be heterozygous. The homozygous half, AA and aa , would not segregate in the next generation, but the heterozygous half, $2Aa$, would give 50 per cent homozygous and 50 per cent heterozygous plants. In other words, if an F_1 is Aa , heterozygosity in F_2 will be one-half; in F_3 , one-quarter; in F_4 , one-eighth; and so on. Homozygosity, of course, will be the converse of this, with the F_2 , one-half; the F_3 , three-quarters; and the F_4 , seven-eighths. The proportion of homozygosity for this locus is given by the formula $(2^m - 1)/2^m$, where m is the number of segregating generations. All other heterozygous loci would approach homozygosity at the same rate. Thus, the formula will give the proportion of homozygosity, no matter how many loci were heterozygous initially. It should be kept in mind that this

formula refers to loci; it does not give the number of plants that would be homozygous. Homozygosis in different segregating generations is illustrated in Fig. 10.1.

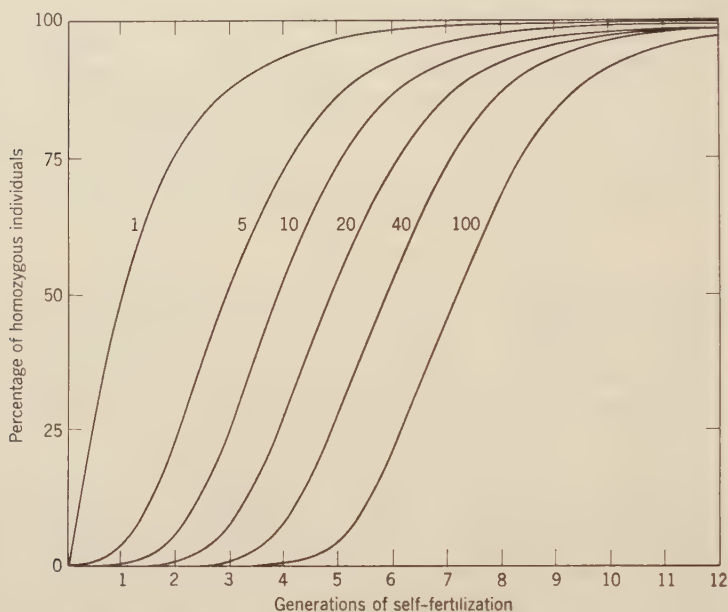


Figure 10.1 Percentage of homozygous individuals in different segregating generations of a self-pollinating hybrid population, assuming different numbers of heterozygous loci in F_1 . The percentage of homozygosis is given by the curve for one heterozygous locus. (From R. W. Allard, *Principles of Plant Breeding*, Wiley, New York, 1960.)

Homozygous genotypes. The proportion of homozygous plants, however, is of particular interest to the plant breeder, since such types usually serve as the basis of new varieties. The number of homozygous genotypes is given by the formula $[(2^m - 1)/2^m]^n$, where m is the number of segregating generations, and n the number of segregating loci. Assume that two loci are heterozygous in an F_1 plant. In F_2 , $[(2^1 - 1)/2^1]^2$, or $1/4$ of the plants will be homozygous. In F_3 , the values will be $9/16$; in F_4 , $49/64$; and in F_5 , $225/256$. Note that for one locus, there are two homozygous genotypes; for two loci, four homozygous genotypes; and for n loci, 2^n homozygous genotypes (Table 10.1).

Linkage will not affect the level of heterozygosity, which applies to

loci, but it does effect the proportion of homozygous genotypes in two respects. First, it has the effect of reducing n in the formula, with two tightly linked genes behaving as if they were at one locus. Second, the different homozygous genotypes will not be present in equal proportions, those involving linked genes being more frequent.

The genic composition in any inbred generation can be obtained by expanding the binomial $[1 + (2^m - 1)]^n$, in which n is the number of gene pairs involved and m represents the number of generations of selfing.⁴ In the expanded binomial, the first exponent in each term gives the number of heterozygous loci; the second, the number of homozygous loci. With a population originally heterozygous for four gene pairs ($n = 4$) and selfed for five generations ($m = 5$), the situation is

$$[1 + (2^5 - 1)]^4 = (1 + 31)^4 = 1^4 + 4(1)^3(31) + 6(1)^2(31)^2 + 4(1)(31)^3 + (31)^4$$

Expressed as homozygous and heterozygous loci this gives in F_6 the results tabulated in Table 10.4. In this population, 88 per cent will be

Table 10.4 Composition of a population in F_6 from an F_1 plant heterozygous at four loci.

HOMOZYGOUS LOCI	HETEROZYGOUS LOCI	PLANTS	
		Number	Per Cent
0	4	1	0.000095
1	3	124	0.0118
2	2	5,766	0.55
3	1	119,164	11.36
4	0	923,521	88.07

homozygous, in contrast with one out of 16, or 6.25 per cent, in the F_2 . In each case the homozygous individuals will be equally divided among the 16 possible homozygotes. If inbreeding is continued until homozygosity is complete, there will be 16 genotypes, in contrast to 81 in the F_2 (see Table 10.1).

By increasing the number of heterozygous loci in the F_1 to 10 and carrying the population to F_6 , one can get some idea of the changes taking place in a real hybrid population. By using the formula $[1 + (2^1 - 1)]^{10}$ for the F_2 and $[1 + (2^5 - 1)]^{10}$ for the F_6 , the number of homozygous loci were calculated and are presented in Table 10.5.

In F_2 there will be 1,024 different homozygous genotypes, each of which

Table 10.5 Composition in F_2 and F_6 of a population derived from an F_1 heterozygous at ten loci.

HOMOZYGOUS LOCI	F_2		F_6	
	Number	Per Cent	Number	Per Cent
0	1	0.1	1	9 ⁻¹⁴
1	10	1.0	310	2 ⁻¹¹
2	45	4.4	43,295	4 ⁻⁹
3	120	11.7	3,547,920	3 ⁻⁷
4	210	20.5	193,939,410	2 ⁻⁵
5	252	24.6	7,329,062,560	6 ⁻⁴
6	210	20.5	186,375,773,010	16 ⁻³
7	120	11.7	3,305,513,693,320	0.3
8	45	4.4	38,382,096,684,845	3.4
9	10	1.0	264,396,221,666,710	23.5
10	1	0.1	819,628,286,980,801	72.8
	1024	100.0	1,125,906,021,359,278	100.0

should be present once if 1,048,576 plants are grown. (These values were calculated using formulas 2^{10} and 4^{10} , respectively, given in Table 10.1.) It is obvious that most of the homozygous genotypes will not occur, let alone be identified, in F_2 populations of 1,000 to 5,000 plants, which are the number usually grown.

In F_6 , on the other hand, 72.8 per cent of the population is homozygous at all 10 loci, and another 23.5 per cent is homozygous at 9 loci. It would be theoretically possible to have all homozygotes represented in a population of 1,407 ($1,024 \times 100/72.8$) F_6 plants.

The proportions of homozygous genotypes in different segregating generations of a cross heterozygous in F_1 for different numbers of loci are given in Fig. 10.1. It is interesting to note that after 12 segregating generations, the percentage of homozygosity is not greatly different whether 5 or 100 heterozygous loci are involved.

For several reasons these calculations cannot be exact. First, there is little reason to believe that all genotypes will be equally productive. In some cases at least, heterozygotes will be more productive than homozygotes, with the result that homozygosity will be attained more slowly than expected. Finally, it is unlikely that values will be like those calculated, because of sampling differences.

It is clear how powerful selfing must be if over a few generations it can produce mostly homozygous types in the population. It is also clear how much easier it will be for the plant breeder to find desirable homozy-

gous types in later generations of hybrid populations of self-pollinated crops.

Shortcuts in the production of homozygous genotypes. Since several generations are required to achieve almost complete homozygosity in a hybrid population, it is not surprising to know that plant breeders have sought homozygosity through other procedures. One obvious method was the doubling of a haploid that develops from an unfertilized egg (Chapter 21). No one has been able to stimulate unfertilized eggs to develop as they are required, so this method of producing homozygous genotypes holds little promise.

It has been suggested by Burnham that translocation stocks be used in the production of homozygous, or nearly homozygous, lines.² While the method is attractive theoretically, it is not yet being used by plant breeders, and will not be discussed here.

Hybridization in Cross-pollinated Crops

Hybridization in cross-pollinated crops will be governed by the same laws that have been promulgated previously, in the following circumstances: (1) parents are homozygous for the genes under consideration, and (2) hybrid populations are inbred. When other circumstances prevail, the laws change; these changes are discussed in later chapters.

It is sufficient here to point out that varieties of cross-pollinated crops are heterozygous for many, perhaps most, loci. In addition, inbreeding often has deleterious effects on hybrid populations, making evaluations of the genotypes difficult or impossible on the basis of their appearance. Following hybridization the plant breeder does not search for one or a few successful homozygous genotypes; rather, he searches for an assembly or pool of genes that will cooperate to produce in each generation a collection of similar but heterozygous phenotypes.

The Successful Gene Combination

The successful variety of a self-pollinated crop is a single homozygous genotype, or a group of relatively few homozygous genotypes. Such homozygous genotypes are assemblies of paired identical genes at different loci that cooperate, or interact, well together and with the environment. Furthermore, these cooperating genes have the ability, by virtue of their paired nature, to reproduce the corporate structure faithfully in the next generation.

In the cross-pollinated crop the successful gene combination is one in which excellent cooperation or interaction exists, not only between genes at different loci but also between alleles at the same locus. In other words,

the sites of gene interaction are greatly increased in cross-pollinated crops. Unfortunately, because the genes are not present as identical pairs, such a successful genotype cannot reproduce itself sexually.

In this dilemma in cross-pollinated crops, where a very successful genotype cannot reproduce itself sexually, there are two ways out. Nature has chosen one, which is the route of asexual reproduction through vegetative propagation or apomixis. Man has found another: producing hybrid varieties from inbred lines.

Résumé

Because a successful variety is a rare combination of genes, it is not easy to develop. Not only is the combination a rare event; it is difficult to identify. Furthermore, it is more difficult as better varieties are developed. It is like the chances of getting a straight flush in cards: 40 are possible, some are better than others, and all are much better than most combinations of 5 cards.

Fortunately, in self-pollinated crops there is a rapid movement in each segregating generation toward homozygosity. This reduces greatly the size of the population that must be examined and yields plants that will breed true.

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BREEDING SELF-POLLINATED
CROPS BY HYBRIDIZATION
AND PEDIGREE SELECTION



In carrying out the improvement of cereals, the selecting of varieties may be considered an important step; and the object, in all probability, will be sooner accomplished and better controlled by first creating a diversity, which can easily be effected by crossing. . . . Crossing tends to produce variation in kinds not given to sporting, and in this respect it has much advantage over the system of improvement by merely selecting from the crops of the farm. A new and important source of variation is opened up by crossing, but a judicious improver of the cereals will never overlook this interesting proceeding. Always cross with the seedlings which inherit in the greatest degree the properties you wish a cereal to possess.¹⁵

SHIRREFF, 1873

SHIRREFF wrote this advice after spending a career in the improvement of cereals in Scotland. By the turn of the century many cereal breeders

felt that they had exhausted the possibilities of improvement of grain crops through introduction and selection and, stimulated by the rediscovery of Mendel's laws, they were turning to hybridization. Flower fanciers had been making crosses even earlier.

During the first 50 years of this century, hybridization became the primary source of new varieties of self-pollinated crops. This is well illustrated by examining the breeding method used for the main wheat varieties of the period 1919-1959.¹² In 1919 only 4 out of 12 had been developed from crosses, and in 1959, 14 out of 16. The same would be true of many important field and vegetable crops.

The term "hybrid variety" was used for many years to identify varieties developed from hybridization. The term is now generally used for varieties that express hybrid vigor, are highly heterozygous, and are derived at will as F_1 from particular parental stocks (see Chapter 18).

Following a cross, the hybrid populations may be handled by two methods or variations thereof. In one, to be discussed in the present chapter, a pedigree system is followed, where detailed records are kept of all plants in segregating generations. The second, called the bulk method, permits the segregating generations to be handled in bulk. It is discussed in the following chapter.

Success in breeding through hybridization will be more likely if the following considerations are observed: (1) the objectives are clearly defined; (2) the parents selected have a high probability of meeting these objectives; (3) appropriate methods of hybridization are used; and (4) hybrid populations are managed properly.

Objectives

The importance of clearly defined objectives cannot be overemphasized in modern plant breeding, even though some past successes appear to have been the result of more or less random crosses. Close study of the crop is necessary in order to detect weaknesses that limit productivity or characteristics that limit acceptability. Factors limiting the productivity of existing varieties are usually well known, or they can be identified by special methods devised by plant breeders or other plant scientists.

Usually, it will be desirable to maintain most of the characteristics of successful varieties in the area. Therefore, objectives will be concerned with the improvement of existing types in one or a few characters. Almost without exception, wheat breeders of the entire North American Continent have had resistance to stem rust as a primary objective. Another rather specific objective in wheat breeding in most areas of North America has been greater earliness. Now, most wheat breeders are working toward shorter and stronger stemmed wheats, better able to utilize higher

levels of fertilizer applications. Similar objectives have prevailed for other crops.

Sometimes the plant breeder may be concerned with raising general productivity, which cannot be related to such specific characters as those mentioned above. Where improvement in yield per se is the main objective, the project is complicated by the fact that yield has a low heritability. Nevertheless some varieties have become popular simply because of their ability to yield well, apparently because they are able to adapt to a wide range of conditions. Thorne wheat, for example, was grown on almost four times as many acres as any other soft red winter variety in 1949, even though it was susceptible to most of the important diseases of the area.¹³ Its popularity was due to its high yield and stiff straw. The Seneca variety, which is replacing Thorne, is a sister selection with a slightly stiffer straw and higher yield, but otherwise similar to Thorne.

Choice of Parents

A contemplated variety is often planned as a replacement for one already established. As a consequence, the existing variety will be a logical choice as one parent. In any case, one parent should be selected on the basis of proved performance in the area of projected use. The second parent, of course, must complement the first in the character under improvement. When there is a choice, the variety exhibiting the character in the most intense form should be used. This should be done even if it exceeds the intensity desired, for some intensity is usually lost in the new genic environment.

Occasionally a third, or even a fourth, parent may be needed to supply the characters desired. This is almost certain to increase genetic variability, with corresponding increase in the difficulties of managing the segregating generations. The alternative would be to make the improvements in two or more steps instead of one.

A search of the literature will often reveal the names of varieties being used by other plant breeders having the same objectives. Direct collaboration with plant breeders working on the same crop will achieve the same thing. The Food and Agriculture Organization (FAO), with headquarters in Rome, Italy, has acted as a clearing-house for information on varieties, and has published summaries of such information. FAO has also published lists of plant breeders, stating the crops of interest to each.

Occasionally the second variety must be found the hard way. If resistance to a disease or an insect is required and none is known, exhaustive tests of a large number of varieties may be necessary. Schaller and Wiebe,¹⁴ needing resistance to net blotch, *Helminthosporium teres*, tested the entire world collection of over 4,500 varieties of barley maintained by the

U.S. Department of Agriculture and found 75 to be resistant. Some of the latter were included in barley-breeding programs.

If the objective is to improve yield without adjusting any specific characters, the second parent must be chosen without much knowledge as to whether it will complement the first. One approach would be to select a second high-yielding parent from the same area of adaptation. In such case, the two parents would be quite likely to have many genes governing productivity in common. This would be especially true in a long-established production area where closely related types have provided most of the germ plasm.

Another approach would be to choose the second parent from a totally different region of adaptation, with the idea of bringing together some major genes for yield from two regions. Some evidence supports this approach. Marquis, the very successful variety of hard red spring wheat, was a cross of Red Fife from eastern Europe and Hard Red Calcutta from India. All the present varieties of the hard spring wheat area can trace their origins back to Marquis (Fig. 11.1).

Varieties have been evaluated as parents from their contribution to the performance of hybrid populations in early generations, but such evaluations have not given consistent results. A discussion of early-generation evaluations is given at the end of the next chapter.

Making Crosses

Even with the most difficult crops, making the cross takes much less of the total time in a breeding program than any other single operation. This rather simple operation requires removal of the anthers from the flower of the female parent. Later, when the flower is mature enough to be receptive, pollen is transferred from an appropriate male flower. Sometimes the time of planting of the parents must be adjusted so that the flowering periods will overlap. Hybrids may be made in the field or in the greenhouse. Under most circumstances few hybrid seeds will be needed.

If the parents are pure lines, records need not be kept of the individual plants involved in each cross because all plants of each parent will have the same genotype. However, varieties of self-pollinated crops often are mixtures of types phenotypically similar but with different genotypes. In this situation a number of crosses should be made, with the parents of each clearly recorded and provisions made to obtain seed from each parental plant. Care in obtaining such records is particularly important if the crosses are to be used in genetic studies.

Emasculation. The anthers are usually removed with forceps (Fig. 11.2). If they are very small, they may be removed by suction. Since anthers are more susceptible to temperature than are stigmas, hot- and cold-water

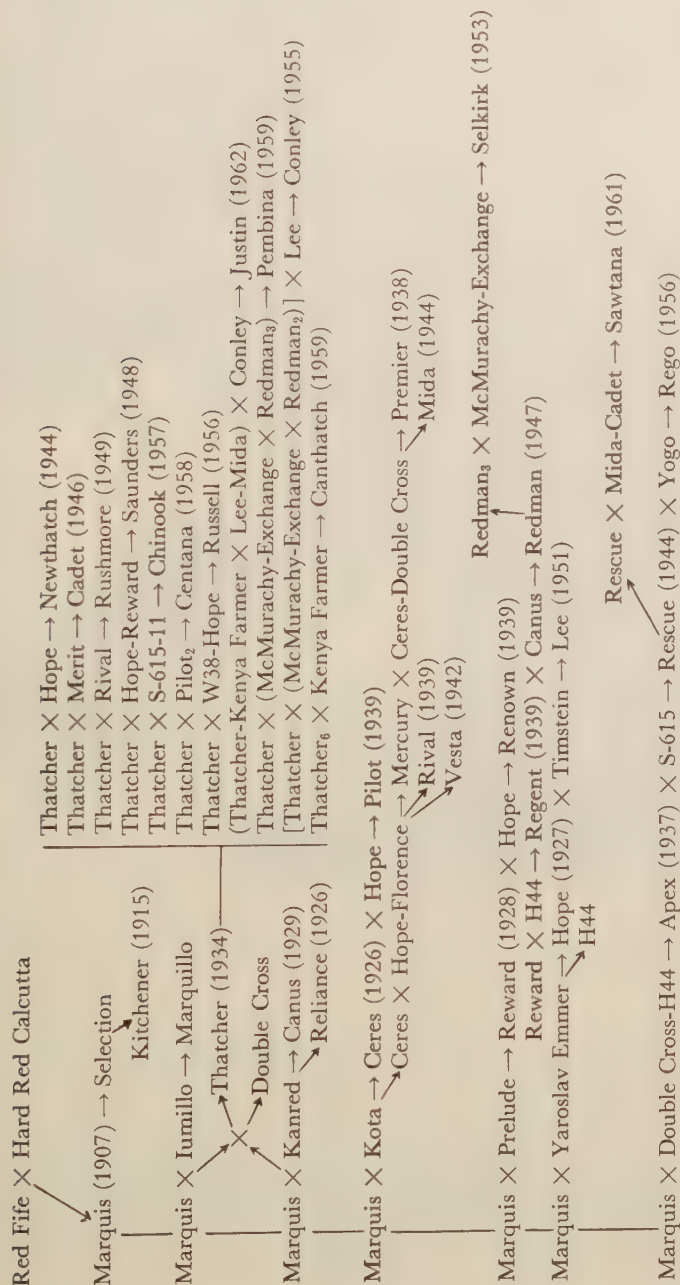


Figure 11.1 Pedigree of hard red spring wheat varieties of Canada and the United States. Symbols X and → between varieties indicate crosses, and subscripts are the number of backcross generations. Release year in parentheses. Marquis at one time occupied much of the hard red spring wheat area, and its germ plasm is still present in all varieties. (From Bayles and Clark³ and Briggles and Reitz⁵)



Figure 11.2 Emasculation of wheat. Note that part of the head has been removed. Glassine bags cover emasculated and recently pollinated heads. (From W. E. Clark, Winnipeg, Canada.)

methods of emasculation have been developed. Such methods are cumbersome to use, especially in the field.

Anthers are most easily removed as they approach maturity, being tougher then and easier to handle without breaking. Moreover, the flower is more durable and less subject to injury. Nevertheless, some injury usually occurs, causing some failure of flower development. Some crops, such as legumes, are very sensitive to mutilation.

Instruments must be kept free of pollen. This may be done by dipping in alcohol, flaming, or drawing the instruments between wetted lips.

Any flowers that have already shed pollen should be discarded, as should flowers too immature for easy emasculation. All emasculated flowers will then be at about the same stage of development and may be pollinated at the same time.

The emasculated flowers are covered to prevent accidental pollination. Glassine bags are widely used, though more porous material is advisable wherever extreme heat might cause excessive moisture to accumulate.

Finally, the plant is tagged to record the cross and the date of emasculation. If the best time of day for emasculation or pollination is not yet established, noting the time of these operations may later prove useful in judging results.

Pollination. In general, pollination is a less exacting process than emasculation. Pollen may be transferred by breaking a ripe anther over the stigma. In many cases pollen may be collected in bulk and transferred with a camel's-hair brush or a small spatula made by flattening the end of an ordinary dissecting needle. Exposed stigmas may be pollinated by shaking a pollen-shedding flower over them. If flowers are visited by insects, the male flowers must be bagged unless pollinations can be made before insect activity begins in the morning.

Pollinations are usually made the day after emasculation, but the time lapse may vary with the species. Environment strongly influences the timing of emasculation and pollination, so it is usually necessary to experiment with different times for each operation. Details of emasculation and pollination techniques for several crops are given by Hayes et al.⁷ and Poehlman.¹⁰

Handling Hybrid Populations by the Pedigree Method

The pedigree method, as the name indicates, provides a record of the lines of descent of all individuals or lines in each generation. Appropriate notes are recorded for each plant or family saved for propagation. These may be simple notations of vigor, maturity date, resistance to some disease or insect, or other useful information. Notes for advanced generations may include preliminary yield data. The accumulation of information becomes increasingly important when decisions must be made as to which lines to eliminate. Only a limited number of lines can be handled in the final evaluation.

The information on line relationships which the pedigree method provides in each generation gives the plant breeder a positive means of sampling the maximum number of lines of diverse descent. For example, if

the breeder chooses two lines that are judged about equal in potential, he would save one each from diverse origin rather than two from closely related families. In fact, the opportunity to sample families of diverse lines of descent is one of the most valuable features of this method. The pedigree method gives the plant breeder his greatest opportunity to practice his skill as a selectionist. Many times the pedigree method provides a means of studying the inheritance of some important character, thereby adding to the store of genetic knowledge of the crop.

In handling hybrid populations of self-pollinated crops, the changing genetic structure must be kept constantly in mind. One important point to consider is that the maximum potential of a cross (that is, the greatest range of segregates) is obtained in F_2 ; generations thereafter will repeat more or less the characteristics of the F_2 plants. Another point is that heterozygosity will decrease rapidly, so that progenies of single-plant selections from the F_2 to F_5 generations are progressively less likely to segregate. This means that the breeder is concerned with single-plant performance in early generations, and line (or family) performance thereafter.

There is abundant evidence that heterosis occurs in self-pollinated plants. This will complicate or bias operations in early generations of a pedigree program. Vigorous plants will be favored by the plant breeder in F_2 and segregating families, and this will tend to preserve heterozygosity. In addition, vigorous plants in families may be the result of outcrosses in the previous year. The breeding behavior, however, of the selected plants will indicate the presence and level of heterozygosity.

Procedures that may be followed in various generations in developing a new variety are given in Fig. 11.3 and described in the next section. Harrington has given a similar outline, which will differ from this in details.⁶ Descriptions of breeding procedures at the Svalof Station in Sweden have been summarized by Åkerman and MacKey.¹ The suggested population sizes and plant spacings in the subsequent discussion are for cereals.

Generations of Single-Plant Evaluations (Segregating)

F_1 . Enough F_1 plants should be grown both to produce the desired F_2 population and to give sufficient reserve to plant a similar F_2 in case of crop failure. F_1 plants are compared with parental varieties to make sure that they are hybrids.

F_2 . For convenience in examining plants, the seeds from F_1 plants of cereals are spaced 4 to 6 in. apart in rows 1 to 2 ft apart. Other species are spaced in accordance with their plant development. The number of F_2 plants should be 10 to 20 times the desired number of F_3 families.

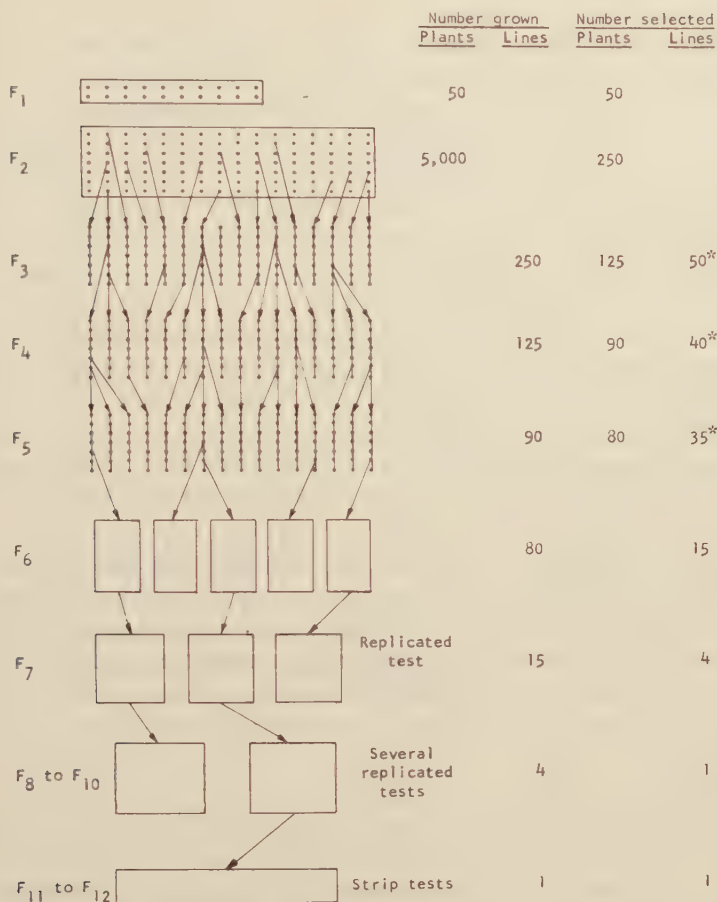


Figure 11.3 Diagram of pedigree method of handling hybrid populations. Dots indicate populations grown as single plants; dots joined by lines represent families or lines examined as single plants; rectangles are selections grown in bulk; arrows show the route of materials from one year to the next; and asterisks are the number of lines or families giving selected plants.

The wider the cross, the wider should be the ratio. A manageable number would be 5,000 plants, which would occupy about 1/20 acre if there were two plants per square foot. At least every tenth row should be sown to a check variety, which may be one or the other of the parents or a successful commercial variety.

The F₂ population provides the first opportunity to practice selection. Obviously, undesirable plants will often be removed as they are identi-

fied. From among the remaining plants will be selected the most vigorous plants that show the characteristics sought in the new variety.

It is here that the beginning plant breeder will need to develop a ruthlessness toward unpromising plants. The breeder who fails to discard ruthlessly will be overwhelmed by materials that will impair his future as an effective plant breeder. As was clearly shown in Chapter 10, one can hope to sample only a small part of the potential of any cross.

Assume that 300 plants are harvested and that 50 of these are discarded after laboratory examination for seed size, shape, color, and plumpness.

F_3 . Seed from the 250 individual F_2 plants are space-planted, normally at intervals somewhat closer than those for the F_2 . Each F_3 family (or line) should be large enough to indicate its full potential.

If 30 plants are grown at 4-in. spacings, a 10-ft row will be sufficient for each F_3 family. A check variety should be included in at least every tenth row. A total of 275 rows will then be grown in the example, which will occupy slightly over 1/20 acre.

The potentials of the cross will have become apparent. If the two parents have nicked well, there will be a wealth of material to select from. Selection is made on a single-plant basis, emphasizing families that are uniformly good while not neglecting outstanding individuals in otherwise unattractive families. The number of selections should rarely exceed the total number of F_3 families. It may be assumed that 150 selections are taken from 50 families in the example and that 25 of these are discarded after laboratory study.

F_4 . This generation is similar to F_3 , with 125 families and 12 check varieties, and will occupy 1,370 sq ft, or 1/30 acre. Some cereal breeders prefer a three-row plot 4 to 5 ft long because they feel that it gives them a better visual evaluation of a family than can be obtained from a single longer row.

Usually, single-plant selections are made, even though many of the families will appear quite homogeneous. The F_4 offers the first good opportunity to reduce population size. This is based on a combination of pedigree and visual evaluation. If F_4 families stem back to the same F_2 plant and have appeared similar in F_3 and F_4 , some of the similar materials may be discarded. It is assumed that 100 plants are harvested from 40 families and that these are reduced to 90 in the laboratory.

F_5 . This generation is similar to F_4 except that larger plots may be used and plant spacings may approximate those of field conditions. Selections are based on pedigree, visual appearance, and yield if plots are large enough. If 90 selections are grown in three-row plots 10 ft long, with 10 check plots, the area occupied will be about 1/15 acre.

As in F_4 , about 100 plants may be taken and then reduced to 80 in the laboratory. In making selections it is assumed that plants are homozygous and that they will breed true. Consequently, selections should be made from families that appear uniform.

Generations of Line Evaluations (Nonsegregating)

F_6 . In this generation the basis of selection shifts from single plants to single families; plots are harvested in bulk and not as single plants. As a consequence, planting rates approximate as closely as possible those of commercial plantings. Plot size should be large enough to permit a good visual evaluation of each family and perhaps to obtain a measure of yield. If abundant seed is available, plots may be replicated. With 80 selections the nursery will be similar to F_5 .

Selection, as in F_5 , takes into account both pedigree and measurements of plot performance. Probably only 15 of the most promising and uniform families should be kept after visual examinations, yield measurements, and laboratory analyses.

F_7 . At this stage, or perhaps a year earlier, the category "family" will no longer be used because it implies variability. The term "line" may be used, but more often this is replaced by "selection." The 15 selections, with check varieties and perhaps with selections from other crosses, are sown in a replicated test, using standard-sized test plots. A relatively simple experimental design such as the randomized block (Chapter 30) is preferable at this stage, since entries can be discarded without invalidating the statistical analyses of the data.

Probably four or five selections will survive the testing program, which will include rather precise evaluations of quality.

F_8 to F_{10} . For a minimum of three years, and sometimes for as many as five, tests like those in F_7 will be continued. Usually tests will be extended to different locations in the proposed area of production. This will usually narrow the number of selections down to one and perhaps to only one or two from several crosses.

F_{11} and F_{12} . These years are devoted to strip tests and seed increase of the selection picked to be a new variety. The strip tests may involve plots of an acre or more in size, which are sown and harvested with farm equipment. During this period the selection is given a variety name and arrangements are made for its approval by certifying agencies (see Chapter 29).

This example indicates that 12 years are required to produce a variety, 5 of them with the focus on single plants, and 7 on populations. It should be realized that the schedule is flexible; the period of single-plant evaluations may range from 4 to 8 years, and that of population tests from

6 to 8 years. Thus, a variety might be developed over a 10- to 16-year period.

Plant breeding obviously takes time. In 1892, Saunders made the cross from which Marquis was selected. Fifteen years later the variety was released. A similar number of years has been required to develop many other successful cereal varieties.

Modifications of the Pedigree Method

An excellent review of modifications in the pedigree method has been presented by Andrus.² These modifications have been designed to (1) slow up the approach to complete homozygosity in early generations and thereby (2) increase the opportunity for superior genetic recombinations to be achieved. Established or suggested procedures include (1) crossing of superior selections in early generations, and (2) crossing of selections at random in early generations.

It is obvious that these modifications impose on the population the breeding system of cross-pollinated plants. When crosses can be made easily, these departures from the pedigree method appear to offer the plant breeder the best chance of achieving superior gene combinations, particularly where linkage is involved. On the other hand, when crosses are difficult to make, or where they yield a small number of seed, these modifications would be impractical. More research is needed to indicate the superiority of these modifications of the conventional pedigree method.

Early Generation Evaluations

The pedigree method of breeding permits more opportunity than any other method for evaluating materials of a cross, if the breeder knows his crop and is skilled in estimating field performance from single-plant behavior. Without this knowledge and skill it is doubtful that the work involved in the pedigree method can be justified, particularly when the bulk method permits the achievement of homozygosity with much less work. The question, then, is: What can be accomplished by selection in early generations when a cross is handled and examined as single plants?

For qualitative characters, such as resistance to disease (in the presence of the disease), earliness, height, flower color, or fruit morphology, the pedigree method permits the early elimination of types of no future value. In this way it conserves space and time for materials of promise.

Plant breeders often resort to special techniques that identify desirable plants in early generations. Such techniques include the following:

1. Artificial disease epidemics, under greenhouse or field conditions.
2. Cold temperature tests, usually in special chambers.
3. Microtests of quality, often requiring only a few grams.

Early generation tests are not unique to the pedigree method; they are used wherever feasible in all breeding programs. The plant breeder often seeks the aid of specialists in other fields such as chemistry, plant physiology, and plant pathology.

Quantitative characters (yield in particular) are difficult to evaluate in early generations. One problem is that single-plant performance under spaced conditions is not necessarily related to population performance under close spacings. With high levels of heterozygosity in early generations, the problem is even more complicated. However, the fact remains that most plant breeders will make their selections among the group of single plants that appear to yield the most.

Kalton,⁸ working with soybeans, found that seed-yield measurements of F_2 plants were of little value in predicting yield potentialities. On the other hand, Raeber and Weber¹¹ found in the same crop that superior single-plant selections in F_5 gave better yields as F_6 lines than did random selections. Even better, though only slightly so, were pedigree selections taken in F_5 from space-planted progeny of F_4 lines with superior performance. On the basis of experiments comparing visual estimates with actual measurements, Boyce et al.⁴ concluded that selection by visual estimate was as successful as selection by weight in raising the mean yields of progenies in early generations.

The earliest attempts at yield evaluations in early generations by using yield tests were those of Nilsson-Ehle in Sweden in the early years of this century.⁹ In a cross of two wheat varieties, 165 F_3 plants were taken from 30 F_3 families and, after their removal, the 10 best F_3 families were harvested in bulk. The next year the progeny of the 165 single plants were grown as F_4 families, and the 10 bulked F_3 families were included in yield tests. A similar procedure was followed in F_4 . Similarly, Raeber and Weber felt that the greatest genetic advance could be made by growing F_3 lines in both replicated yield trials and a separate space-planted nursery.¹¹ Agronomic performance in the replicated yield trials would identify superior lines, and single-plant selections would be made from these same lines in the space-planted plots. They suggested that this method be followed in subsequent generations also.

Résumé

The merit of the pedigree method must be evaluated against the bulk method to be discussed in the next chapter. The primary advantages of

the pedigree method are: (1) it permits elimination of a great deal of unpromising material in early generations; (2) it permits the evaluation of selections on the basis of several years' performance; and (3) it permits a rapid approach to homozygosity, where single-plant selections are made in uniform single-plant progenies. These advantages apply primarily to qualitative characters that can be measured visually.

The principal objection is that the amount of material is limited because of the time devoted to individual plant evaluations. Even more time-consuming is the task of space-planting nurseries and harvesting individual plants. Where several crosses are being explored as sources of superior genotypes, the time factor will often prohibit consideration of the pedigree method. On the other hand, when a plant breeder is trying to get the most out of one or a few crosses in the shortest possible time, the pedigree method is the best choice.

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12

BULK POPULATION METHODS OF BREEDING SELF- POLLINATED PLANTS



Still another course of procedure in crossing work, especially with autumn wheat, has begun to be practised at Svalöf, viz., the creating of populations. Two known sorts are crossed and the whole progeny from all second and succeeding generations is sown together en masse. . . . The method in question allows nature, with the help of man, to gradually weed out the weaker combinations so that after a few generations only tried and constant forms remain.¹⁸

NEWMAN, 1912

THE PROCEDURE referred to by Newman was developed by Nilsson-Ehle in 1908 when he crossed the winter hardy wheat variety Squarehead with

the high-yielding Stand-Up. He grew the F_2 and several succeeding generations in bulk, before making single-plant selections. Newman noted that (1) large populations could be grown in each generation, thereby increasing the possibility of many more gene combinations; (2) populations could be grown under conditions of low winter temperatures where only the hardy would survive, thereby permitting the entire effort to be devoted to the winter hardy segregants; (3) very little work was required for any one cross, which would permit several crosses to be carried forward; and (4) selections from later generations would breed true.

The term *mass* is sometimes used as a synonym for bulk population. The essential difference between the mass and pedigree methods is one of management; for both methods the same careful consideration must be given to objectives and the choice of parents.

The bulk-population method was used by nature and by man in the early development of cultivated plants. Interestingly enough, it followed the adoption of the pedigree method in modern plant breeding.

Procedures in the Bulk Method

The bulk method of handling hybrid populations is illustrated in Fig. 12.1. In contrast to the pedigree method (Fig. 11.3), the early generations are grown as bulk populations. The number of bulked generations will vary with the plant breeder and with the nature of the cross. Some plant breeders, who feel that absolute homozygosity is not desirable, prefer to make single-plant or head selections in F_5 or F_6 . Others make their selections in F_7 or F_8 or even later. Where parents are similar, the number of bulked generations may be less.

The last bulk generation, when single-plant selections are made, will normally have the plants space-planted, as in the F_2 of pedigree populations. Thereafter, as illustrated in Figs. 11.3 and 12.1, the bulk and pedigree methods are essentially the same.

Some plant breeders prefer to space-plant their bulk hybrid populations and to select and bulk only the better plants in each generation. This procedure has sometimes been called the *modified mass method*. Following this procedure in bulk hybrid barley populations, Atkins found that lines obtained from selected populations were on the average only slightly higher in yield.¹ Selected populations were also slightly later and slightly taller, though no conscious selection was practiced for these characteristics. This modified mass method would be recommended for characters having high heritability.

The bulk and pedigree methods are not mutually exclusive. Harrington proposed and used the *mass-pedigree method*, in which he grew hybrid populations in bulk until circumstances were favorable for the expression

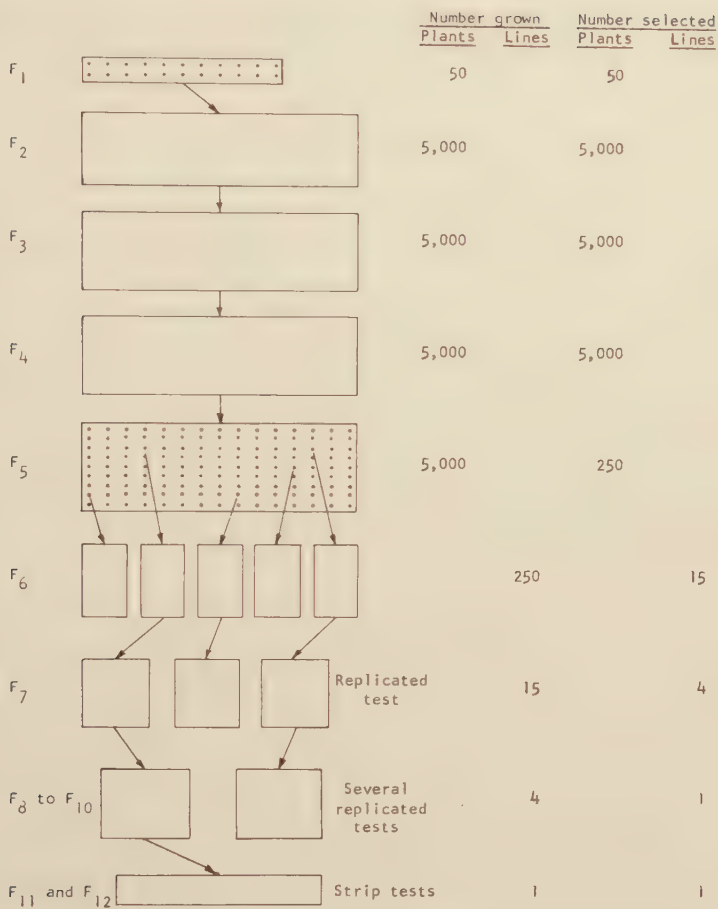


Figure 12.1 Diagram of the bulk method of handling hybrid populations. Dots indicate populations grown and examined as single plants; rectangles show populations grown as bulk populations, up to F_5 as mixtures, and thereafter as pure lines; and arrows indicate the route of materials from one year to the next.

of important characters, when he made single-plant selections and carried the populations forward by the pedigree method.¹⁰ Selections would be made for straw strength in wet years, drought and heat resistance in very dry years, and resistance to disease when a severe natural epidemic occurred. Others have preferred to use the pedigree method in early generations in order to eliminate undesirable types quickly, and then to bulk the better plants into one population and follow the bulk method thereafter.

Florell suggested that backcrossing be used for one or two generations following a complex cross, before carrying the hybrid population forward in bulk.⁴

Role of Natural Selection in Bulk Populations

Usually, hybrid populations are handled in bulk only until a high level of homozygosity is achieved, usually not beyond F_8 . In this case, natural selection has a very short time in which to act on homozygous genotypes.

Some plant breeders give natural selection a longer time in which to operate. They feel that natural selection will favor genotypes that are best adapted to their environment and which have the highest yielding ability. They are not thinking so much of genotypes better able to survive the calamities of the environment, such as disease, severe frosts, or very high temperatures, but rather of genotypes that perform better under normal growing conditions. There is increasing evidence that natural selection is effective in this respect if allowed to operate over long periods of time.

Natural selection among cultivated varieties. Interest in bulk methods of breeding was stimulated by the results obtained with a mixture of barley varieties, investigated by Harlan and Martini.⁸ The mixture was made up of equal numbers of seed of 11 varieties, some known to be well adapted to some areas and others known to be poorly adapted. The mixture was grown for a variable number of years at ten experiment stations in different parts of the United States, and a census taken each year. For each planting, a census was obtained by growing out 500 spaced plants the following year. The final census for each location is given in Table 12.1. Coast and Trebi were paired in the census because they are so similar in appearance.

It is interesting to note in Table 12.1 that one or a few varieties dominated the mixture at most locations. Usually there was good agreement between the success of a variety in the mixture and its status as a commercial variety.

The popularity of Coast as a variety on the Pacific Coast and Trebi in the northern intermountain areas and farther east is reflected by the success of the Coast-Trebi combination in the mixture. Except for one location, this combination held its own or became one of the dominant components of the mixture. At Moro, Oregon, White Smyrna, a two-row variety, very well adapted to arid conditions, practically eliminated all varieties, including the Coast-Trebi combination.

At only two locations was there poor agreement between the success of a variety in the mixture and its success in commercial production. At

Table 12.1 Final census in a mixture of 11 barley varieties grown 4 to 12 years at different locations; based on a total of 500 plants.⁸

VARIETY	ARLINGTON, VA. 1928	ITHACA, N.Y. 1936	ST. PAUL, MINN. 1934	FARGO, N. DAK. 1930	NORTH					PULLMAN, WASH. 1930	MORO, ORE. 1934	DAVIS, CALIF. 1928
					PLATTE, NEBR. 1932	MOCCASIN, MONT. 1936	ABERDEEN, IDAHO 1936					
YEARS	4	12	10	6	8	12	12	6	10	4		
Coast and Trebi	446	57	83	156	224	87	210	150	6	362		
Gatami	13	9	15	20	7	58	10	1	0	1		
Smooth Awn	6	52	14	23	12	25	0	5	1	0		
Lion	11	3	27	14	13	37	2	3	0	8		
Meloy	4	0	0	0	7	4	8	6	0	27		
White Smyrna	4	0	4	17	194	241	157	276	489	65		
Hannchen	4	34	305	152	13	19	90	30	4	34		
Svanhals	11	2	50	80	26	8	18	23	0	2		
Deficiens	0	0	0	1	3	0	2	5	0	1		
Manchuria	1	343	2	37	1	21	3	1	0	0		

Ithaca, New York, the dominant variety in the mixture was Manchuria, whereas the commonly grown variety was Alpha, a two-row type. At St. Paul, Minnesota, where Manchuria was commonly grown, the two-row Hannchen variety was dominant in the mixture. There was good evidence, however, that Manchuria had good yield potential in New York, and Hannchen in Minnesota. Surveying the results of their research as a whole, Harlan and Martini concluded that the success of a variety in a mixture could be used as a measure of adaptation and yielding ability under commercial conditions. Laude and Swanson obtained a similar

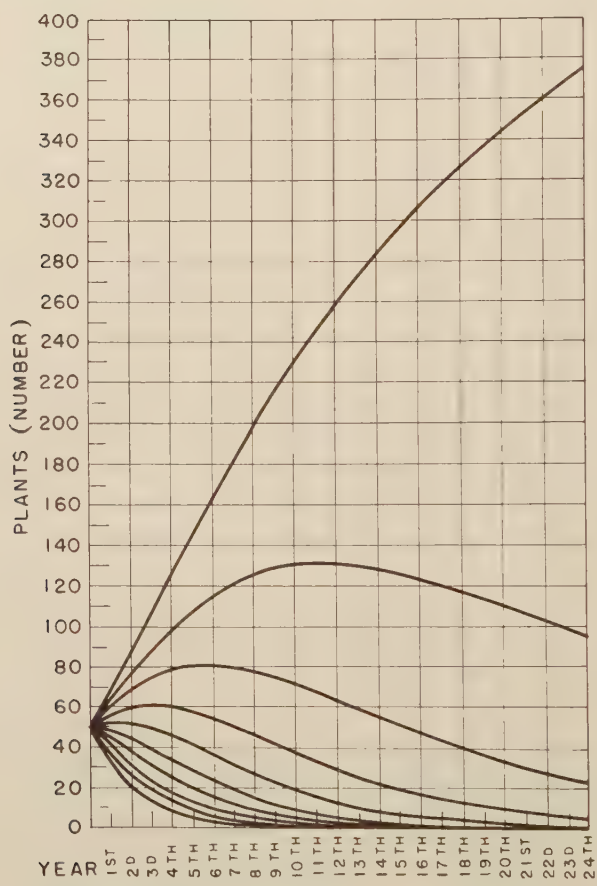


Figure 12.2 Theoretical curves of natural selection based on an equal mixture of 10 varieties differing by five kernels each in their productivity, the poorest plant producing 45 seeds. (Harlan and Martini.⁸)

response in mixtures of two winter wheat varieties;¹⁴ the well-adapted variety Kanred quickly displaced the variety Currell at one location and the variety Harvest Queen at another.

At each location, Harlan and Martini studied the pattern in the increase and disappearance of varieties over a period of years. Actual and theoretical values were compared. Theoretical values were obtained by assuming different levels of seed production for each of ten varieties. They assumed that the best variety would contribute 90 seeds per plant to the next generation, the next 85, and so on to the poorest, which would contribute 45 seeds. Curves for the ten varieties are given in Fig. 12.2. Note that poor varieties, as expected, quickly drop to low levels or disappear. Note also that the best variety increases almost linearly until it constitutes most of the mixture. In between there is a group of varieties that increases while competing with poorer types, and then decreases as it competes with better types. There was good agreement between the theoretical curves and those actually obtained.

Suneson and Wiebe studied the changes in mixtures of barley varieties, all of which were known to be well adapted to Davis, California, where the study was located.^{21,25} The average yield of the varieties (Table 12.2)

Table 12.2 Proportion of each variety in varietal mixtures of barley grown at Davis, Calif., from successive seeding of a mixture originally containing equal numbers of seed.²⁵

VARIETY	PERCENTAGE OF PLANTS OF EACH VARIETY IN MIXTURE ^a										YIELD, bu/acre 1933-1941
	1933	1934	1935	1936	1937	1938	1939	1940	1941	1948 ^b	
Atlas	25.4	38.1	47.4	42.8	49.2	54.4	47.7	63.2	65.5	88.0	73.3
Club Mariout	24.7	23.4	18.6	22.7	24.3	20.1	27.6	17.3	18.8	10.5	69.9
Hero	24.7	20.5	15.9	12.5	12.2	9.2	13.7	8.3	7.7	0.7	70.2
Vaughn	25.2	18.0	18.1	19.9	14.3	16.2	11.1	11.3	7.5	0.4	79.0

^a Disparity from 100 per cent total represents other varieties or natural hybrids.

^b Suneson continued the mixture for seven more years, with the final results indicated.²¹

is evidence that all were almost equally good as commercial varieties.

Surprisingly, however, a mixture of the four varieties in equal proportions did not stabilize at levels equivalent to the average yields in separate tests of each (Table 12.2). Mixtures and the variety test plots were grown in adjacent areas. The variety Atlas increased until, after eight years, it constituted 65.5 per cent of the mixture and 88.0 per cent after 15 years. These results suggest that survival in a mixture is not a criterion

of the yielding ability of a variety. However, there was good agreement between the survival of Atlas in the mixture and its acceptance by farmers. Survival, in measuring competitive ability, was perhaps measuring intangible qualities important in commercial production.

Similar data reported by Suneson and Wiebe from mixtures of five varieties of wheat grown for a five-year period failed to correlate very well with grower preference.²⁵ With soybean varieties in Iowa the results were contradictory; the highest-yielding variety in nursery tests was the leading variety in two bulks and the trailing variety in a third.¹⁷ These contradictions clearly point out the importance of learning more about the nature of survival in competitive mixtures.

Natural selection in bulk hybrid populations. It is not surprising that Harlan and his associates followed up their studies of mixtures of barley varieties with studies of changes in hybrid populations.⁹ Unfortunately, they did not study the changes taking place in hybrid populations from separate crosses of barley varieties, but emphasized the results from composite populations, which will be discussed presently. Actually, there are few data clearly showing changes in single hybrid populations in response to the pressures of natural selection.

Bulk hybrid populations of soybeans in Iowa responded to two successive late seasons with an increase in the proportion of late plants, but the trend toward lateness was interrupted by an early killing frost in the third season.²⁹ F_2 to F_4 bulk hybrid populations of wheat shifted slightly but significantly toward later maturity in northern Iowa where hot, dry weather after heading did not favor earlier types.²⁷ It was evident in both of these studies that natural selection was affecting the composition of bulk populations. The evidence from composite populations is even more convincing.

Composite Crosses

The first proposal for composite crosses was made by Love when he stated that several crosses could be composited.¹⁵ It was Harlan, however (Fig. 12.3), and his associates who gave prominence to this method of breeding and provided data to describe its operation.^{7,9} This method required that the F_2 of several crosses be bulked into one composite population, frequently termed a "composite cross." They chose 28 varieties as parents, which should give 378 different crosses (actually, 379 were made). This composite was grown without selection up to F_8 , when 2,921 selections were made. These were compared with an equal number of selections obtained from the individual crosses by the pedigree method. The average yields of the two series were: for the composite selections, 480.4 g per plot; for the pedigree selections, 463.4 g per plot. The best



Figure 12.3 Miss Mary Martini and Dr. H. V. Harlan examining barley plants in a nursery at Sacaton, Arizona, 1938. (From *One Man's Life with Barley*, by H. V. Harlan, by permission of the publisher, Exposition Press. Courtesy Dr. J. R. Harlan, Oklahoma State University.)

selections from each series were included in tests of a second year, with the following results:

	<i>Number</i>	<i>Avg. Yield, g</i>
Pedigree selections	965	534.1
Composite selections	1,269	540.7

At first glance these data seem to indicate that the composite was a more efficient technique, but Harlan and Martini pointed out a few complicating factors that plant breeders must consider in such comparisons. Selections from some of the separate crosses, and particularly from the crosses involving two-row varieties, were very low in yield. These were maintained and included in the separate crosses, but over the several years in the composite, they were undoubtedly reduced in number and very few appeared among the selections from the composite. Their absence would naturally push upward the average yield of the selections from the composite. The fact remains, however, that the composite was not inferior to the sum of the separate crosses, and it involved a great

deal less work. If several crosses are bulked together, they may be planted over an area of $\frac{1}{4}$ to 1 acre, with all operations being done with ordinary field equipment.

Suneson grew the Harlan and Martini composite cross at Davis for 29 generations in bulk.²² Yielding ability in relation to Atlas has been measured periodically, as shown in Table 12.3.

Table 12.3 Yields of Composite Cross II^a in various generations in comparison with Atlas.²²

TEST YEARS	GENERATIONS	YIELD, bu/acre		PER CENT OF ATLAS
		<i>Bulk</i>	<i>Atlas</i>	
1937-1938	F ₃ -F ₄	58.3	86.2	67.6
1933-1934	F ₇ -F ₈	71.3	83.8	85.1
1937-1940	F ₁₁ -F ₁₄	72.6	81.7	88.8
1941-1946	F ₁₅ -F ₂₀	75.1	70.8	106.1
1947-1949	F ₂₁ -F ₂₅	53.0	52.3	101.3
1951-1955	F ₂₆ -F ₂₉	50.8	49.1	103.5

^a Composite Cross II, C.I. 5461, evolved from the hybrid described earlier.⁹

Yields were determined in small-plot trials involving 10 to 20 paired comparisons. During the early segregating generations, the composite was conspicuously inferior to Atlas in yielding ability and general agronomic appearance. It was not until the F₁₅-F₂₀ generations that the bulk consisted rather uniformly of types similar to those grown commercially in California and became about equivalent to Atlas in yields.

Suneson and Stevens reported on 356 selections made in the F₁₂.²⁴ Not one desirable selection yielding more than Atlas was recovered. From 50 selections made in F₂₀, there were two promising lines, one of which has outyielded Atlas in each of the seven years they have been in advanced nursery trials. Yield advantage has been 37 per cent, and test weight has averaged 3.5 lb higher. In the F₂₄, 66 selections were made. From limited yield data it appears that 9 of the 66 will be superior to Atlas in yield. The top 3 have a 56 per cent yield advantage. Suneson suggests that there be a minimum of 15 generations of natural selection.²² Thereafter, there can be repeated recourse to one of three methods of breeding: (1) continue as a bulk population with prospects of natural selection producing significant gains in yield; (2) make cyclic hybrid recombinations with intervening natural selection to give a kind of recurrent selection; or (3) resort to conventional selection and testing.

This appears to be the only case where natural selection has been permitted to operate on a bulk population over a long period. These

data indicate that natural selection will favor the segregants that have good general adaptation and productivity.

Making a composite cross. A composite cross may be derived in a variety of ways. Harlan et al. crossed 28 barley varieties in all combinations, and composited the same amount of F_2 seed from each cross.⁹ This procedure permits the germ plasm of each variety to be combined with that of every other variety, but it still means, if cross-pollination is excluded, that all hybrid plants can have germ plasm from no more than two varieties; there is no opportunity to combine germ plasm from three varieties or more. Another method, assuming 16 parental varieties, would be to make 8 crosses, including all varieties, and then to cross the F_1 plants in 4 crosses. In turn, the F_1 of these crosses would be combined, and finally a single cross would be made, each parent having germ plasm from 8 varieties.

Suneson's suggestion of crosses of successful selections in a composite has merit.²² Such hybrids would then constitute a new composite. Again, it should be clear that such hybrids will bring together the genes from no more than four parents. A large number of selections should be used in hybridizations, in case a number of the successful hybrids stem from the same single cross.

Use of male sterility. Undoubtedly cross-pollination, even at a very low level, will significantly increase the number of gene combinations in a composite hybrid population. This will be particularly true where the composite is carried over many generations. The most potent means of achieving a high level of outcrossing is through some system of male sterility. The recessive gene *ms* for complete male sterility has been used in barley composites by Suneson.²⁰ If ten different varieties are crossed with a male-sterile plant, the ten F_1 will be fertile, but one-quarter of the composite F_2 will be sterile. By propagating from male-sterile plants, and assuming that the pollen comes from male-fertile plants in the population, an equilibrium will be reached in F_4 with half of it male-sterile. This is shown below in diagrammatic form.

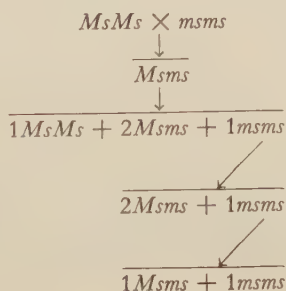
Each of 10 varieties (M_sM_s) crossed
to male-sterile plants

F_1 : All fertile

F_2 : 25% male-sterile
(pollen from M_sM_s and M_sms)

F_3 : 33% male-sterile
(pollen from M_sms)

F_4 : 50% male-sterile



Suneson and Wiebe pooled eight to ten seeds from each of the 6,200 spring barleys in the world collection maintained by the U.S. Dept. of Agriculture, and grew the composite in rows alternating with rows of Composite Cross XIV and Composite Cross XV having equal numbers of *Msms* and *msms* plants.²⁶ *Msms* plants were removed as they were recognized. With a seed set of only 19 per cent, 29 lb of seed were obtained from 7,500 male-sterile plants. From $\frac{1}{4}$ acre of F_1 plants, 700 lbs of seed were obtained, and the entire lot was made available for free distribution. Seed has gone to plant breeders over the entire world. A similar composite was developed in wheat, and the seed from F_1 plants also has had worldwide distribution.²³

When the plant breeder feels that the possibilities of gene recombinations have been fully exploited, the male-sterile gene can be eliminated by propagating from fertile plants that breed true for fertility. Where male sterility of this type is available in a self-pollinated crop, the plant breeder has two powerful techniques at hand, one to promote abundant gene combinations and one to develop pure lines.

Early-Generation Evaluations

Early-generation evaluations of hybrid populations have had two purposes: (1) It was thought that they might indicate the varieties most likely to be useful as parents in further crosses; and (2) it was hoped that they would indicate crosses most likely to produce superior selections in later generations.

Ideally, these evaluations should be done in F_1 . However, this generation has not given promising results, primarily because (1) the number of crossed seeds available have not been sufficient in many crops to permit the usual type of yield test; (2) yield results from widely spaced F_1 plants have not correlated well with yields from closely spaced plants; and (3) heterosis in the F_1 has complicated the results.

Yield evaluations have been conducted from F_2 to F_6 on bulk hybrid populations of a number of crops including barley,^{6,9,12,27} wheat,^{5,11} oats,² and soybeans.^{13,16,29} Results from these studies have been contradictory, even if one crop is considered. One problem with such tests is that they indicate average yield only; they will not identify crosses that produce superior segregates if the latter are counterbalanced by many that are inferior. Inasmuch as yield has a low heritability, results from yield tests of bulk hybrid populations in one year at one location will not be reliable in predicting either their yields in a similar test in another year or the yields of selections taken from the hybrid populations in later generations. The reliability of predictions will be reduced further if they are made from data obtained in an abnormal year. On the

other hand, early-generation predictions for characters with high heritabilities, such as height or maturity, have been relatively accurate.

Recently, the *diallel cross* has been proposed as a method of evaluating varieties as parents in crosses.³⁰ In the diallel cross, all varieties under consideration are crossed one with another. Then data in F_1 and F_2 from all crosses of one variety are averaged and compared with similar data from all crosses of the other varieties. Detailed results have been given in several studies, but will not be considered here. The problem with such studies is that they have required a great deal of work in making crosses to provide the F_1 plants, in growing nurseries with plants carefully spaced, and in collecting data from individual plants. With more than ten parents, the number of crosses becomes unmanageable. For example, with 15 and 20 parental varieties, the number of crosses will be 105 and 190, respectively.

A Comparison of the Pedigree and Bulk Methods

Each of the pedigree and bulk methods of breeding has its strong advocates, with opinions based on long experience in plant breeding. Experimental evidence on the subject is scarce and contradictory. Two studies on soybeans indicated that there was essentially no difference in the average performance of superior selections obtained from bulk populations and from pedigree populations.^{19,28}

Copp, on the basis of 26 years of wheat breeding in New Zealand, where the two methods have been carried out simultaneously, concluded that the pedigree method is superior where disease is not a limiting factor.³ Selections from the bulk populations were usually made in F_{10} . Selection throughout the pedigree cultures was similar to that outlined in this chapter. Five named varieties were obtained by the pedigree method, but none by the bulk method of breeding. Dr. V. H. Florell of the U.S. Dept. of Agriculture, in contrast, was particularly successful with a cross of Atlas $\times$ Vaughn barley made in 1927 and grown in bulk until 1934.⁴ Forty-five of the most promising selections from this cross were distributed to several of the western experiment stations. From these, four varieties have been released, Arivat by Arizona and Beecher by Colorado in 1940; Glacier by Montana in 1943; and Gem by Idaho in 1947.

Résumé

Under two conditions plant breeders have chosen the bulk method of handling hybrid populations in preference to the pedigree method. The first of these is when it is necessary to handle large amounts of segregating

material, either from several crosses or from a few complex crosses. The second condition is when natural or artificial selection pressures will eliminate large proportions of undesirable plants in segregating populations. The fact that both methods are used, sometimes by the same plant breeder, testifies to the effectiveness of both methods of breeding. Actually, for any hybrid population, the choice is not one method to the exclusion of the other. Plant breeders have devised many different combinations of both methods.

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13

THE BACKCROSS METHOD OF BREEDING



The science of genetics has been of great value to the plant breeder in that it has given him a clearer conception of his problems and a better understanding of the processes involved. However, there have been a few cases where he has been able to make direct use of specific genetic principles to set up a scientific plant breeding program. Such a program should enable him to predict accurately the nature of the variety which he will breed, or having produced such a variety, he could, if need should arise, repeat the breeding operations and obtain exactly the same variety. Within certain limits the backcross offers the plant breeder such an exact method.

BRIGGS, 1938

AS BRIGGS POINTED OUT, the backcross method of breeding gives results that are both predictable and repeatable. It is the only plant-breeding

method with these qualifications. As such it is being adopted increasingly by plant breeders. On the other hand, it has important limitations which have restricted its use.

In Chapter 2 introgression was listed as one method by which crop species evolve. In this method certain characteristics are transferred from species B to species A without changing the integrity of species A. It occurs in nature when the products of a cross of the two species are crossed repeatedly over several generations to species A. The repeated crosses to species A means that the population takes on the characteristics of A. However, by chance or through natural selection, some characteristics of B survive the process and are added to species A. When this process is precisely controlled, it is called the backcross method of breeding. Species A is called the *recurrent parent* and species B is the *donor parent*. The characteristics or genes of B added to A are termed *characteristics or genes under transfer*.

Livestock breeders used backcross breeding for perhaps a century, naming it *line breeding*, so it is a little surprising that plant breeders did not adopt the method at an earlier date. It was first proposed by Harlan and Pope as a method of breeding small grains,⁶ and in the same year Briggs used the method as a means of adding disease resistance to wheat and barley varieties in California.³ It is now used by many plant breeders in the improvement of both self- and cross-pollinated crops, usually as a supplement to other methods of breeding or in combination with them.

In the backcross method a successful variety or inbred line is the recurrent parent. The recurrent parent is crossed to another variety that has a desirable character not present in the recurrent parent. Products of the cross carrying the gene or genes under transfer in F_1 or later generations are backcrossed to the recurrent parent (abbreviated BC^1). The process is again repeated with the products of this backcross (Fig. 13.1). Usually five to six backcrosses are made, but the number may be as high as ten.

The number of times that a recurrent parent is used in a backcross program is indicated either by a superscript or subscript. In Fig. 13.1 the population after the third backcross would be symbolized $A^4 \times B$ or $A_4 \times B$; the original cross of A to B and three backcrosses = A^4 or A_4 . The last population of Fig. 13.1 would be symbolized $A^6 \times B F_2$ or $A_6 \times B F_2$. A further abbreviation for the latter would be $A^6B F_2$, or $A_6B F_2$.

Backcrossing may be considered from two points of view: (1) the recurrent parent and its recovery; and (2) the character under transfer and its recovery.

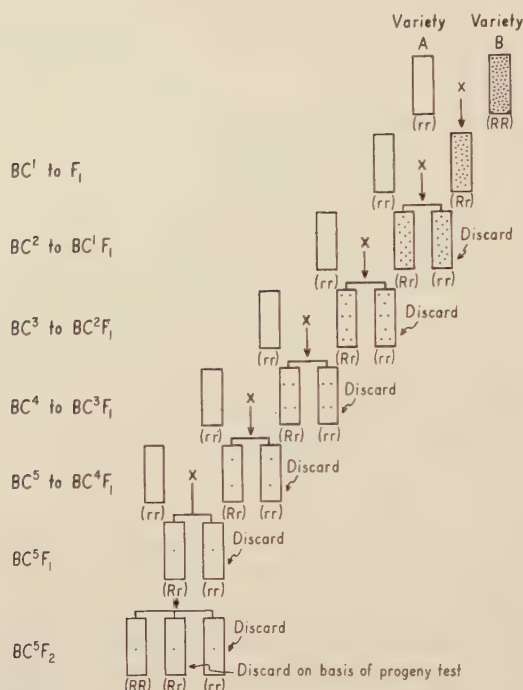


Figure 13.1 Diagram of the backcross method being used to transfer a characteristic produced by gene R in donor variety B to recurrent variety A. Genotypes are in parentheses. The average proportion of B (represented by numbers of dots) in the hybrid population is reduced by one-half with each backcross. Because R is dominant to r , plants of genotype Rr can be recognized after each backcross and used for the next backcross. Plants of genotype rr are discarded. See text for symbols.

Recurrent Parent and Its Recovery

Basic to the successful use of the backcross method of breeding is the existence of a *superior variety* with proven performance. Usually, such a variety is available in most areas with an advanced system of agriculture. Often the variety is deficient in one or a few respects; it may be susceptible to a race of a disease, weak stemmed, or slightly late. Farmers have become accustomed to the culture of the variety, and the processor to its utilization. The merit of preserving established varieties has been apparent in Mexico,¹ Canada,⁷ and Australia.⁹

Reference has been made to successful wheat varieties such as Marquis (Fig. 11.1) in the hard red spring wheat area, Turkey in the hard red winter wheat area (p. 23), and Thorne in the soft red winter wheat area (p. 135). Similar examples of other crops could be given for other areas. From the Chapter 8 discussion of the characteristics of good varieties, it is evident that they are rare combinations of genes in good balance with the environment. Once achieved, the backcross method provides a breeding procedure that will preserve this combination of genes and permit the addition of any number of genes in stepwise fashion to correct its deficiencies.

With every cross or backcross to the recurrent parent, the average proportion of the germ plasm from the donor variety is reduced one-half; this is represented by numbers of dots in Fig. 13.1. Assuming that the F_1 from the original cross had one-half its germ plasm from the donor parent, the proportion in the $BC^1 F_1$ will be one-quarter. If the number of crosses and backcrosses to the recurrent parent is n , the proportion of germ plasm from the donor parent is $(1/2)^n$. After five backcrosses it would be $(1/2)^6 = 1/64$; in Fig. 13.1 this is represented by one dot out of 64 from the donor variety B.

The *proportion of homozygosity* is given by the same formula as that used in the selfing series (p. 127), namely:

$$\frac{2^m - 1}{2^m}$$

where m is the number of backcrosses. After five backcrosses the proportion would be $(2^5 - 1)/2^5 = 31/32$. On the average, the number of homozygous loci in the population will be $31/32$, or 96.9 per cent. The distinction from the selfing series is that under backcrossing the homozygous loci are those of the recurrent parent and are not different combinations of loci from both parents.

The *proportion of homozygous genotypes* in the population is also given by the appropriate formula of the selfing series (see p. 128), which is

$$\left(\frac{2^m - 1}{2^m} \right)^n$$

if m is the number of backcrosses and n the number of heterozygous loci in the F_1 of the original cross. Using the example from the selfing series of ten heterozygous loci, and assuming that five backcrosses were made, 72.8 per cent of $BC^5 F_1$ would be homozygous for the ten loci of the recurrent parent. All homozygous genotypes would be those of the recurrent parent. If one more backcross were made, the number of genotypes like that of the recurrent parent would be 85.4 per cent.

The numbers of backcrosses that are used in breeding programs vary mostly over a range of three to ten. Where ten are used, almost complete identity with the recurrent parent is achieved. Briggs used five or six backcrosses most frequently. However, he selected strongly for the recurrent type in early generations of a backcross program, and felt that by so doing he accomplished the equivalent of two additional backcrosses. After the third backcross, selection was ineffective because all the plants were so much alike. Pugsley felt that there was little to be gained by more than four backcrosses, as judged from experience gained in incorporating rust resistance into ten varieties of wheat in South Australia.⁸

Some breeders prefer to backcross three times at the most, which is sufficient to reconstitute the recurrent parent in large part, but leaves considerable variability in which to select for improvements on the recurrent parent in adaptation and yield.⁷ After the third backcross the breeding material is handled by the pedigree or bulk methods.

Where the recurrent parent is being recovered completely with several backcrosses, it is advisable during the last one to cross to several plants of the recurrent parent. This is done under the assumption that the recurrent parent is made up of several slightly different genotypes.

Character Under Transfer and Its Recovery

Where a choice exists, it is advisable to select the characteristic for transfer in its most intense form. This is particularly true if the characteristic has a low heritability. Even where a characteristic is simply inherited, the more intense expression should be used.

In breeding bunt-resistant varieties of wheat at the California Agricultural Experiment Station, the Martin variety was chosen as the donor parent because it consistently remained free of bunt under heavy inoculation, in contrast to 70 to 90 per cent infection in susceptible varieties. Many of the backcross-derived lines showed as much as 3 to 4 per cent infection. Briggs showed that this was due to modifying factors.² Further study showed that at these low-infection rates, the disease could not maintain itself.¹⁶ Another example is provided by experience with hessian fly. Though Dawson, the donor parent, showed only 2 per cent infestation, the Big Club 43 recurrent parent, recovered by backcrossing, had 4 to 6 per cent infestation. However, once Big Club 43 was sown to the area where hessian fly was present, the insect disappeared within a short time.

In breeding Calrose, a medium-grain rice variety, where the short-grain variety Calora was the recurrent parent, J. W. Jones and L. L. Davis chose Lady Wright, a long-grain variety, as the donor parent. As expected, a good deal of seed length was lost in the transfer, but the re-

covered Calrose variety has been quite successful because it preserved in large part the yielding ability of Calora.

Where the donor variety has two characters to be transferred, it is usually more efficient to transfer each character in separate programs. When the transfers are completed, the characters may then be combined by crossing the recovered types.

Where a character is determined by a dominant gene, it is relatively easy to transfer with a backcross in each generation (Fig. 13.1). This has been true in many cases with disease resistance, where backcross generations have been grown under conditions of natural or artificial infection.

If the character under transfer is due to a recessive gene, it is often advisable to grow backcross generations to F_2 , to permit the identification of the homozygous recessive genotype. An alternative procedure is to backcross twice or even three times in successive generations, and then to grow out the products of the second or third backcross to find plants homozygous for the recessive gene under transfer.

Where a quantitative character is under transfer, each backcross generation may be grown through to F_3 lines before the next backcross is made. In addition, if its heritability is low and several genes are involved, backcross F_2 and F_3 populations will have to be large.

Linkage may complicate backcross breeding. The complication occurs when the gene under transfer is linked to an undesirable gene or block of genes. Selection for the desirable gene will enforce selection for the undesirable gene, the intensity of the selection for the undesirable gene being directly proportional to the tightness of the linkage. Where the effect of the undesirable gene is conspicuous, a selfing series following a cross is more effective in breaking linkage because the opportunity exists for breaks in linkage in both the male and female gametes, whereas in backcrossing it cannot occur in the recurrent parent. However, where selection is not possible for breaks in linkage, either because the effects of the undesirable gene or genes are not expressed under the conditions of selfing and backcrossing or their heritability is low, backcrossing is more effective in breaking linkages than selfing.⁵

Examples of Backcross Breeding

An example of rapid transfer of stem rust resistance from one variety of wheat to another is provided by Pugsley.^{8,9} The recurrent parent was Insignia, susceptible to a new physiologic race of rust in South Australia. A dominant gene for resistance was possessed by Gabo. The procedure was as follows (I = Insignia, G = Gabo, and superscripts indicate number of crosses and backcrosses to Insignia):

1. Insignia $\times$ Gabo (IG), Jan. 18, 1945.
2. IG F_1 (resistant) $\times$ Insignia (BC^1), June 4, 1945.
3. I²G F_1 (resistant segregate) $\times$ Insignia (BC^2), Nov. 7, 1945.
4. I³G F_1 (resistant segregate) $\times$ Insignia (BC^3), March 12, 1946.
5. I⁴G F_1 (resistant segregate) $\times$ Insignia (BC^4), Sept. 27, 1946.
6. I⁵G F_1 resistant plants harvested, February, 1947.
7. I⁵G F_2 resistant plants harvested, June, 1947.
8. I⁵G F_3 lines from F_2 plants: one homozygous resistant line harvested and bulked, November, 1947.
9. F_3 line increased as new rust-resistant strain, December, 1947.

To this period of two years should be added time for growing parents for the original cross plus time for testing the new backcross strain for yield and quality. Since one dominant gene was responsible for resistance, 50 per cent of the F_1 segregates were resistant in the pedigrees I²G, I³G, I⁴G, and I⁵G.

The pattern of backcross breeding used by Briggs in the development of Baart 38 is different from that used by Pugsley, primarily because of the need to carry backcross populations to F_4 in some cases to identify high levels of stem rust resistance.⁵ Baart is susceptible to bunt and stem rust. The need for bunt resistance in the western United States was very real because seed treatment had never given perfect control. Seed treatment reduced yield losses to a low level, but there was enough bunt in the grain to reduce market grade and price. The need for stem-rust resistance was even greater. Therefore, the latter phase of the project was advanced as rapidly as possible. The summer crop was used in testing for resistance to stem rust, since the crop headed and matured under conditions of temperature and humidity that were especially favorable to this disease. Growing two crops a year reduced the time required for transferring rust resistance from Hope. The summer crop was planted in early July and harvested in late October. The spring, or normal, crop was planted in November or December and harvested in early June. The steps taken in transferring stem-rust resistance from Hope to Baart and merging this with Baart 35 to form Baart 38 are shown in Table 13.1.

Development of Baart 38 began in 1930 when Baart was crossed to Hope, the source of rust resistance. A backcross was made in each of the three succeeding generations following the original cross. The Hope $\times$ Baart⁴ population was grown to F_4 with selection for rust resistance and Baart characteristics. A rust-resistant segregate of the F_4 was then backcrossed to Baart.

The backcross of the next generation was to Baart 35. Baart 35 was a bunt-resistant version of Baart developed in a separate backcrossing pro-

Table 13.1 The development of Baart 38 wheat.⁵

YEAR	NURSERY	GENERATION	REMARKS	NUMBER OF NURSERY ROWS ^a	NUMBER OF PLANTS USED FOR CROSSINGS ^b	NUMBER OF HYBRID SEEDS MADE
1930	Spring	Hope × Baart	Original cross	1	1	20
1931	Spring	Hope × Baart ²	First backcross	1	4	49
1931	Summer	Hope × Baart ³	Rust test; 3 heterozygous resistant plants used for second backcross	4	3	66
1932	Spring	Hope × Baart ⁴	All 60 plants were used for third backcross	3	60	904
1932	Summer	F ₁	Rust test; 31 heterozygous resistant plants selected	60		
1933	Spring	F ₂	Without benefit of rust test, selected 405 plants on basis of agronomic characters	31		
1933	Summer	F ₃	Rust test; selected 180 plants from homozygous resistant rows	405		
1934	Summer	Hope × Baart ⁵	Subjected F ₄ to rust, selected 14 plants for fourth backcross	180	14	240
1935	Spring	(Hope × Baart ⁵) × Baart 35 ^c	Selected 28 plants for fifth backcross to Baart 35	14	28	588
1935	Summer	F ₁	Selected 290 plants	28		
1936	Spring	F ₂	Inoculated with bunt, saved 1056 bunt-free plants	290		
1936	Summer	F ₃	Rust test; grew 1056 lines in 3-row blocks, 340 were homozygous for resistance	3,168		
1937	Spring	F ₃	Remnant seed of 340 stem-rust resistant lines inoculated with bunt; 157 were homozygous for bunt resistance. These were bulked as Baart 38	1,020		

^a Rows of Baart grown for checks and parents excluded. Rows were 16 ft long.^b Excludes Baart parent.^c Baart 35 is a bunt-resistant strain of Baart developed independently from Martin × Baart⁷.

gram. This illustrates an important feature of the backcross technique. It provides for the stepwise improvement of a variety and the use of the most recently improved version as the recurrent parent. In the present example the final version was resistant to both bunt and stem rust.

From the fifth backcross the material was carried through F_1 and F_2 to F_3 lines. In the first planting of F_3 lines during the summer of 1936, rust-resistant lines were identified. Remnant seed from planting these rust-resistant lines was inoculated with bunt spores and sown during the winter of 1937. The 157 lines that were resistant to bunt were bulked to form Baart 38.

Baart 38 required eight years for its development, more than twice the time used by Pugsley for rust-resistant Insignia. However, 8 years is a much shorter period than the 12 to 16 years required with the pedigree or bulk methods.

It will be noted that there were only five backcrosses to Baart. This is less than the usual number used by Briggs. Subsequently, two more backcrosses were made, and the new version of Baart was released as Baart 46. The number appended to Briggs' varieties is the year of seed increase prior to release.

Knight has added disease and insect resistance to Sakel, a cotton variety with desirable fiber qualities and good adaptation to Sudan.¹³ Eight major genes for resistance to the blackarm disease (*Xanthomonas malvacearum*) were identified and transferred to Sakel strains through backcrossing. Resistance to jassid (*Empoasca*), an insect, has also been added. Resistance to jassid depends on a dense growth of long hairs on the lower leaf surface. Thomas has reviewed the role of backcrossing in the improvement of cotton, tomatoes, and potatoes.¹³

Cross-pollinated Crops

Backcross breeding has been used much less with cross-pollinated crops than with self-pollinated crops, though the genetic principles apply equally well. Two features of cross-pollinated crops must be kept in mind: (1) their heterogeneity and heterozygosity will require that large numbers of plants of the recurrent parent be used in each backcross; and (2) it may be difficult or impossible to grow backcross generations as selfed F_3 lines to identify more accurately the plants carrying a character under transfer.

Backcross breeding of alfalfa. Stanford and Houston used the backcross method in developing the Caliverde variety of alfalfa, which is resistant to bacterial wilt, mildew, and leaf spot.¹¹ The project was set up to transfer resistance to bacterial wilt from a Turkestan strain to the California Common variety. Resistance to mildew and leaf spot was ob-

tained from a selected strain of California Common, which was used in one backcross.

California Common met the qualifications of a recurrent parent very well. It had its origin as an introduction from Chile, made nearly a hundred years ago. It has since been molded by natural selection until it now represents a type of exceptional merit, though it is susceptible to bacterial wilt and other diseases. In making each backcross, 125 plants of California Common were used as the female parent, so that its full range of variability would be under transfer.

Improvement of inbred lines. Another application of backcross breeding to cross-pollinated crops is in improving inbred lines used in hybrid varieties. For such purposes, procedures are the same as in self-pollinated crops.

Evaluation of the Backcross Method of Breeding

The backcross method has been most useful in the transfer of characters with high heritability. In most cases resistance to disease is highly heritable, often being determined by one or a few genes with clear expression in the presence of the disease. Resistance to disease is a spectacular improvement in crop plants, usually making the difference between success and disaster. In the light of this situation the backcross method has made an important contribution to the improvement of cereal crops in California. This contribution has been reviewed in some detail.^{5,12} Pugsley believes that breeding for disease resistance in cereals should be based entirely on the backcross technique.⁹

Backcrossing is not limited to breeding for disease resistance. Suneson developed an awned version of the wheat variety Onas, which was released as Onas 53 because it was higher yielding than Onas (see pp. 79-80). Walkof has used backcrossing to add earliness to tomatoes.¹⁴ Some of the features of the backcross method of breeding are summarized below.

Accumulation of desired characters. It has been pointed out that Baart 38 was a product of two backcross programs, one to add bunt resistance and the other to add stem-rust resistance. Another example would be Big Club 43 (Fig. 13.2). It will be noted that Big Club was the first recurrent parent, followed by Big Club 37 resistant to bunt, and finally Big Club 40 resistant to hessian fly. The final product, Big Club 43, was resistant to bunt, hessian fly, and stem rust.

Independence of backcross breeding from environment. Backcross breeding may be done in any environment where the plant will grow and where the character under transfer will express itself; the evaluation of agronomic performance is unnecessary. Therefore the greenhouse and

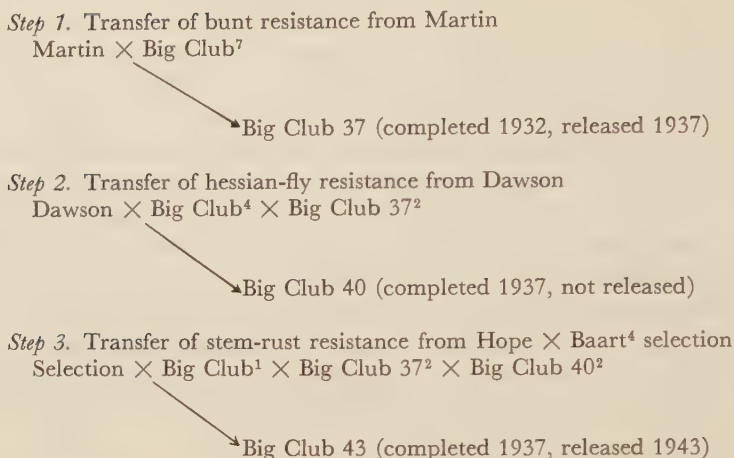


Figure 13.2 The improvement of Big Club, a well-adapted variety in some regions of California, by successive steps through backcrossing. (Briggs.⁴)

off-seasons may be used in advancing generations and in evaluating the character under transfer. This is illustrated by a program concerned with adding nematode resistance to the Ventura variety of the standard lima bean, adapted to the fog belt along the coast of southern California. It was done more efficiently at Davis, where facilities were available, than could possibly have been done 400 miles away. It was no handicap that the Davis area is totally unsuited to production of this variety.

Evaluation trials. An important feature of the backcross method, emphasized by Briggs, is that backcross-derived varieties can be safely released to growers without evaluation of yield, adaptation, or quality.³ That practice has been followed, and its validity has since been confirmed. Suneson reported on comparisons of nine backcross varieties of wheat produced at the California Agricultural Experiment Station at Davis up to 1944.¹² His comparisons were made in tests over nine western states of the United States, but comparisons were excluded where bunt, stem rust or hessian fly attacked the unimproved variety. Suneson concluded that these comparisons failed to show any general significant difference in yield.

From an average of more than 60 tests, Baart 38 excelled in test weight by 0.33 lb. That difference has a high mathematical probability of being real, but amounts to only 0.6 per cent. Baart 38 was slightly inferior to Baart in baking quality. This deficiency has since been removed by the addition of two more backcrosses in the production of Baart 46.

Atlas 46, which is resistant to mildew and scald, was compared by Schaller with Atlas barley, its prototype.¹⁰ In the absence of disease there was no difference in yield.

Résumé

The backcross method of plant breeding has three requirements: (1) a satisfactory recurrent parent must be available which needs improvement in only one or very few characters; (2) donor parents must also be available with the characteristics needed in the recurrent parent, preferably with one or a few genes determining the inheritance of each desired characteristic; and (3) the number of backcrosses should be sufficient to reconstitute the recurrent parent.

Its particular advantages are: (1) because improvements are made in a stepwise fashion, previous gains are not lost; (2) the breeding program may be independent of the environment; (3) evaluations of backcross-derived varieties are not necessary; (4) it is rapid; (5) it requires a small number of plants; (6) it is predictable; and (7) it is repeatable. Its greatest use has been in the development of disease-resistant varieties.

There is only one disadvantage to backcrossing, but it is important. It does not permit the achievement of unusual combinations of genes from two or more varieties. If such a combination of genes is obtained from a program using the pedigree or bulk population methods, the performance of such a variety may be on a plane different from that of the recurrent parent of a backcross program. However, the backcross method is adaptable in part to this situation. Enough backcrosses may be made to recover essentially the recurrent parent, and yet leave enough variability for exploitation by other methods of breeding.

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CROSS-POLLINATED CROPS



A Mendelian population is a reproductive community of sexual and cross-fertilizing individuals which share in a common gene pool.

DOBZHANSKY, 1950

IN SELF-POLLINATED CROPS attention was centered on homozygous genotypes. With cross-pollinated crops one must think in terms of heterozygous genotypes, each one different to some degree from all others in the population. This heterozygosity and variability is, of course, a consequence of outcrossing, which permits a whole new assembly of gene recombinations to appear in each generation. On the average, these assemblies will be similar from generation to generation.

When evaluating a cross-pollinated plant, the plant breeder is not too much interested in the genotype of the plant itself because it is heterozygous and will not breed true. Instead, he is interested in the genotypes of the gametes that the plant will contribute to make the next generation. The success of these gametes will in large measure depend upon how well they combine with gametes produced by several other plants. Actually, much of the effort that goes into the improvement of cross-pollinated crops involves measurements of the value of gametes.

The Gene Pool

A useful concept in considering cross-pollinated crops is that of the "gene pool" proposed by Dobzhansky.^{1,2} During meiosis at the end of sporophytic generation, the genes are put back into the pool. There they remain until the end of the gametophytic generation, when they are withdrawn as gametes combine at fertilization to form the next sporophytic generation. The next generation is never quite the same assembly of genotypes as the previous one, at least within the limits of most populations. Assuming that many loci are represented by more than one allele, and perhaps by several, the number of possible genotypes is almost limitless. A gene pool may be thought of as a stable entity, that produces genotypes performing on the average about the same from generation to generation. When the word genotype is used, unless otherwise specified, it refers to the genotype of the sporophyte.

The frequency of genes in the pool, however, is determined in large part by their fate in the genotype. If they contribute to successful genotypes producing abundant seed, such genes will increase in the pool. Conversely, if they handicap genotypes, their frequency will decrease. It is the role of the plant breeder to manage gene frequencies so as to yield the most successful genotypes.

Linkage, of course, restricts the free combination of genes. Undesirable genes may be carried in the gene pool if they are tightly linked to successful genes. Likewise, selection against an undesirable gene will reduce the frequency of a desirable gene closely linked to it. There is some evidence, however, that natural selection through long periods of time has favored linkages that would benefit the species.

When exposed to a constant environment, the gene pool comes to equilibrium, with gene and genotype frequencies remaining constant within the limits of random fluctuations. The gene and genotype frequencies are, however, not independent entities. As Dobzhansky has said, they are *coadapted*. Changes in the frequencies of some genes or genotypes will result in changes in others; these in turn cause other changes and the result is a new equilibrium and a new coadaptation.

The fate of a gene in a population is determined by its effect (1) in combination with itself, that is in the homozygous state; (2) in combination with other alleles at the same locus; (3) in combination with genes at another locus, or epistatic effects; and (4) in combination with closely linked genes. It is obvious that the management of gene pools is a difficult undertaking.

Hardy-Weinberg Law

Independently, Hardy³ in England and Weinberg⁵ in Germany developed the Hardy-Weinberg law. Given the frequency of two alleles, this law states the frequency of genotypes at equilibrium. If the frequency of an allele A is q and a is $1 - q$, the frequency of the genotypes will be

$$q^2AA + 2q(1 - q)Aa + (1 - q)^2aa$$

This law assumes that (1) there are only two alleles; (2) selection pressure for both alleles is the same; (3) mating is at random; (4) there is no differential migration of alleles into or out of the population; and (5) the rate of mutation from A to a is the same as a to A . This formula provides a useful base from which to consider cross-pollinated populations, even though the assumptions rarely apply, in total at least, to any population. Actually, it is modifications in the equilibrium that are of concern to plant breeders, since these are the bases of change and improvement. The effects of selection are so important that an entire chapter is devoted to them.

Some examples of the use of this formula are as follows: If the frequency of $A = q = 0.9$ and $a = 1 - q = 0.1$, the frequency of the genotypes therefrom will be $(0.9)^2AA + 2(0.9)(0.1)Aa + (0.1)^2aa = 0.81AA + 0.18Aa + 0.01aa$. Conversely, if the frequency of aa is known, it is possible to calculate the frequency of AA , Aa , q , and $1 - q$. If $aa = 0.04$, the frequency of $a = 1 - q = \sqrt{0.04} = 0.2$. The frequency of $A = q = 1 - 0.2 = 0.8$, and the frequencies of AA and Aa are 0.64 and 0.32, respectively.

How soon does a population reach equilibrium? This is an important question, and one that is easily answered. It is reached in one generation of random mating, in any mixture of different genotypes. In other words, a mixture of different genotypes growing together would give in the next generation a population that was in equilibrium, but *only for individual loci*. Where more than one locus is involved, equilibrium is achieved in a matter of a few generations, provided linkage is not involved.

Several alleles at one locus. The Hardy-Weinberg formula assumes only two alleles at a locus. Actually, there will often be more, but the

formula can be extended to handle this situation. If the frequency of three alleles, A , A_1 , and A_2 , are represented by p , q , and r , respectively, and $p + q + r = 1$, the formula becomes

$$p^2AA + q^2A_1A_1 + r^2A_2A_2 + 2pqAA_1 + 2prAA_2 + 2qrA_1A_2$$

If $p = 0.2$, $q = 0.3$ and $r = 0.5$, the frequencies become: $0.04AA + 0.09A_1A_1 + 0.25A_2A_2 + 0.12AA_1 + 0.20AA_2 + 0.30A_1A_2$. With more alleles the proportion of homozygous genotypes tends to decrease. With two alleles in equal amounts, the proportion of homozygotes is 0.50, with three 0.33, four 0.25, and five 0.20. If there were any advantage to heterozygosis per se, natural selection would tend to increase the number of alleles at each locus.

Nonrandom mating. In the model population considered above, it was assumed that mating was entirely at random. This would be expected to occur only in very large populations. Even in these, purely chance assortment may at times produce significant differentiation. In smaller populations such as those the plant breeder will be working with, the random change from generation to generation is likely to be continuous. This has been referred to as the *Sewall Wright effect* or *genetic drift*.⁶

Starting with a population in which A and a are equal, then $q = 1 - q = 0.5$. Wright has shown that the frequencies of A and a will fluctuate up and down by chance and that the smaller the population, the greater the fluctuation.⁶ If q changes from 0.5 to 0.6, a new gene frequency is established, which leads to a population ratio of $0.36AA:0.48Aa:0.16aa$, with an increase in homozygosity from 50 to 52 per cent. The ratio of $AA:aa$ changes from 1:1 to 2.25:1. The process is reversible as long as the value of p is between 1 and 0 because variation is by chance. If q should become 1, the population would become homozygous for AA , and if $1 - q = 1$, it would be aa . Either might reappear as a mutation.

Mutations. Mutations are important in producing variability in populations over a long period of time. The rate of accumulation of mutant genes in a population is related to their frequency and their survival value. For the sake of simplicity, only gene mutations will be considered.

The mutation frequency is quite variable for any given gene. For example, Stadler found that frequencies in eight genes of corn ranged from 0 to 492 mutations per million gametes.⁴ Others have found similar frequencies. Also, it should be borne in mind that mutations are reversible. Their equilibrium in populations will be illustrated by a few examples.²

An example may be considered in which AA is the ancestral form; only mutations from A to a occur, and AA , Aa , and aa are equally adapted. Under these circumstances the population eventually will become homozygous for aa because, with each mutation, q decreases and $1 - q$ in-

creases. If the reverse mutation occurs with equal frequency, the equilibrium of the population will not be disturbed. However, the ratios in the two directions usually are different.

Another example may be used, namely, where the mutation of A to a (u) is opposed by the reverse mutation a to A (v). If these occur at the ratio of $0.000002u$ to $0.000001v$, the equilibrium value of q will equal $v/(u + v)$ or $0.000001/(0.000002 + 0.000001)$, or 0.33 . Then $1 - q$ becomes 0.67 . This means that 33 per cent of the chromosomes will carry the gene A and 67 per cent the gene a . The genotypes will occur in the ratio of $1AA:4Aa:4aa$.

Finally, we should consider the situation where the recessive mutant is a lethal character, such as albinism in corn. The equilibrium value of a completely recessive lethal (a) has been shown to equal the square root of the mutation rate of the gene (A) producing that lethal, or the frequency of a , $1 - q = \sqrt{u}$. Thus, if the recessive mutation occurs at the rate of four in a million, two-tenths of 1 per cent of the chromosomes will eventually carry it.

Integration. Rarely, if ever, is a genetic population completely isolated from other populations with which it can hybridize or integrate. Integration might occur in a closely related population or in one that differs to the extent of belonging to a different species. Hybridization will disturb gene equilibria through the introduction of new alleles into a population by changing frequencies of established alleles, and by providing for new interactions of alleles at different loci.

Features of Cross-Pollinated Crops

Heterogeneity. From the discussion of the genetic structure of cross-pollinated crops, it may perhaps be assumed that varieties of such crops would be highly heterogeneous, in comparison with varieties of self-pollinated crops, which consist of one or a few genotypes. The difference, however, is not so great as might be expected. Often, varieties of cross-pollinated crops present a rather uniform appearance under field conditions. It is only when the plants are grown under spaced conditions that the heterogeneity is apparent.

Heterozygosity. Heterozygosity is imposed on populations by cross-pollination. It is obvious in selfed progenies of single plants also, when segregation will occur. Their heterozygous nature will permit some part of the variability to be hidden, only to be exposed when segregation occurs in the next generation. Heterozygosity, as will be seen later, is involved in the expression of heterosis.

Inbreeding. Imposing homozygosity by inbreeding in a cross-pollinated crop usually depresses vigor, and may expose many recessive genes with

conspicuous effects. A later chapter deals with both inbreeding and heterozygosity in some detail.

Enforced hybridity. Many cross-pollinating species have some means to enforce cross-pollination. It may involve the separation of the sexes into different flowers or into different plants, or pollen may simply be nonfunctional under selfing due to some system of incompatibility. This is considered in the next chapter.

Résumé

A cross-pollinated crop may be considered as a population drawing upon a common gene pool to form each new generation of genotypes. Each genotype in a population is different to some degree from all others, each is highly heterozygous, each is largely outcrossed, and each gives a variable progeny. Plant breeding of cross-pollinated crops has taken two directions; it has changed the frequencies of genes in the pool so that they combine to produce a higher frequency of superior genotypes, or it has developed inbred lines which combine to produce vigorous hybrids.

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15

CONTROL OF CROSS- POLLINATION



The improvement of plants and animals by breeding is based essentially upon changing the natural breeding system of the species.

LEWIS, 1956

THE MORE OBVIOUS morphological features enforcing cross-pollination have already been noted in Chapter 4, and some of these will be discussed in more detail here, having in mind their genetic basis or their utilization by the plant breeder. Greater emphasis, however, will be given to genetic systems favoring cross-pollination, often referred to as incompatibility systems. A thorough knowledge of the breeding system of a crop species is essential, since improvement procedures will sooner or later require ma-

nipulation of the breeding system. It is even more essential with the increased use of hybrid and synthetic varieties.

Male sterility, a relatively unimportant means of pollination control in natural species, has become an important tool of the plant breeder in the production of hybrid varieties. It is so important that it is being exploited in both the self- and cross-pollinated plants.

Separation of Male and Female Flowers

Dioecy. Among the higher plants, in contrast to the higher animals, the separation of sexes in different individuals is rare. Some important dioecious cultivated plants are dates, hops, asparagus, spinach, and hemp. This system of mating is positive in its effect; all seed must be a consequence of cross-pollination. Occasionally, most dioecious species will produce a monoecious type, and a plant breeder may increase the frequency of such types through selection.

At about the time that the date was first cultivated several thousand years ago, it was realized that the orchard need have very few male plants. In modern orchards the proportion of male trees is three or four to a hundred females. Though naturally wind-pollinated, the usual practice is to collect pollen by hand and apply it to the inflorescences of the female plants. The male plant will determine in part the quality of the fruit, so it is necessary in the improvement of this crop to consider both sexes.

In hops, the female inflorescence is used commercially. Highest quality is achieved when hops are unfertilized and produce no seed. Consequently, male plants cannot be found in most hop yards. Where seed hops are produced, it is necessary to have about 1 male plant to every 100 females. In a breeding program, little attention need be given the development of a male plant except that when it is used, it should produce abundant pollen and should flower at the same time as the female plant. Any program of improvement through hybridization, however, will depend as much on the male as the female.

Both hops and dates are perennial and may be clonally propagated, which has facilitated the perpetuation of desirable types. In annual crops such as spinach or hemp, there are approximately equal numbers of male and female plants, and both are used commercially. This is true also of asparagus, a perennial, which is established from seed. Both the male and female plants appear to be about equal in quality, though there is good evidence that the female asparagus plant is more productive than the male.

Improvements in dioecious crops propagated by seed have been limited mostly to mass selection. More recently, controlled hybridization and

selection have been used. Inbreeding has been achieved by brother-sister matings. This, of course, is much less efficient than selfing in promoting homozygosity. Where monoecious plants occur, they may be selfed. While this will hasten the approach to the homozygous condition, it will impress the monoecious characteristic on the progeny. It would be a blind alley to the breeder interested in retaining the dioecious character.

Monoecy. From most breeding standpoints, monoecious species are handled as though they had both sexes in the same flower. Selfing procedures are facilitated if the two sexes are on the same inflorescence, but care should be taken to move pollen inside the selfing bag from the male to female flowers. In corn, where the male and female flowers are on different inflorescences, two selfing bags are required, and pollen must be transferred by hand. Crossing is greatly facilitated where the flowers are separate, particularly where the sexes are on different inflorescences. Their separation in corn has been exploited in producing hybrid varieties, to the extent that removal of the tassels is done mechanically, at least in large part.

Incompatibility

It is obvious that dioecy and monoecy are inefficient systems of producing seed, since seed production is restricted to certain plants, certain inflorescences, or certain flowers. A much more efficient system is that involving some form of self-incompatibility. Often the systems are as effective as dioecy in preventing self-pollination, yet all plants and flowers produce seed. Sometimes, however, the genetic systems enforcing cross-pollination may be associated with morphological features that facilitate cross-pollination.

East¹³ has estimated that more than 3,000 plant species distributed among 20 families have some system of incompatibility, but Lewis²⁵ thinks that this is a low estimate. It is present in many cultivated species. Lewis classified incompatibility systems into two main groups, heteromorphic and homomorphic. The homomorphic group is further divided into gametophytic and sporophytic types.

Heteromorphic incompatibility. This system depends on the relative lengths of the stamens and the style. Darwin listed 38 genera having heterostyled species, and described in detail the situation in *Primula sinensis*.⁹ Subsequently, Lewis worked out its genetic basis. One type has long styles and short anthers, termed *pin*, and the other has short styles and long anthers, termed *thrum* (see Figs. 15.1 and 15.2). *Pin* has the genotype *ss* and *thrum* has *Ss*, with *S* completely dominant to *s*. Compatible crosses are

Pin (ss) $\times$ thrum (Ss) $\rightarrow$ 1 pin (ss):1 thrum (Ss)
Thrum (Ss) $\times$ pin (ss) $\rightarrow$ 1 pin (ss):1 thrum (Ss)

Incompatible crosses (pin $\times$ pin and thrum $\times$ thrum) do not occur, so that no line breeds true for the genotypes ss or SS .

The main effects of the genes S and s in producing self-incompatibility is not on the relative lengths of the styles and stamens but on incompatibility reactions of the style and pollen tube. Pollen from the genotype ss will grow in the style of a plant of the genotype Ss , and vice versa. In other words, the genotype of the plant producing the pollen determines the pollen behavior; hence it is said that pollen behavior is under sporophytic control. For example, in the style of genotype Ss , pollen of genotype s will grow if it comes from a plant of genotype ss , but not if it comes from a plant of genotype Ss .

Gametophytic incompatibility. The gametophytic or oppositional system was first found in *Nicotiana sanderae* by East and Mangelsdorf.¹⁴ Since then it has been found in many species, including the following

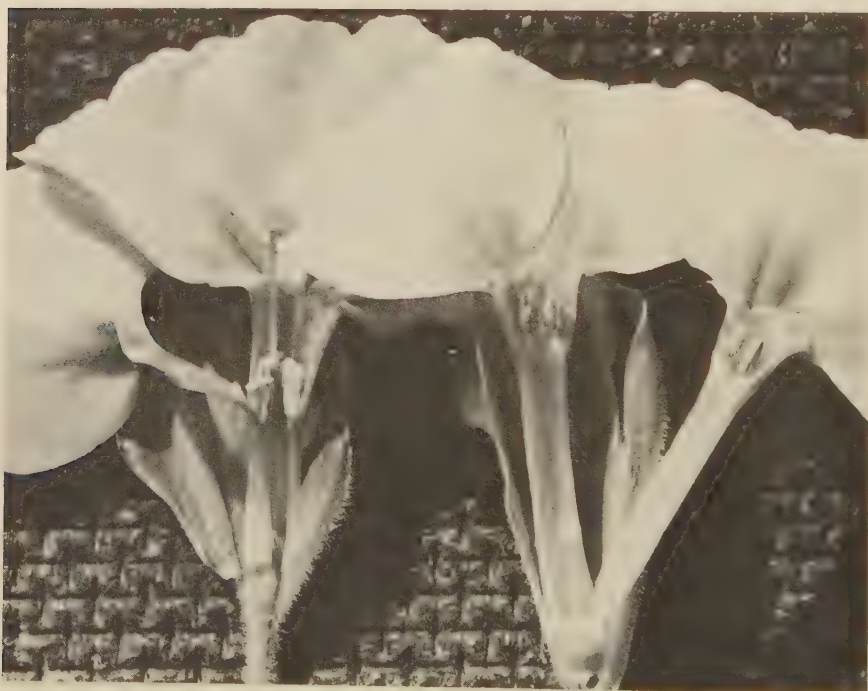


Figure 15.1 Flowers of *Primula sinensis*. On the left the pin type with long style and stamen attached deep in the corolla tube; on the right the thrum type with short style and anthers high in the corolla tube.

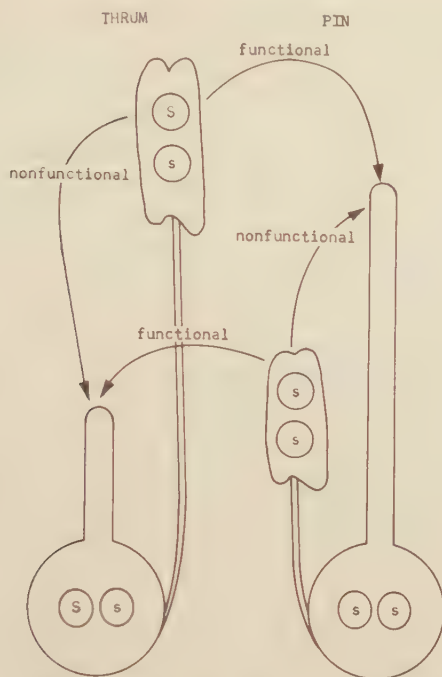


Figure 15.2 Heteromorphic system of incompatibility. Also sporophytic, since *S* is dominant to *s*, and both *S* and *s* pollen of plant with genotype *Ss* behave the same. Both flowers are self-sterile, and in crossing produce equal numbers of *Ss* (thrum) and *ss* (pin) genotypes.

cultivated crops: red clover, white clover, alsike clover, and yellow sweet clover.

A series of alleles at one locus determines whether pollen will be functional or nonfunctional. These alleles have been termed $S_1, S_2, S_3, \dots, S_n$. Diploid stylar tissue will normally have any two alleles of the series, but never the same two. A single pollen grain will have only one. When the stylar tissue has the same gene as the pollen grain, the development of the pollen tube is inhibited. Pollen of two genotypes will be produced by any plant, both inhibited in their development in styles of the plant producing them. Considering the fertility relationships of two different plants, three levels of crossing will occur (Table 15.1): (1) fully incompatible, which is similar to selfing; (2) half the pollen compatible; and (3) fully compatible, in which all pollen grains of each plant

Table 15.1 Three levels of cross-compatibility under the gametophytic and sporophytic systems of self-incompatibility.

SYSTEM	CROSS		MALE GAMETES		GENOTYPES OF PROGENY
	<i>Female</i>	<i>Male</i>	<i>Functional</i>	<i>Non- functional</i>	
<i>Gametophytic</i>					
<i>Both plants with the same genotype: no pollen functional</i>	$S_1S_2 \times S_1S_2$		None	All	None
<i>Plants differing in one allele: one-half of pollen functional</i>	$S_1S_2 \times S_1S_3$		S_3	S_1	S_1S_3, S_2S_3
	$S_1S_3 \times S_1S_2$		S_2	S_1	S_1S_2, S_2S_3
<i>Plants differing in both alleles: all pollen functional</i>	$S_1S_2 \times S_3S_4$		S_3, S_4	None	$S_1S_3, S_2S_3, S_1S_4, S_2S_4$
	$S_3S_4 \times S_1S_2$		S_1, S_2	None	$S_1S_3, S_2S_3, S_1S_4, S_2S_4$
<i>Sporophytic^a</i>					
<i>Both plants with the same genotype: no pollen functional</i>	$S_1S_2 \times S_1S_2$		None	All	None
<i>Plants differing in one allele: all or none of pollen functional</i>	$S_1S_2 \times S_2S_3$		None	S_2, S_3	None
	$S_2S_3 \times S_1S_2$		S_1, S_2	None	$S_1S_2, S_1S_3, S_2S_2, S_2S_3$
<i>Plants differing in both alleles: all pollen functional</i>	$S_1S_2 \times S_3S_4$		S_3, S_4	None	$S_1S_3, S_2S_3, S_1S_4, S_2S_4$
	$S_3S_4 \times S_1S_2$		S_1, S_2	None	$S_1S_3, S_2S_3, S_1S_4, S_2S_4$

^a Assuming the order of decreasing dominance in the pollen to be S_1, S_2, S_3 , and S_4 , and no dominance in the style.

are functional on the other. With gametophytic incompatibility, reciprocal crosses give the same breeding behavior, though not the same genotypes if the parents have an allele in common.

The ability of the pollen to function is determined by its own genotype and not by the plant that produces it. This is the reason for the term "gametophytic incompatibility."

East and Yarnell found an S allele that was self-compatible, and termed it S_t .¹⁵ A plant with this allele in the homozygous or heterozygous condition is self-fertile. Later, Anderson and de Winton found another S allele able to inhibit pollen tubes with the S_t allele.²

Sporophytic incompatibility. In the sporophytic system the incompatibility relationship of pollen is determined by the plant producing it, and in this respect is similar to the situation in heterostyled plants described above. The first analysis of this situation was made in *Crepis foetida* by Hughes and Babcock²⁰ and in *Parthenium argentatum* by Gerstel.¹⁸ While sporophytic incompatibility has been identified in other species such as broccoli,²⁹ radish,^{3,36} and kale,⁴² it appears to be more limited in occurrence than gametophytic incompatibility.

As in gametophytic incompatibility, the sporophytic is usually determined by a series of alleles at a single locus. Since the alleles may show dominance or individual action or competition, the genetic explanation may be very complex. A comparison with the gametophytic system is given in Table 15.1, where it is assumed that there is dominance in the pollen and individual action in the style. Note that where plants have one allele in common both reciprocal differences and homozygous types may occur, neither of which is possible in the gametophytic system.

Physiology of incompatibility. Cytological studies have indicated that incompatibility may be expressed in three different ways: (1) the germination of pollen is decreased; (2) the germination of the pollen is normal, but pollen tubes are inhibited in the style; and (3) the pollen tubes grow normally, and gametes reach the ovules, but no seed develops.³⁷ The chemistry of incompatibility, at least in some cases, seems to involve some sort of antigen-antibody reaction.

Broccoli provides an example of the first type, where the germination of pollen is decreased. Sears found that the incompatibility reaction occurred in the stigma, and that its removal, totally or in part, permitted a normally incompatible flower to produce seed.³⁷ Even maceration of the stigmatic surface was sufficient.

An example of the second type is provided by species of *Nicotiana*.¹² In this genus, the pollen germinates normally under selfing, but the pollen tube is inhibited in part as it penetrates the stigma; it then grows slowly in the stylar tissue until the flower falls because fertilization has not occurred. Inhibition of pollen-tube development may occur in other parts of the style in other species, retardation in red clover taking place a little over halfway down the style.³⁸ In still other species the pollen tubes may burst before reaching the bottom of the style.

The third type, with incompatibility manifested after fertilization, is rare. In *Gasteria*, pollen germination and tube development were not affected in incompatible pollinations.³⁷ Degeneration occurred after fertilization when the endosperm was in the two nucleate stage and the embryo was uninucleate. Degeneration occurred first in the integuments

and proceeded thereafter as if no fertilization had occurred. In cacao (*Theobroma cacao*) the male nucleus fails to pair with the polar nucleus to form the endosperm.

Induced mutations. Gametophytic self-incompatibility systems have been much easier to change through mutation than have sporophytic systems. By application of X-rays, P³², and chemical mutagens to pollen mother cells, Lewis was able to obtain mutations of the *S* alleles of *Prunus avium* to self-fertile, *S_f*, types.²⁵ Mutations were easy to identify on selfed plants, simply because the mutated pollen effected fertilization and seed was produced. Such seeds on a plant of genotype *S₁S₂* would be *S₁S_f* and *S₂S_f*. Stable mutations were obtained only from irradiations applied during the resting stage prior to meiosis, and were dominant to incompatibility alleles. Stable self-fertile alleles could be handled easily and transferred to other genotypes in the following manner:

$$\begin{aligned} S_1S_2 \times S_1S_f \text{ (male)} &\rightarrow S_1S_f + S_2S_f \text{ (all fertile)} \\ S_1S_2 \times S_3S_f \text{ (male)} &\rightarrow S_1S_3 + S_2S_3 \text{ (both self-sterile) and} \\ &\quad S_1S_f + S_2S_f \text{ (self-fertile)} \end{aligned}$$

Mutagenic agents have not changed incompatibility alleles from one to another. The reason for this is not known.

Substances involved in sporophytic incompatibility are developed before meiosis, so that irradiation of pollen mother cells will have no effect on fertility relationships. Irradiation at an earlier stage may have no immediate effect on the pollen because the allele for self-compatibility is recessive.

Effects of polyploidy. Where incompatibility is determined sporophytically, it is not changed greatly by doubling the chromosome number. This is to be expected because the diploid already operates under a system where two different alleles may interact to give the different expressions of incompatibility. Raising the number to four permits more of the same type of situation found in the diploid. With a tetraploid it is possible to have four different incompatibility alleles present in the style, each inhibiting a different pollen. This means that the cross-compatibility would be reduced. For the same level of cross-compatibility, a larger number of incompatibility alleles should be present in the tetraploid than in the diploid.

With the gametophytic system the situation is quite different.^{24,25,26} When a pollen grain carries two different alleles in a tetraploid, they may interact to neutralize the incompatibility reaction. In other words, pollen carrying such combinations of different alleles may be functional on styles carrying the same alleles; that is, selfing is possible. Where pollen grains carry the same two alleles, interaction is not possible; such

pollen grains will not function on styles carrying the same allele. The general effect of autotetraploidy is to weaken the incompatibility system, and to eliminate it completely in some species. An example is provided by the pear, which is self-incompatible as a diploid but compatible as an autotetraploid. The same has been true in alsike clover and white clover,^{4,5,6} and red clover.³²

Incompatibility and plant breeding. In general, self-incompatibility is a handicap to the plant breeder because of the restriction it places on inbreeding. As a consequence, much effort has been devoted to finding means or developing mutations that permit self-fertility. Some of the techniques employed to permit self-pollination are: (1) removal of the stigmatic surface, as described above; (2) pollination in the early bud stage, apparently before the inhibiting substance is formed; and (3) lowering the temperatures, which either slows down the development of the inhibiting substance or gives the pollen tube a longer time in which to grow.

An unexpected advance in cherry breeding occurred as a consequence of inbreeding cherry trees following the achievement of self-fertility through mutations.²⁶ One of the plants obtained through inbreeding was two days earlier than the earliest variety known, which suggested that inbreeding might yield greater variability than crossing. Lewis feels that the improvement of several cultivated plants has followed the loss or weakening of their incompatibility systems. In this regard he compared two species of *Primula* cultivated for about 80 years: *P. sinensis* yields some seeds on selfing and has tremendous variation in flower color, form, habit and leaf; but *P. obconica*, which is completely self-incompatible, has little variation. Likewise, the garden snapdragon has exhibited many varieties following the loss of its incompatibility system.

It is apparent that a second consequence of self-compatibility will be inbreeding depression. The inbreeding depression may be tolerated in breeding materials, but it is likely that it will be necessary to restore incompatibility to commercial varieties, at least those grown from seed.

Where seed is not desired in the fruit, as in the pineapple, self-incompatibility is essential. It is desirable also in certain ornamental plants where blooming will occur over a longer period if seed production does not occur. Of course, these examples require both that the plants be propagated clonally and that other clones of the same species not be present to provide pollen.

Other clonally propagated crops such as cherry, apples, pears, and several other tree fruits will not produce fruit without fertilization. This has required the presence in the orchard of a few trees of a second variety to provide pollen. Where two varieties are present, cross-pollination is

very largely dependent upon bees, which often have to be introduced to the orchard at considerable expense. Even then, in cold wet weather, their activity may be reduced to a point where cross-pollination is seriously reduced and low yields result. The introduction of self-fertility to such clonally reproduced crops would greatly simplify the pollination process and would permit the planting of large blocks to a single variety.

Male Sterility

Male sterility is not a widely occurring phenomenon under natural conditions, but has been observed frequently in cultivated plants. In fact, in almost all crops, where a diligent search has been made, some form of male sterility has been found. Its increasing use in the production of hybrid varieties makes it necessary for the plant breeder to be familiar with its genetic basis and its expression.

As pointed out by Gabelman, male sterility may take many forms.¹⁷ It may include situations where: (1) the pollen is absent or extremely scarce, termed pollen sterility; (2) there is severe malformation or absence of the stamens or male flowers, called staminal sterility; and (3) the pollen is normal but fails to dehisce, known as positional or structural sterility. Usually, however, male sterility means pollen sterility. Male sterility may result from gene action, a combination of genes and cytoplasm, or from cytoplasmic influences alone.

Genetic male sterility. Genetic male sterility is determined usually by a single recessive gene, often termed *ms*. This form of male sterility has been found in several crops, among them tomatoes,³⁴ barley,⁴¹ lima bean,¹ and potato.³⁰

The propagation of genetically male-sterile types requires special procedures. Assuming that the male-sterile plant has the genotype *msms*, it is necessary to use pollen from a heterozygote, *Msms*. In this case, half the progeny will be male-sterile (*msms*) and half male-fertile (*Msms*). In other words, in such a population grown under isolation, if only the male-sterile plants are harvested, the progeny will always be 50 per cent male-sterile (see p. 157).

The recognition of male-sterile types may present a problem, and this applies to all types. In barley they are obvious (Fig. 15.3) because the lemma and palea often remain open for some time after anthesis, or they may set less than the normal amount of seed. Where abundant pollen is available, as in a cross-pollinated species, male-sterile plants may set seed in the normal manner. Their identification often requires close examination of the anthers to learn whether or not anthesis is taking place.

Cytoplasmic-genetic male sterility. Sterility depends upon the inter-

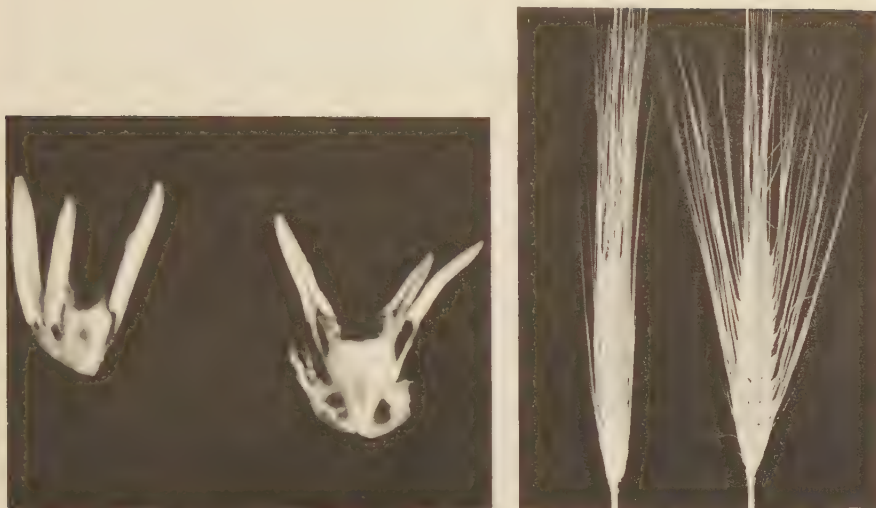


Figure 15.3 Genetic male sterility in barley. Right: male-sterile head with lemmas and paleas spread apart slightly, and shriveled stamens. Left: normal head and stamens. Stamens enlarged four times and heads reduced one-half.

action of a particular gene or genes with a particular cytoplasm, with neither the cytoplasmic factor nor the genetic factors alone being sufficient to cause male sterility. There are many symbols representing this situation. Those used here will be as follows: fertile cytoplasm never showing any interaction with nuclear factors will be represented by (F), and cytoplasm reacting with a nuclear factor or factors to produce male sterility by (S); where a single gene restores fertility to sterile (S) cytoplasm, it will be symbolized by *Rf* (for fertility restorer; it is assumed that the fertility-restoring gene is dominant). The nuclear symbol *RfRf* will sometimes be used to indicate the nuclear genetic situation restoring fertility, even when the number of genes involved is not known.

Jones and Davis have reported on the use of cytoplasmic-genetic male sterility in the production of hybrid onions.²³ Hybrid onion varieties are single-cross F_1 hybrids produced by crossing a male-sterile female parent with a fertile male (Fig. 15.4). The genotype of the hybrid may be *Rfrf* (S), or *rfrf* (S), the former male-fertile and the latter male-sterile. The male sterility of the hybrid variety is unimportant in the onion because the bulb is the commercial crop. Where the hybrid is used for seed production, it should be male-fertile. Cytoplasmic male sterility in corn,^{11,21,35} sorghum,²⁷ beets,³¹ carrots,⁴³ and flax⁸ has a similar basis; both nuclear and cytoplasmic factors are involved.

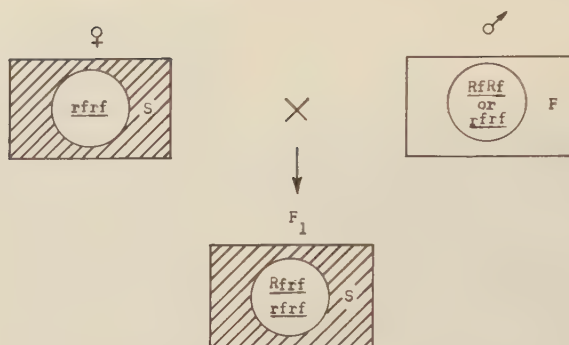


Figure 15.4 Method of producing hybrid seed of onions for commercial production. The rectangles and circles represent cells and nuclei respectively. (Adapted from Jones and Davis.²³)

Once the nature of cytoplasmic-genetic male sterility has been identified, it may be combined at will with other characteristics. Its addition to a desirable inbred line is a simple matter of backcrossing, in which the desirable line is used as the recurrent parent and the male-sterile line as the donor parent.

Chase suggested that androgenesis be used to add male sterility to successful male-fertile inbred lines.⁷ Subsequently, this was done by Goodsell.¹⁹ He used pollen of a male-fertile inbred line, N-6, on a male-sterile plant. In a few cases, seed was produced by androgenesis (the development of a sperm nucleus to form a haploid embryo). From such seed was obtained a plant that was a haploid version of N-6, but it had male-sterile cytoplasm. Pollination of the haploid with pollen from diploid N-6 gave a few seeds in a diploid sector of the ear. These seeds gave plants that were exactly like N-6, but male sterile. This technique made possible the production of male-sterile versions of inbred lines in two generations. The frequency of paternal haploids was about 1 per 80,000 seedlings, so the technique was feasible only where the self-fertile inbred line carried a recessive character that could be readily identified in the seedling stage.

The maintenance of the desirable male-sterile line is a simple matter of crossing it in each generation to a male-fertile version of the desirable line with the constitution *rfrf* (*F*).

Cytoplasmic-genetic male sterility in corn has been found in different materials, two being used commercially.^{11,39} The Texas source (designated as the T-type) was isolated from Golden June, a variety of dent

corn grown in the southwestern area of the United States. The S-type was identified by Jenkins of the U.S. Department of Agriculture. A detailed discussion of cytoplasmic-genetic pollen sterility in corn has been provided by Duvick.¹¹

These two types of cytoplasmic sterility react differently to genes restoring fertility, though crosses of both types to some inbred lines will give male-fertile progeny. Plant breeders have incorporated both sources of male sterility into successful inbred lines of corn, though the T-type is used most widely. Backcrossing, in the manner described above, has been used to make the transfer.

Of the 3 billion corn plants with Texas cytoplasm and nonrestorer genes that are grown in the United States each year, none has been reported to be pollen fertile.¹¹ In only two cases have fertile types been found in male-sterile types with S-type cytoplasm.²¹

To the corn breeder and to the farmer it is important that the sterile cytoplasm have no adverse effect on yield. Experimental data with respect to this effect have been contradictory, some indicating that the sterile cytoplasm increased the number of barren stalks and degree of root lodging,¹⁰ and others showing that there was no consistent difference.^{16,22,28} Still other data demonstrate a stimulation to yield from the interaction of sterile cytoplasm and the fertility restoring gene.⁴⁰ Certainly, the data are sufficient to permit a plant breeder to incorporate cytoplasmic male sterility into corn inbreds, with confidence that in most cases the hybrids derived from them will be equal in performance to hybrids obtained from fertile inbreds. The actual utilization of male-sterile lines in hybrid corn production will be discussed in Chapter 18.

The economic value of cytoplasmic-genetic male sterility in hybrid varieties has encouraged plant breeders to search for it systematically. As pointed out by Jones, it has usually followed the combination of nuclear factors of one species, or of a distinct type within a species, with the cytoplasm of another.²¹ This has been true in tobacco, flax, onions, sorghum, corn, wheat, and evening primroses.

Cytoplasmic male sterility. A form of male sterility dependent upon the cytoplasm alone was first described in corn by Rhoades.³³ This was probably similar to the cytoplasmic-genetic male sterility just described, except that fertility-restoring genes were not present in his stocks. The male-sterile plant would be *rfrf* (S), and the male-fertile plant *rfrf* (F).

Résumé

The precise control of the mating system of crop plants has had profound effects on plant breeding. Of greatest immediate interest has been the exploitation of male-sterility in the production of hybrid varieties

in both the cross- and self-pollinated crops. However, the developments of greatest significance to plant breeding arising out of the control of pollination will be the merging of breeding techniques for cross- and self-pollinated crops. On the one hand, breeders of self-pollinated crops are interested in the use of male-sterility in augmenting cross-pollination and with it gene recombination in their materials. On the other hand, the breeders of obligately cross-pollinated crops have sought modifications of the mating system that would permit selfing.

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16

SELECTION IN CROSS- POLLINATED CROPS



The response of a population to selection must depend on three sets of factors. The first of these includes the types and strengths of the selective forces themselves. Secondly, . . . the action and interaction of the genes in producing their effects must be concerned. Thirdly, we have the amount, distribution and system of genetic variability in the population.

MATHER, 1955 *

IN A GENERAL WAY, cross- and self-pollinated plants respond similarly to mass selection. Both tend to move in the direction of selection pressure, and both will retain considerable variability. It is when single-plant

* From K. Mather, *Cold Spring Harbor Symposia on Quantitative Biology*, 20 (1955), 158.

selection is practiced that the mating system expresses itself in a conspicuous way. The self-pollinated plant will produce a uniform progeny, whereas the cross-pollinated plant will not. This was frustrating to the early breeders of cross-pollinated crops when a very desirable single plant was identified.

Discussion in this chapter will be limited to the use of mass selection, with and without progeny tests. Later chapters will deal with the selection of inbred lines, with systems of recurrent selection, and with the selection of the components of synthetic varieties.

Effects of Selection on One Locus

The effectiveness of selection for or against an allele at a single locus depends on its dominance. If the homozygous genotype aa can be distinguished from AA and Aa , it is obvious that selection for aa and against AA and Aa will eliminate the gene A from the population in one generation if eliminations are made before flowering.

An example of this is branching in sunflowers. Often, commercial varieties become contaminated with a type that has several branches at the top of the plant, each bearing a small head. Fortunately the branched character is due to a dominant gene Br .⁹ By eliminating genotypes $BrBr$ and $Brbr$ before the plants open their flowers, the gene Br can be removed quickly and easily.

But suppose an attempt is being made to eliminate a recessive gene a from a population where the genotypes AA and Aa are indistinguishable. How effective is continuous selection for AA and Aa and elimination of aa before it produces gametes? This will be determined in large part by the frequency of a , or the value of $1 - q$, in the Hardy-Weinberg equilibrium $q^2AA + 2q(1 - q)Aa + (1 - q)^2aa$. Assuming that $1 - q$ is 0.8, the frequency of aa will be 0.64 and Aa will be 0.32, or the proportions of a in genotypes aa and Aa are 0.64 and 0.16, respectively, out of a total of 0.80. The proportion of allele a removed with aa will be $0.64/0.80 = 80$ per cent. If $q = 0.2$, the frequency of genotype aa will be 0.04, and the proportion of gene a removed with aa will be $0.04/0.20 = 20$ per cent. Actually, the proportion of allele a removed with genotype aa is $1 - q$. This will explain why selection against a recessive allele a by the removal of the genotype aa is very effective if the frequency of a is high, but ineffective if the frequency is low. It is virtually impossible to remove a recessive allele completely by selection because, at very low levels, the allele is mostly obscured by its dominant allele. Assuming that the frequency of a recessive allele a is 0.5 in F_2 , and aa genotypes are removed in F_2 and all succeeding generations, the frequencies of a will be as follows: in F_3 , 0.333; in F_4 , 0.250; in F_5 , 0.200; in F_{10} , 0.100; in F_{50} , 0.020; and in F_{100} , 0.010.⁵

Again the sunflower provides an example. Occasionally in breeding materials and commercial fields, plants appear with all flowers of the head modified to form a mass of vegetative tissue. Such heads are due to a recessive gene. Even though they produce no seed or gametes, the gene responsible persists at a very low level, being carried forward to the next generation by heterozygous plants.

The extent to which a genotype is removed from a population is termed the *selection coefficient*, and is symbolized by s . This is incorporated into the Hardy-Weinberg formula in the following way:

$$q^2AA + 2q(1 - q)Aa + (1 - q)^2(1 - s)aa$$

This assumes that the reproductive rate of each genotype is 1, and that selection is directed against the genotype aa . Since $s(1 - q)^2$ plants of genotype aa are removed from the population, the total for the Hardy-Weinberg formula is no longer 1 but $1 - s(1 - q)^2$. Where no plants of genotype aa reproduce, the selection coefficient becomes 1, or the value of $(1 - q)^2(1 - s)$ is 0. The selection coefficient is 1 for the example of the sunflower plants with vegetative heads.

Techniques of Mass Selection

Terms that have been applied to systems of mass selection are *selection intensity* and *selection differential*. Selection intensity, v , is the percentage of plants saved to provide seed for the next generation. It is a term that could apply to both a qualitative and a quantitative character, though it is usually used only for the latter. In Fig. 16.1 the selection in-

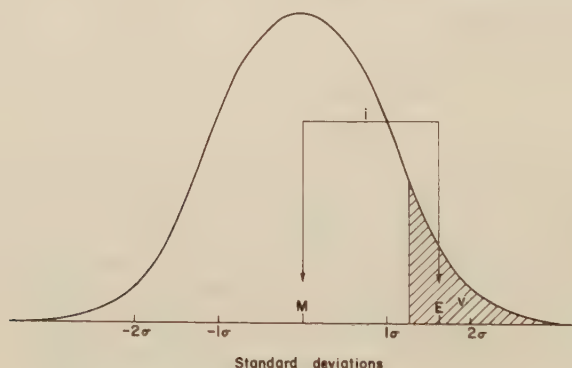


Figure 16.1 Representation of selection for a quantitative character, where it is distributed normally. M = population mean; E = mean of selections; v = selection intensity = 10%; i = selection differential = difference between M and E in standard deviation units.

tensity is 10 per cent, and is represented by the shaded area. Note in this figure that if the plants saved are only those that excel the mean by the standard deviation, the selection intensity is 16 per cent; if they excel the mean by twice the standard deviation, the selection intensity is 2.5 per cent. The selection differential, i , is the difference between the population mean and the mean of the selected plants.

Mass selection can take many forms, all of which involve the harvest of a bulked lot of seed from several plants. Usually it has been based on the phenotype, but sometimes it has been based on the genotype as determined from a progeny test.

Phenotypic mass selection. In this form of mass selection, desirable plants are selected on the basis of their appearance. The basis of selection could be a qualitative character, such as flower color and reaction to disease, or a quantitative character, such as height, earliness, and seed yield.

The stage of plant development when selections are made will have a bearing on the advance made by mass selection. If made before flowering, the rejected plants can be removed from the population, and only the selected plants will mate and contribute genes to the next generation. If made after flowering, the selected plants will have already crossed to all plants in the population, and many genes will come from the discarded plants. These genes will add inertia to the advance under selection. Mass selection of annual plants after flowering will always suffer from this inertia. With biennial or perennial plants, evaluations may be made in one year, and selected plants can be used for seed production the following year.

A refinement of the latter method, used by Fryer for alfalfa, is called *maternal line selection*.⁶ His procedure involved a sequence of four-year cycles, with each cycle as follows:

1. First year: Plant 80 open-pollinated progenies of single plants in separate rows.
2. Second year: Discard progenies or plants with poor seed production.
3. Third year: Discard further, leaving 100 plants from 30 to 40 progeny rows.
4. Fourth year: Discard 20 more plants, leaving only 80 to intercross. Seed from these 80 plants are used to plant the progeny rows for the second cycle.

To reduce environmental effects, Gardner used a procedure that may be called *stratified mass selection*.⁷ He grew a mass population of the corn variety Hays Golden and stratified it into subpopulations of 40

plants each. In each subpopulation he picked the four best plants. This meant that all environments of the experimental area were sampled equally. Over a four-year selection program he was able to advance the yield of Hays Golden from 79.3 to 97.4 bushels per acre.

Progeny testing. Progeny tests of selections of cross-pollinated crops were first used by Louis de Vilmorin of France in the improvement of sugar beets during the latter half of the nineteenth century.^{3,20} Vilmorin selected beets carefully on the basis of their size, shape, and sugar content. His procedures are shown in diagrammatic form in Fig. 16.2. It will be noted that evaluations are completed in two generations (four years), but selected materials may be subjected to further tests before final selections are made and bulked together for commercial use. This method, or one similar to it, may be used in both development and maintenance of varieties. Using the progeny test, Vilmorin raised sugar con-

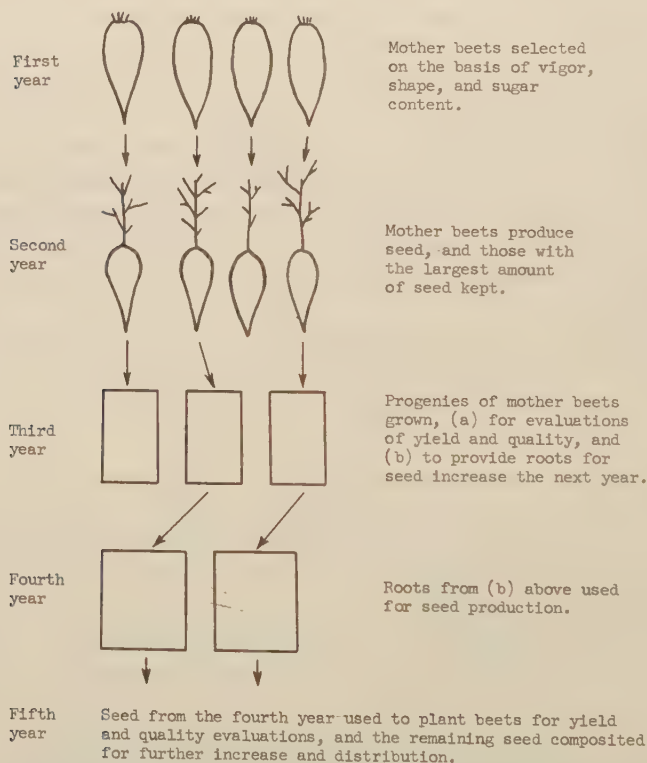


Figure 16.2 European method of producing sugar beet seed which was developed from Vilmorin's progeny test. (From Coons.³)

tent from 5 or 6 per cent to 16 or 18 per cent, about that of present-day varieties.

Detailed pedigrees of mother beets are kept and often can be traced back many generations. The pedigree, of course, gives only the female side of the inheritance; the male side is unknown.

Ear-to-row breeding in corn was introduced by C. G. Hopkins in 1897, and was patterned after the Vilmorin method described above. In this method, as usually practiced (Fig. 16.3), superior ears are picked from an

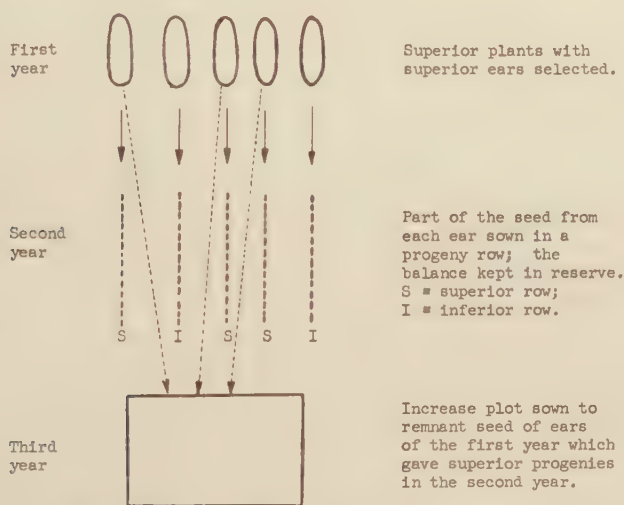


Figure 16.3 Ear-to-row method of breeding corn.

open-pollinated population, probably a named variety. In the next year, a portion of the seed from each ear is sown in a progeny row, and the balance is kept in reserve. The superior progeny rows are identified, and remnant planting seed of these from the previous year is then composited. Thus the cycle is completed in two years. Another cycle can commence in the composite when it is grown in the third year, and cycles can be repeated as long as there is progress in the selection program.

Using this method, Hopkins was quite successful in improving corn seed in oil and protein content over a period of a few years, and continued use of the method effected further change in these characters (pp. 204–206). For yield, however, the results have been disappointing.⁸ One serious shortcoming of the method was in the nature of the progeny test. Because it was usually limited to one row at one location, it did not give an adequate evaluation of the genotype of the parent plant.

Lonnquist has described a refinement of the ear-to-row method, which may be termed *ear-to-row-to-ear* breeding.¹³ This method evaluates progenies in several locations and shortens each cycle to one year (Fig. 16.4).

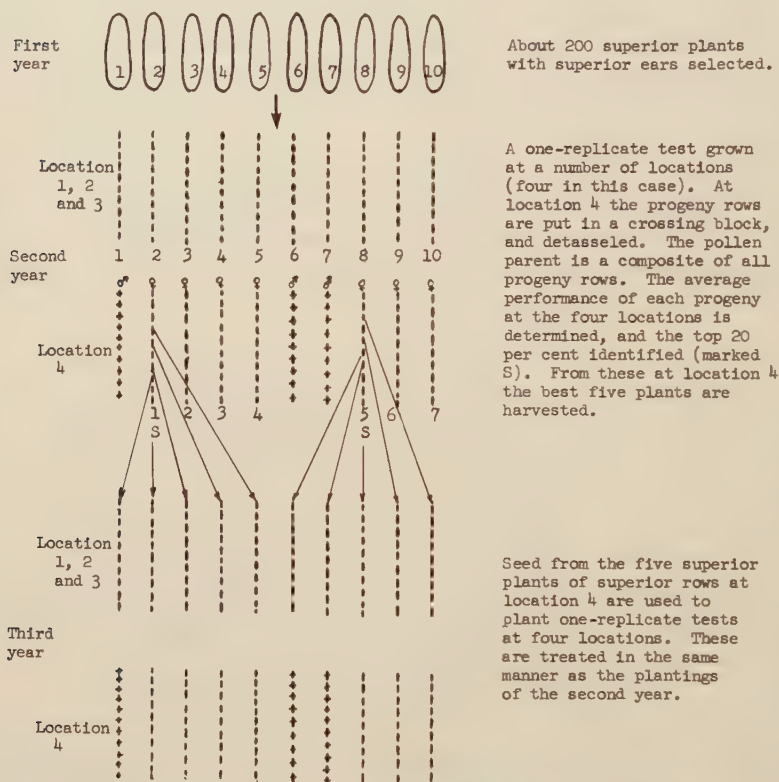


Figure 16.4 Mass selection procedures used in the ear-to-row-to-ear selection program. (After Lonnquist.¹³)

From each ear, progeny rows are grown in a single replicate at different locations representing the range of environment to be used by the intended variety.

The replicate at one location is handled as a crossing block, with four detasseled progeny rows alternating with two rows serving as a pollen parent. The pollen parent is a composite of equal numbers of plants from each progeny row. This permits the maximum opportunity for each progeny row to cross to all other progeny rows. Ears from the five best plants in each progeny row are identified at harvest. These identi-

fied ears are kept from only the top 20 per cent of the progenies, as judged by their average performance at all locations. Seed from these ears are sown the next year in progeny rows in the manner described above.

Composite of selfed lines. The combination of selfed lines is rarely used as a method of improving cross-pollinated crops, even in crops that will produce some selfed seed. The reasons are obvious. In the first place, selfed progenies usually are depressed in vigor, making it difficult or impossible to rate them as components of a composite. Another reason is that there will be a tendency to favor plants that self readily, and a composite from such plants may show more selfing than is desirable for the maximum expression of vigor. In fact, it has been found in alfalfa that the yields of the progenies of three groups of plants averaging 85, 51, and 23 per cent self-fertility gave average forage yields of 6.04, 6.35, and 6.59 tons per acre, respectively.¹⁹

Dr. M. L. Kinman has maintained an inbred line of sunflowers at College Station, Texas, by selfing a large number of plants and saving only those plants that produce a small number of seed.* In this way he has selected against self-fertile plants. The inbred line was used as the female parent in the production of a single-cross hybrid variety. As such, it was important that a high level of self-sterility be preserved in the line. Where self-fertile types were used as the female parent in a single cross, most of the seed from this parent was a result of selfing and not of crossing to the male parent.

Selfing is a useful method of purging a population quickly of one or a few undesirable recessive alleles. Undesirable alleles would be those conditioning the susceptibility to disease, or producing obviously abnormal morphological characteristics.

Single-plant selections. Only rarely have single-plant selections of cross-pollinated crops been used as varieties. The reason is that one plant does not provide a large enough reservoir of alleles, even assuming that seed is the product of crosses to a large number of plants.

Mass Selection of Corn

Prior to the discovery of the New World, the Indians had undoubtedly practiced mass selection of corn for several centuries. Essentially all modern endosperm types had been developed, among them the dent, flint, flour, and sweet types. The distributions of the different types, of course, were much different than they are now. The corn plant of today is little different from that of pre-Columbian time, the present varieties differing mainly in their uniformity. The principal contribution of the white man

* Personal communication.

has been the development of dent varieties as we know them. In Chapter 2 it was pointed out that the modern dents were developed by early settlers in the Midwest from crosses of the flints, which they brought with them, to local types with conspicuous denting.¹ An enormous amount of variability must have been provided by these crosses.

Nearly every corn grower was a selectionist to the extent that he gave some attention to the ears he used for seed. Ears were favored that were 8 to 10 in. long and which had 16 to 18 rows of long, yellow kernels with slight denting at the tip (these are the characteristics of modern dent varieties). The more progressive farmers made their selections in the field, where plant height, maturity, vigor, and other characteristics were taken into account. The variations in the ideals and methods of selection of different farmers and professional corn breeders resulted in widely different varietal types and differences among strains of varieties. Environmental differences from one area to another were also reflected as differences between and within varieties. Normally, enough seed was selected to plant a commercial field, which meant that the number of plants selected was large enough to prevent inbreeding.

Although mass selection was effective in establishing and maintaining

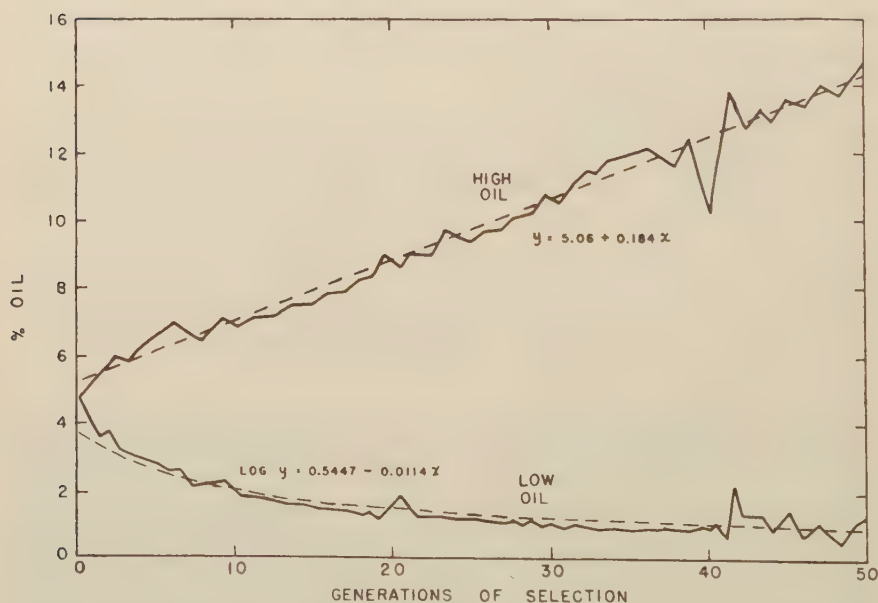


Figure 16.5 Effect of 50 generations of selection on oil content of corn. Actual data indicated by solid lines; fitted trend lines are shown as broken lines. (Woodworth et al.²¹)

most of the open-pollinated varieties of corn, it did little to improve yields during the early years of the present century. It was believed that mass selection methods of breeding had exhausted all the genetic variability that would contribute to yield, and that a permanent yield plateau had been reached. At the same time, interest in hybrid varieties had developed, and for this reason alone many corn breeders lost interest in mass selection methods.

Recent work at several stations indicates that mass selection has merit in improving corn if (1) a good deal of variability is present, (2) populations are exposed to the range of environment found in the area of intended use, and (3) progeny tests (in ear-to-row methods) are made more precise.

Selection for oil and protein content. A classical selection experiment in corn was commenced in 1896 by C. G. Hopkins at the Illinois Station, and the results over a 50-year period were reported in 1952.²¹ Through mass selection in one open-pollinated variety, Burr White, an attempt was made to develop four separate populations with kernels that were (1) high in oil, (2) low in oil, (3) high in protein, and (4) low in protein. (See Figs. 16.5 and 16.6). Ear-to-row selection methods were used for the

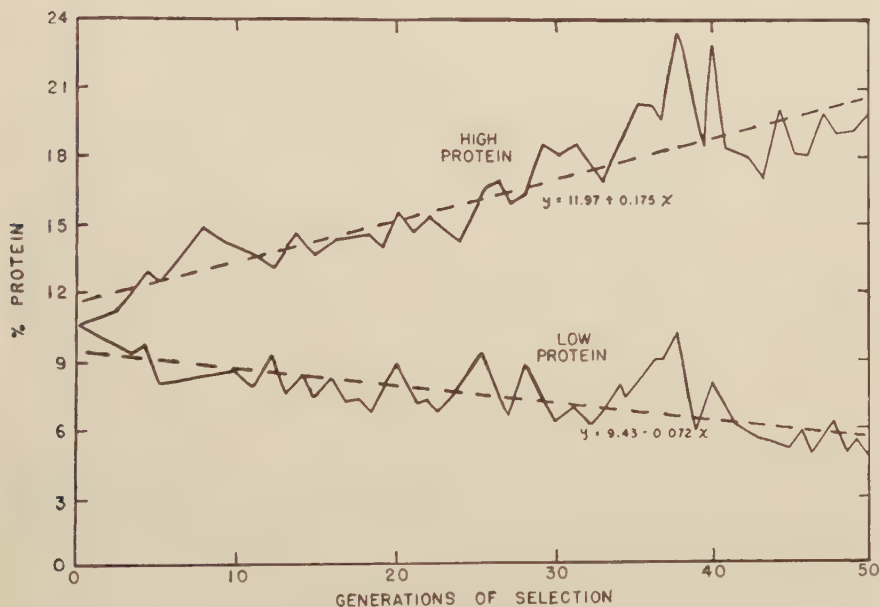


Figure 16.6 Effects of 50 generations of selection on protein content of corn. Actual data indicated by solid lines; fitted trend lines are shown as broken lines. (Woodworth et al.²¹)

first 28 generations. Thereafter, because of the difficulty in obtaining isolation, the methods of handling the four populations were modified. Each year 60 ears were selected from each strain and, after analyses, the 12 most extreme types in the direction of selection were saved. Two adjacent plots were sown from these 12 ears, each plot from the composited seed of six ears. Bulked pollen from one plot was used to pollinate plants of the other plot, and vice versa. At harvest, 30 ears were taken from each plot.

Selection for high oil gave steady improvement through the entire period, raising the level from 4.70 per cent to above 15 per cent. But selection for low oil resulted in no change after 30 years, when it leveled off at 1 per cent. Selection for high protein, which began at about 11 per cent, seemed to level off after 40 years at about 20 per cent, and low protein continued steadily downward to about 5 per cent. It was obvious that the results of selection were different for different characters. Selection continued to be effective in changing some characters over a 50-year period.

Selected populations were 50 per cent lower in yield than the unselected variety, and about equal to good inbred lines. The ear and kernel characteristics, which were distinctly different in the four strains, are given in Table 16.1.

Table 16.1 Oil and protein improvement of corn through selection.²¹

CONTENT	EAR	KERNEL
High Oil	Relatively small	Small with large germ
Low Oil	Large, with low row number	Very large, deeply dented, with a small germ
High Protein	Small, with large cobs	Hard, flinty, and translucent, with medium dent
Low Protein	Large, with relatively high row number	Long and starchy, with little or no dent

The high oil and high protein strains are being used by the Illinois Station and other stations in breeding programs to develop inbred lines, and eventually hybrid varieties, with higher protein and oil contents than present commercial varieties.

Responses to Selection

Inasmuch as selection is a feature of all plant breeding programs, and often the feature that determines success or failure, it is important that

some consolidation of the responses to selection be made at this point. This will be a discussion of factors that affect response.

Amount of variability present. Some form of mass selection has always been very effective in the development of distinctive or improved varieties of crop plants in which a great deal of variability is present. Often this is true of an unimproved variety such as a land variety. After a period of improvement by selection, variability will decrease and advances will be slower and more difficult to detect.

Proportion of variability free or potential. Free and potential variability are terms used by Mather and his associates.^{14,15,16} When variability is free, it is immediately available. This is variability that is expressed by different combinations of chromosomes or chromosome arms present in the population. It is variability that is freed without crossovers at particular locations or several locations on the chromosome. Responses to such free variability are usually rapid, often a feature of early stages of a selection experiment. Potential variability is released slowly because it is released only as the alleles of polygenes are regrouped, often through several crossovers of a single chromosome pair. With several generations of crossing over, polygene combinations may be achieved and increased in the population such that selection will continue to give a response, even over a period of 50 or more generations.

Amount of inbreeding. With intense selection pressure (v is very small in Fig. 16.1), the population of selected plants may be so small that a certain amount of inbreeding will occur. This will reduce the vigor of succeeding generations. In addition, with the small populations associated with intense selection pressure, genetic drift (see p. 20) may easily change the frequency of some alleles, and as a consequence the characteristics of the population will change.

Correspondence between genotype and phenotype. It is obvious that good correspondence between genotype and phenotype will result in good response to selection. This is the same as saying that response will be good if heritability is high. Because heritability has been low for many characters of importance to the plant breeder, the progeny test has been the only reliable measure of the genotype.

Correlated responses. Intense selection for one character will sometimes bring a response in another character. Often, both in cross-pollinated plants and animals, fertility has been associated with strong selection for some character. When Mather and Harrison selected separately for increased and decreased bristle number on the ventral surfaces of the fourth and fifth abdominal segments of the fruit fly, *Drosophila melanogaster*, fertility decreased.¹⁶ This was not due to pleiotropic effects of polygenes giving high bristle number because, by relaxing selection

pressure for a short period and renewing it again, high bristle number and fertility could be combined. The decrease in fertility was attributed to polygenes that were linked to polygenes accumulating in response to selection for bristle number.

The 50-year selection experiment in corn, described above, offers several examples of responses in ear and kernel characteristics associated with selection for low and high levels of oil and protein. It was also noted that the high oil strain was the earliest and shortest of the four strains and that the low oil strain was the latest and tallest. The high protein and low oil strains tended to tiller strongly.

Stepwise responses. Occasionally, strong selection pressures will not change a population for several generations and then there will be a sudden response for a few generations, after which the population will plateau again in spite of continued selection. Mather and Harrison obtained this type of response while selecting for bristle number in the fruit fly.¹⁶ Selection raised bristle number from 36 to an average of 53 to 54 in 28 generations, and then there was no further response until the 82nd generation. From the 82nd to the 86th generations, bristle number increased to about 56, where it maintained a plateau until the 110th generation. Again, between the 110th and 116th generations, there was an increase to 63 bristles, where the number again reached a plateau. The authors believed that the sudden responses were due to rare cross-overs, perhaps near the centromere, which released new genetic variability.

Natural versus artificial selection. From previous discussions of bulk or composite populations of self-pollinated crops, it appeared that natural selection would be successful in increasing the proportion of well-adapted types in the population. Certainly types that produced abundant seed would increase in the population, and increased seed production was often the objective of the plant breeder. With cross-pollinated crops, the results will be the same; alleles that contribute to poor adaptation or to low seed yield will decrease in the gene pool. Under different environments, natural selection will develop different gene pools.

Artificial selection reconstitutes the gene pool and changes the frequency of all the alleles in the population. Artificial selection tends to satisfy the needs of man: It may be made to gain higher forage yield and not seed production, to increase root yields in sugar beets and many vegetable crops, or to produce a specific quality. Because artificial selection has largely been a matter of changing frequencies of alleles, often against the pressure of natural selection, there always exists the possibility of changes in the genetic constitution of a mass-selected variety. This change in the gene pool in response to natural selection has been

termed *genetic shift*. It is an extremely important phenomenon in crops that are grown for some purpose other than seed production.

Stanford, Laude, and their associates have studied the genetic shifts in Pilgrim, a variety of ladino clover, which is a cross-pollinated forage crop. Using 21 parent clones, they developed identical plantings of Pilgrim at three different locations with different climates (Table 16.2).

Table 16.2 Effect of location on the composition of Pilgrim, a variety of ladino clover.^{11,17}

LOCATION	LATITUDE, DEG. N	MEAN TEMP., °F		MEAN FROST- FREE SEASON, DAYS	PER CENT OF PLANTS WITH CLASSIFIED CHARACTERISTICS			
		January	July		<i>Red Fleck</i>	<i>Enzyme</i>	<i>Gluco- side</i>	<i>Petal V-mark</i>
Davis, Calif.	38	45.4	75.3	242	0.02	21.45	19.79	86.27
Aberdeen, Idaho	43	19.8	69.9	102	2.69	2.98	12.38	90.22
Prosser, Wash.	46	31.0	72.2	169	18.12	4.08	9.68	85.09

Seed from these locations was grown at Davis, and about 500 plants from each seed source were examined. Plants were classified for the presence of the following characters: (1) red flecking of leaves, produced by a dominant gene in one clone; (2) a hydrolyzing enzyme, linamarase, controlled by a dominant gene present in one clone; (3) cyanogenetic glucosides, also controlled by a dominant gene, and present in six clones; and (4) a white V-mark on the leaf, which was present in all but one clone. The high frequency of red fleck in plants obtained from seed grown at Prosser was not unexpected because the red-flecked clone was rated second highest in vegetative vigor and flower production. At Aberdeen this clone had been below average in floral production; at Davis it was eighteenth in floral development. At Davis the clone producing the enzyme linamarase was very productive, and at Prosser and Aberdeen was less than average in productivity; these productivities are reflected in the frequencies of plants in the census having the enzyme present. The clones having the glucoside produced less than expected at all three locations, particularly at Aberdeen and Prosser. The V-mark was about as expected at all locations. These data clearly show that environment can have profound effects upon the composition of an open-pollinated variety in one generation.

The mean date at which the original Pilgrim clones first flowered at Davis was June 25. Succeeding generations at Davis had mean flowering

dates on June 18, June 11, and June 9.¹⁸ This was an advance of about two weeks in flowering date.

Genetic shift will continue to be a problem when seed stocks of a mass-selected variety are increased in different environments. The problem has been even more acute where seed has been produced out of the area of production. An example is the concentration of seed production of forage crops in the western and southwestern states of the United States for use in other parts of the nation. Almost without exception, there has been a shift in the characteristics of a variety, with each generation of seed production increasing the shift. Usually, the shift has been greater with greater differences in the environments of seed use and seed production. It is likely that the new version will be less desirable than the original in the area of intended use. This has led to strict rules in the certification of seed crops of forage species relative to (1) the number of generations that the seed may be produced out of the area of use, and (2) the area that may be used to produce seed of a particular variety.

Résumé

Mass selection has been the method that nature has leaned heavily upon in the evolution of plants and animals. It has also been the breeding method used by man in the development of most cultivated crops. In the light of the recorded accomplishments it must be rated as an extremely effective breeding method, though often too slow to satisfy the patience of modern man.

Mass selection has been particularly effective where (1) there was a large reservoir of variability and (2) it was applied to a character with high heritability. Where some degree of improvement has already been achieved in a crop, it has not been too effective, particularly where improved yield was an objective. Mass selection has even led to deterioration when the selected population was so small that genetic drift or inbreeding occurred.

Finally, relaxation in the selection pressure has often permitted the population to slip back toward its former level. Genetic shift has occurred, usually toward a less desirable type, if seed is produced outside the area of adaptation.

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INBREEDING AND HETEROSIS



Nature abhors perpetual self-fertilization.

DARWIN, 1877

THE DEPRESSING effects associated with inbreeding in both plants and animals are well known and widely accepted. Its recognition among animals is as old as recorded history. It is generally assumed that the taboo against the marriage of near relatives among ancient civilizations resulted from the observation of the ill effects of inbreeding. This taboo, which is now known to be scientifically sound, was not universal among all civilizations at all times, and inbreeding as close as brother-sister matings was actually practiced and presumably approved on eugenic grounds.

In his excellent account of early ideas on inbreeding and cross-breeding, Zirkle pointed out that two great pillars of European thought, Hebrew morality and Greek philosophy, endorsed inbreeding as a matter of course.²⁶

Observations on inbreeding and cross-breeding in plants are of comparatively recent origin. Koelreuter, the great plant hybridist, who published from 1761 into the nineteenth century, described hybrid vigor in several interspecific crosses. He was impressed by the floral mechanisms that insured cross-pollination and concluded that nature had designed plants to benefit from cross-breeding. While he did not comment on inbreeding, it is obvious that he considered it undesirable.

Charles Darwin concluded that cross-fertilization is generally beneficial and that self-fertilization is injurious.⁷ Working with corn, he was the first to report accurate measurements of responses to inbreeding and cross-breeding. The average seedling height from selfed seed was 17.6 in., and from crossed seed 20.2 in. When mature, their respective heights were 61.6 and 66.5 in., a ratio of 93:100.

Inbreeding

Inbreeding may be defined as any system of mating that will lead to an increase in homozygosity. Normally, cross-pollinated species that are not self-sterile may be inbred by controlled self-pollination. This provides the most rapid approach to homozygosity. Where the sexes are on separate plants or where self-incompatibility systems are present, sib or brother-sister matings are used. The comparative efficiency of these two systems of mating is shown in Fig. 17.1. It will be noted that three

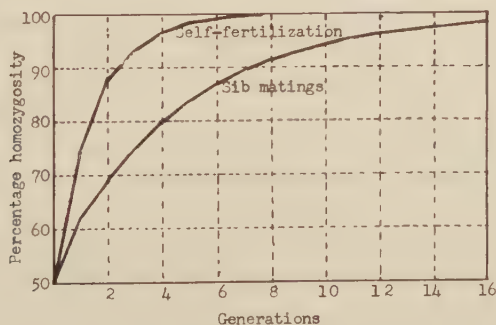


Figure 17.1 Percentage of homozygosity in successive generations under self-fertilization and sib matings. (Adapted from Wright.²⁵)

generations of selfing nearly equal ten generations of sib-matings in homozygosity. Either method will lead to 100 per cent homozygosity.

The progeny of a selfed plant is symbolized by S_1 , the selfed progeny of the latter by S_2 , and so on. Occasionally the symbol $\otimes$ is used to indicate selfed seed.

Effects of inbreeding. The inbreeding experiments with corn, begun independently by East and Shull early in this century and extended by a large number of other investigators, have provided a vast amount of information on inbreeding in this crop. As a consequence, corn will provide the examples for much of this discussion.

A large number of deleterious qualitative characters appears in the early generations following inbreeding. The most frequent of these are chlorophyll-deficient plants. Such deficiencies range in magnitude from small areas of the leaf to the entire plant. For example, when Jenkins grew progenies from 3,750 selfed plants of different Corn Belt varieties, 680, or 18.1 per cent, were segregating for some type of chlorophyll defect.¹⁰

Other characters that occur less frequently include defective endosperm, tasseled seed, and resistance to such diseases as smut, rust, and *Helminthosporium*. It will be recognized that some of these are useful. It should be emphasized that most, if not all, of the above characters were known already to corn breeders. Inbreeding just increases their frequency.

A second result of inbreeding is the development of uniform lines following the increase in homozygosity. This would be expected from the behavior of self-pollinated plants, which has been considered in Chapter 10. In corn this is manifested by lines being uniform in such characters as height, date of tasseling, and ear appearance.

A third feature of inbreeding and the one of greatest concern to the plant breeder is the decline in vigor and the accompanying reduction in productivity. This reduction in vigor and yield is associated with a decrease in height, ear length, and other such characters, but yield has continued to decline beyond any noticeable decline in plant size. Jones reported on plant height and yield for three inbred lines of corn through 30 generations of inbreeding.¹² Measurements were made at five-year intervals. Plant height was reduced from 120 in. to approximately 80 in. by the fifth generation (Fig. 17.2). Through another 25 generations the heights remained essentially the same. There were small differences among lines and some fluctuations during the period of measurement.

The most rapid decline in yield occurred in the first five-year period.

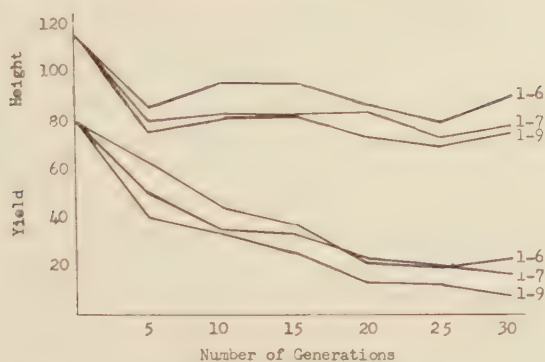


Figure 17.2 A comparison of three maize lines, derived from the variety Leaming, self-fertilized for 30 generations. Height of stalk is measured in inches and yield of grain in bushels per acre. (Adapted from Jones.¹²)

The yield of two of the three lines dropped from 80 bu to approximately 40 bu in five years, but the third (which was consistently the tallest and usually the highest in yield) did not reach 40 bu until the tenth year. By the end of 20 years, yield was down to 20 bu, and the best line showed no further decreases during the last 10 years, but the other two continued the downward trend on a much reduced scale. This response to inbreeding has been a feature of all corn-breeding programs. Indeed, Kiesselbach pointed out that by 1950, none of the inbred lines of corn developed in the United States yielded much more than half as much as the best open-pollinated varieties.

Information on the effects of inbreeding in other crops is much more limited. Alfalfa is even more intolerant of inbreeding than corn. Tysdal et al. found that forage yield declined to 68 per cent and seed yield to 62 per cent in one generation of inbreeding involving 54 inbred lines.²⁴ By the third inbred generation, few plants remained, and only a small number of these set seed, even sparingly. Yields of forage and seed declined to 28 per cent and 8 per cent, respectively, in the four inbreds available in the S_8 .

At the other extreme there are a number of normally cross-pollinated crops that do not exhibit any, or at least very little, adverse reaction to inbreeding. As mentioned in Chapter 4, the cucurbits do not exhibit marked inbreeding degeneration, either in the production of deleterious characters or in reduction of vigor and productivity.

Heterosis

Heterosis, or hybrid vigor, may be defined as a manifestation of heterozygosity, expressed as increased vigor, size, fruitfulness, and resistance to diseases, insects, or climatic extremes. In the plant world, hybrids may be between inbred lines, varieties, or clonal lines. Hybrids between inbred lines are of greatest commercial interest. In order to be successful, the hybrid must be better than the best open-pollinated varieties. Hybrid vigor has been recognized in nearly every crop where experiments have been designed to measure it. This includes self-pollinated as well as cross-pollinated crops.

Genetic Basis of Inbreeding and Heterosis

It was easy for the student of genetics to understand how genes for deleterious characters such as chlorophyll deficiencies can exist at low levels in a heterozygous population (Chapter 16) and appear following selfing as homozygous recessives at much higher frequencies. Although there are a number of different types of chlorophyll deficiencies, each usually is conditioned by a single gene difference. The variability from line to line, and uniformity within each line, for such characters as maturity and ear location on the plant can be explained on a similar genetic basis.

There is greater difficulty in explaining the decline in vigor and productivity, in which a ceiling in the yield of inbred lines of corn seems to be fixed at about 50 per cent of the variety from which they are derived. The genetic explanation has to show why inbreeding depression occurs, and why F_1 hybrids frequently excel in vigor not only the inbreds used to make the cross, but also the open-pollinated variety from which they come.

Overdominance or heterozygosity theory of heterosis. The first theory of heterosis was proposed by Shull and East independently in 1908. It was restated later by Shull¹⁹ as follows: "Hybridity itself,—the union of unlike elements, the state of being heterozygous—has . . . a stimulating effect upon the physiological activities of the organism which effect disappears as rapidly as continuous inbreeding reduces the progenies to homozygous types." For this he proposed the term "heterosis." Although it is recognized that heterosis is the manifestation of the physiological activities of the organism, the theory implied, although not specifically stated in Mendelian terms, that the heterozygote was superior to either homozygote. This was a new concept of interaction at a single locus and did not conform to the accepted views of simple dominance involving two alleles. Later, East extended this theory to include a series of alleles,

a_1, a_2, a_3, a_4 , etc., at a single locus.⁸ These diverged in an increasing series in their function. Heterosis in the heterozygote increased with greater differences in the combined alleles in a series $a_1a_2 < a_1a_3 < a_1a_4$, etc. This would provide for varying degrees of overdominance, depending on the particular alleles present. All heterozygotes showed more vigor than homozygotes.

At the time East and Shull first proposed this theory, there was no direct evidence of a locus at which the heterozygote exceeded either homozygote. Stadler²¹ cited the following examples with R alleles in corn in which the heterozygous plant is more extensively pigmented than either homozygote:

R^oR^o	colored aleurone, colorless anthers
$r^r r^r$	colorless aleurone, colored anthers
$R^o r^r$	colored aleurone, colored anthers

Stadler pointed out that if the R alleles were to affect the synthesis of some substance important to general cell metabolism in the same manner in which they affect the synthesis of anthocyanin, it is probable that many heterozygotes of different R alleles would be more vigorous plants than either parental homozygote.

Quinby and Karper have reported that two alleles at the same locus contribute to higher yields in sorghum.¹⁷ Three genotypes from a cross of Dwarf Yellow milo (intermediate in maturity and having genotype $MaMa_2ma_2ma_3ma_3$) and Sooner milo (early and having genotype $mamama_2ma_2ma_3ma_3$) were compared in F_3 , after the genotypes were determined from F_4 progeny tests (Table 17.1). The heterozygote was

Table 17.1 Comparison of different genotypes of milo for plant characters and yield when sown June 20, 1944, at Chillicothe, Texas.^a

GENOTYPE ^b	NUMBER OF PLANTS	NUMBER OF DAYS TO ANTHESIS	NUMBER OF STALKS	HEAD WEIGHT, g	STOVER WEIGHT, g
<i>mama</i>	120	51	2.0	94	83
<i>MaMa</i>	118	70	2.1	130	146
<i>Mama</i>	236	83	2.2	149	174

^a From J. R. Quinby and R. E. Karper, *Amer. J. Botany*, 33 (1946), 716.

^b All plants were homozygous for ma_2 and ma_3 .

distinctly later than the latest parent and higher in yield of both grain and stover.

Where alleles at a single locus appear to have a heterotic effect, it is

difficult to eliminate the possibility of close linkage, giving pseudo-over-dominant effects. For example, if the combination of alleles A and a appear to give a heterotic effect, it is possible that this is due to a gene B linked to a and a gene C linked to A in the following manner:

$$\frac{CAb}{caB}$$

In this situation, heterosis is due to the dominant alleles covering up deleterious effects of the recessive alleles, as in the dominance hypothesis discussed below. One could reason also that the stimulus comes from an interaction between C and B , which would be an epistatic effect.

Dominance theory of heterosis. The second genetic explanation of inbreeding and hybrid vigor arose out of the discovery by Nilsson-Ehle of multiple factors for kernel color in wheat.¹⁶ It will be recalled (p. 91) that for any one of three loci, the heterozygote and the homozygous dominant genotypes are about equally red in color. But when each of two loci has at least one dominant allele present, the intensity of red increases; if this is true of all three loci, the deepest red color is obtained. A similar model may be developed in which the dominant alleles, A , B , and C , contribute equally to heterosis, and the recessive alleles, a , b , and c , do not. It is assumed that dominance is complete.

By using this model, some of the effects of inbreeding may be better visualized. Starting with the genotype $AaBbCc$, having the maximum heterosis, selfing over several generations will render all genotypes homozygous, with 2^3 or 8 genotypes possible, each present in the same amount. Of these eight genotypes, one will have dominant alleles at three loci, three will have them at two loci, three at one locus, and one will have no dominant alleles present. If it is assumed that the dominant allele at each locus, in the homozygous or heterozygous condition, produces one unit of heterosis, then the amount of heterosis produced under selfing will average 1.5 (Table 17.2). In the same table the proportion of different phenotypes under random outcrossing has been calculated, using the Hardy-Weinberg formula for three loci.

It is readily seen that outcrossing greatly reduces the number of individuals having no loci or only one locus with dominant alleles present, and correspondingly increases the number having three loci with a dominant allele present. Average heterosis under outcrossing is 2.25, or 50 per cent, higher than under selfing.

Independently by Bruce² and by Keeble and Pellew¹³ in 1910, an extension of this concept was advanced to explain hybrid vigor. They believed that the accumulation of favorable dominant genes in the F_1 , different ones of which were contributed by each of the two parents,

Table 17.2 Quantitative expression of heterosis at equilibrium under selfing and outcrossing of the genotype *AaBbCc*.

NUMBER OF LOCI HOMOZYGOUS OR HETEROZYGOUS FOR THE DOMINANT ALLELE ^a	PROPORTION OF INDIVIDUALS IN THE POPULATION		HETEROSIS PRODUCED	
	<i>Selfing</i>	<i>Outcrossing</i>	<i>Selfing</i>	<i>Outcrossing</i>
3	0.125	0.4219	0.375	1.2657
2	0.375	0.4219	0.750	0.8438
1	0.375	0.1406	0.375	0.1406
0	0.125	0.0156	0.000	0.0000
Total	1.000	1.0000	1.500	2.2501

^a This is the degree of heterosis.

would provide an adequate explanation. This was in accordance with genetic theory, particularly with reference to the dominant-recessive relationship of qualitative characters that had received most of the attention of geneticists up to that time.

The production of an F_1 hybrid more vigorous than the open-pollinated variety would be explained by making the cross $AAbbcc \times aaBBCC$ with heterosis ratings of 1 and 2, respectively. The F_1 hybrid, $AaBbCc$, would have a rating of 3. It is obvious in this model that the heterosis rating of the F_2 will decrease to 2.25 (see Table 17.2) and stabilize there.

Two objections were raised to the dominant growth-factor hypothesis suggested above, namely, that (1) this situation would lead to a skew distribution in F_2 , which did not occur; and (2) the expected homozygous individuals equaling the open-pollinated varieties in vigor were not recovered. Jones suggested that linkage between favorable and unfavorable genes would suppress skewness and prohibit the recovery of dominant genes in numbers equal to that found in the original variety.¹¹ Later, Collins showed that skewness could not be detected if 20 or more genes were involved.³ It will be apparent also from the discussion in Chapter 10 that very large populations are necessary to permit the recovery of a single genotype with all or almost all dominant genes in the homozygous condition, if segregation is occurring at more than 20 loci.

Epistasis and heterosis. It was quite logical for geneticists first to consider intra-allelic theories to explain heterosis. More recently, inter-allelic gene action or epistasis has been examined. This includes all effects of a gene at one locus on the expression of genes at other loci. Examples include complementary, inhibiting, and duplicate gene interactions.

The interaction of nonallelic genes to produce characters is a well-

known genetic phenomenon. However, no satisfactory case can be cited to show that the interaction leads to a heterotic effect. It will be necessary again to reason from an example of genes with qualitative effects. Two gene pairs, *Acac* and *Lili*, have been identified which are involved in the production of high hydrocyanic acid in white clover.^{1,4} The dominant allele *Ac* governs the production of a glucoside and the dominant gene *Li* the enzyme that converts the glucoside to hydrocyanic acid. A cross of two plants of genotypes *AcAclili* and *acacLiLi*, neither of which have hydrocyanic acid, will give an F_1 , *AcacLili*, with the acid present. Alleles at two loci could be visualized which would produce heterotic effects in a similar way.

Evidence for theories of heterosis. It has been difficult to get direct evidence in support of the dominance and overdominance theories because they both lead to a similar result. Richey and Sprague¹⁸ and Murphy¹⁵ have supplied some data in favor of the dominant gene hypothesis. Inbred lines of corn were improved by reciprocal transfer of genes from each other, termed *convergent improvement*. This involves crossing of two inbred lines, A and B, and then backcrossing the better plants of the segregating generations to both original inbred lines. After a series of backcrosses, both inbred lines should be improved by dominant genes from the other. The derived A and B lines would be termed A(b) and B(a), respectively. In actual fact, the derived lines did exceed the original in vigor. The F_1 between the derived lines should equal the F_1 between the original lines even though it would be less heterozygous. Actually, some of the F_1 's from derived lines equaled or possibly exceeded those from the original lines. If it is assumed that the genotype for line A is *AABBCCddeeff* and line B *aabbccDDEEFF*, the F_1 would be heterozygous at all six loci. Then, if derived lines may be assumed to be improved by the addition of a single dominant gene, their genotypes become *AABBCCDDdeeff* for line A(b) and *aabbCCDDEEFF* for line B(a). The F_1 of these improved lines would be heterozygous at only four loci.

The evidence for overdominance is even less direct. Hull calculated that the yield of hybrid corn is greater than can be accounted for by dominance because it exceeds the sum of the two inbred parents.⁹ This assumed favorable genes acting in an additive fashion.

Crow has considered the adequacy of each of the two theories to account for the observed consequences of inbreeding and crossbreeding.^{5,6} He concluded that the dominance hypothesis is adequate to explain the deterioration that results from inbreeding and the restoration of vigor on outcrossing, but it is difficult to explain how the hybrids could greatly exceed the vigor of the population from which their parents were derived. This excess vigor would need to be accounted for by overdominance.

He points out that only a small proportion of loci would need to contain genes that produced overdominance in the heterozygous condition.

In the discussion thus far, the observed facts have been considered only in terms of alleles. There are good reasons to believe that some of these will turn out to be closely linked gene blocks. If a model is visualized wherein two series of six closely linked alleles combine to produce a heterotic effect (namely, *AbCdEf/aBcDeF*) it will be seen that there will be great difficulty in getting the exact crossovers necessary to bring all the dominants together in a single gamete.

The two primary theories explaining heterosis lead to essentially the same result and are not therefore mutually exclusive. There is no reason to believe that a phenomenon as complex as this should result from a single type of reaction.

Résumé

The values of inbreeding in the improvement of a cross-pollinated crop are as follows: It provides a means of eliminating recessive genes with undesirable effects, and it permits the development of uniform inbred lines that may be combined to produce a uniform and vigorous hybrid.

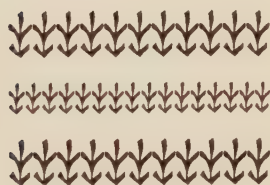
The exploitation of heterosis in hybrid varieties is one of the greatest accomplishments in plant breeding, even though the full genetic explanation must await further research. If the dominance hypothesis turns out to be correct, it should be possible for plant breeders, given enough time, to develop homozygous varieties equal to the present hybrid varieties. On the other hand, if heterosis is due even in part to overdominance, it will prohibit the achievement of homozygous varieties equivalent in performance to hybrid varieties.

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HYBRID VARIETIES



In my opinion hybrid corn is the most far-reaching development in applied biology in this quarter century.

MANGELSDORF, 1951

NO PRODUCT of the plant breeder's art or science has had greater impact on increasing the world's feed or food resources than hybrid varieties. Dramatically successful at first in corn, their use has spread to other crops, including both cross- and self-pollinated species. It is likely that their use will spread to most crops.

Hybrid varieties are those in which F_1 populations are used to produce the commercial crop. Parents of the F_1 may be inbred lines, clones,

varieties, or other populations. Hybrid varieties are used where the increased yields from hybrid vigor will more than pay for their development and the extra cost of planting seed. An added premium in the case of hybrids of inbred lines is uniformity.

Historical Background

Observation on, and knowledge of, inbreeding and crossbreeding in plants and animals prior to the twentieth century has been summarized by Zirkle, and his publication has been liberally used here.⁴⁶ During the nineteenth century, hybrid vigor was recognized and recorded on many occasions where not only different species but also different varieties were involved. Darwin's experience with inbreeding and crossbreeding in corn has already been mentioned in the preceding chapter. In 1880, Beal published the results of his experiments in bringing together stocks of corn grown 100 miles apart. He grew them in alternate rows and detasseled one stock, forcing it to produce hybrid seed. The increase in yield of the hybrid was as high as 51 per cent. Sanborn, in 1890, reported increases of 31 per cent from variety crosses, and recognized that hybrid seed would have to be produced each year. McClure, in 1892, noted that the performance of hybrid stocks could not be predicted from the performance of the parent varieties. As the nineteenth century closed, publications were appearing frequently on the subject of inbreeding and outcrossing in corn. The history of hybrid varieties had largely become the history of hybrid corn.

The importance of corn in the economy of the United States and the large number of persons contributing to its improvement undoubtedly were reasons for the rapid development of knowledge about inbreeding and hybrid vigor in corn. Another equally important reason was the nature of its floral structure. The separation of the female flowers into ears and the male flowers into tassels made crosses easy to make, both by the plant breeder and the seedsman. Pollen was produced in abundance, making many crosses possible from a single plant. Each ear produced many seed from a single pollination. Though inbreeding depression was evident, it was not great enough to preclude the development and commercial use of inbred lines.

It is to the credit of the pioneers in the development of hybrid varieties that the whole pattern for their use in corn was established within two decades of the rediscovery of Mendel's laws. This included genetic explanations for the basis of hybrid vigor. Shull, in 1909,³⁴ and East and Hayes, in 1912,⁸ had given the essential features of producing single-cross varieties, and Jones, in 1918,¹⁷ had proposed the use of double-cross

varieties. There was about a ten-year lag in commercial utilization of the available information.

The impact of hybrid varieties on yields of corn in the United States is obvious from the data of Table 1.1. From the decade 1915-1924 to the decade 1955-1964 corn yields increased 108.5 per cent, due either to the use of hybrid varieties or a combination of hybrid varieties and better cultural practices. The yield of oats, an important crop in the Corn Belt, increased 32.4 per cent over the same period, largely due to improved cultural practices alone. There can be no doubt that the greater increase in corn is due largely to the use of hybrid varieties.

Hybrids from Crosses of Varieties

A detailed study by Richey²⁸ of the yields of 244 variety crosses reported by several corn breeders indicated that 50 per cent of them gave yields higher than the higher yielding parent, and some of the variety hybrids were comparable to modern commercial hybrids in yielding ability. Richey noted that the hybrid vigor of variety crosses was usually greater if the parents came from different sources or were widely different in type. For example, flint $\times$ dent varieties were consistently better than dent $\times$ dent. In the early part of this century, East felt that variety hybrids held greater commercial possibilities than single crosses of inbred lines, if the varieties were carefully selected.¹¹ Undoubtedly, an important factor preventing the commercial acceptance of variety hybrids at that time was technological in nature; it would have required an entirely new system of producing and distributing planting seed.

In recent years, crosses of varieties have not encouraged corn breeders to substitute them for the double-cross hybrids now in use.^{23,33} They have, however, indicated varieties that might be useful as sources of superior inbred lines.

Single Crosses

Hybrid corn, using inbred lines as parents, was first proposed by G. H. Shull in 1909.³⁴ In making this proposal he recognized (1) the necessity of carefully choosing inbred lines on the basis of their performance in hybrid combinations; (2) that inbred lines would reproduce themselves faithfully; (3) that the hybrids from inbred lines would be uniform and the same from year to year; and (4) that there would be economic problems associated with the production of planting seed from low-yielding inbred lines.

The low yields of inbred lines did, in fact, prohibit the widespread use of single crosses. In addition, the seed of the inbred lines was small and

misshapen, and not well suited to mechanical planting. Often, germination was poor and seedlings lacked vigor. In producing single crosses, two rows of the female alternated with one row of the male, so that only two-thirds of the field grew hybrid seed for sale.

It should be recognized that inbred lines do not always combine to give higher yields than the open-pollinated variety from which they came. A random lot of inbred lines will give hybrids above the open-pollinated parent variety in only about 50 per cent of the cases.¹¹

Single crosses are grown commercially where corn is used for canning or freezing. The value of the product, and the importance of uniformity and quality, make the extra cost of the seed worthwhile.

Double Crosses

Jones, in 1918, proposed the use of the double cross to produce hybrid varieties of corn.¹⁷ By double cross is meant the hybridization of two single crosses and the use of the F_1 as the commercial crop (Fig. 18.1). The seed has been comparatively cheap, since it is grown on vigorous, high-yielding, single-cross hybrids. Usually the ratio of the female to male rows in the crossing block for double-cross seed has been 4:1, respectively, so that 80 per cent of the seed field produces seed for sale.

By careful selection of parental inbred lines, it has been possible to produce double-cross hybrids that are about equal to the single crosses in yield. As expected, double crosses have been more variable than single crosses, but this variability has often given them wider adaptation.

Most of the commercial acreage of corn in the United States is grown to double-cross varieties. Three-way crosses, from combinations of a single cross as female and an inbred line as male, are grown to a lesser extent. They are intermediate between single and double crosses in their characteristics.

Inbred Lines

Kiesselbach estimated in 1951 that more than 100,000 inbred lines of corn had been tested in the United States up to that time, with only 60 of them being of value in the production of hybrid varieties. While the number of tested lines has increased considerably since then, the proportion of useful lines is probably even less. This means that the chances of success are very small and are probably decreasing as efforts are made to improve upon the excellent lines that are presently available.

Inbred lines are produced by selfing open-pollinated stocks, which may be varieties or the products of crosses of inbred lines. Selfing is continued for five to seven years, which is long enough to make the lines uniform

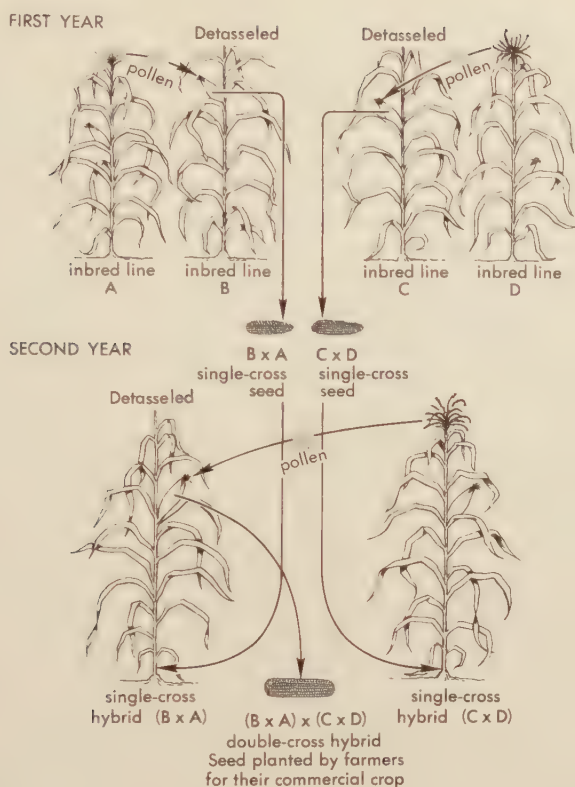


Figure 18.1 Diagram of method of crossing inbred plants and the resulting single crosses to produce double-cross hybrid seed. (Pearson,^{25a})

in most respects, and certainly long enough to permit evaluations of the inbreds as parents for hybrid varieties. Selfing may be continued for a longer period to eliminate small variations. During the period of inbreeding, selection is practiced for desirable agronomic characters such as time of maturity, height, nature of ear, kernel characters, stem strength, and disease resistance. Some attention is given to yielding ability, since a productive inbred line will reduce the cost of growing hybrid seed. This is the first stage in the evaluation of an inbred line. The second stage is its testing as a parent in single crosses, and the third stage is its evaluation in double crosses. Each stage will be considered separately.

Correlation between inbreds and their single-cross hybrids. As early as 1922, Kiesselbach showed a general correlation between the yields of inbreds and their crosses. Subsequent research by Richey, Hayes, Jenkins,

and others has shown that visual selection of inbreds will yield lines with high combining value.^{11,12,38} It is a common practice of corn breeders to evaluate inbred lines carefully before using them in single crosses.

Prediction of general combining ability. At first, inbred lines were tested for productivity or combining ability by crossing all inbreds *inter se*. When it is considered that 435 single crosses, not including reciprocals, may be made from 30 inbred lines, it will be realized that it is utterly impossible to test in this way even a few hundred inbreds. The number of single crosses is given by $n(n-1)/2$, if n is the number of inbred lines. A more efficient method, the inbred-variety or top cross, was suggested by R. L. Davis.⁶ This consists of crossing each inbred line with an open-pollinated variety, which gives a measure of general combining ability.

Jenkins and Brunson provided abundant data to support this method of evaluating inbred lines.¹⁵ They compared the yields of a number of inbreds in top crosses to commercial varieties with the average yields of the same inbreds in a number of single crosses. In other words, they compared inbred lines for their general combining ability and their average combining ability. Correlation coefficients in these comparisons ranged between 0.53 and 0.90. They concluded that the top cross could be safely used to discard half the inbreds. The balance could be tested for specific combining ability.

The choice of the variety to use in the top cross may be difficult. Usually, the best compromise is to select a successful open-pollinated variety from the area where the hybrid will be grown. The inbred lines are customarily grown as the female parents for top crosses and are detasseled. The open-pollinated variety is used in every fourth or fifth row as a pollen source. Should the open-pollinated variety be the female, it is recommended that at least ten plants be used in the cross to each inbred in order to sample adequately the genetic variability of the open-pollinated variety.³⁶ Hayes¹¹ and Sprague³⁸ have reviewed the literature and opinions on alternative materials that might be used to measure general combining ability.

Because of the great expenditure of time and money in the development and maintenance of inbred lines, it was natural that corn breeders should wonder how early in the selfing program a measure could be obtained of general combining ability. Jenkins reported that "inbred lines acquired their individuality as parents of top crosses very early in the inbreeding process and remained relatively stable thereafter."¹⁴ Sprague's data indicated that a measure of general combining ability could be obtained from top crosses made to the original plants selfed in an open-pollinated variety (these are termed S_0 plants).³⁷ As stressed by Sprague,

it is a method for identifying large differences in combining ability and a method for eliminating large volumes of material with no potential for the production of inbred lines. While there is not general agreement among corn breeders as to the value of early testing,^{11,38} it is being widely used as a basis for discarding up to 80 per cent of the materials selected for selfing in open-pollinated materials.

Specific combining ability. Specific combining ability, a term offered by Sprague and Tatum,³⁹ is the ability of inbred lines to combine in specific single, three-way, and double crosses. Of primary concern here is their combining ability in double crosses. In this regard it is important to remember that no matter how good the inbred line is in a single cross, it will have no commercial value if the single cross is not a good parent of a double cross; an inbred line must be evaluated in combinations with three other inbreds. While 30 inbreds may be used to make 435 different single crosses, they may be combined as double crosses in 657,720 different ways, not including reciprocals. This is given by the formula $n(n-1)(n-2)(n-3)/8$, if n is the number of inbred lines. Assuming that top crosses have narrowed the number of inbreds down to 10, there will still be 630 different double-cross combinations possible, far too many to test. It is obvious that some method must be used to predict the specific combining ability of inbreds from their performance in single crosses.

Jenkins¹³ presented data on four methods of estimating the yield potential of four inbred lines in a double cross, which were based on their average yields in (1) the six possible single crosses; (2) the four single crosses not used in making the double cross; (3) all possible combinations with ten other inbreds; and (4) top crosses. The correlations between actual and predicted yields of 42 double crosses using these four methods were 0.75, 0.76, 0.73, and 0.61, respectively. As a consequence of this and other research, it has become an accepted practice to predict the yields of double crosses from the yields of the single crosses not used in the double cross.

The data of Anderson will illustrate the accuracy of this prediction.¹ The yields obtained from all possible single and double crosses from five inbred lines, and the predicted yields of the double crosses, are given in Table 18.1. The predicted yield of the double cross $(23 \times 24)(26 \times 27)$ is the average yield of 23×26 , 23×27 , 24×26 , and 24×27 , which would be $(62.6 + 70.8 + 65.6 + 72.1)/4 = 67.8$ bu per acre. This is very similar to the yield of 68.8 bu per acre that was actually obtained. The agreement between the actual and predicted yields in this experiment is remarkably close.

Richey's analyses of varietal crosses mentioned earlier and data from several other studies indicate that crosses between inbreds from diverse

Table 18.1 Actual single- and double-cross yields of five inbred lines (23, 24, 26, 27, and 28) and predicted yields of double crosses from single-cross yields.¹

CROSS	YIELD PER ACRE, bu		CROSS	YIELD PER ACRE, bu	
	<i>Actual</i>	<i>Predicted</i>		<i>Actual</i>	<i>Predicted</i>
SINGLE CROSSES					
23 × 24	41.7		24 × 27	72.1	
23 × 26	62.6		24 × 28	69.3	
23 × 27	70.8		26 × 27	64.2	
23 × 28	64.4		26 × 28	60.4	
24 × 26	65.6		27 × 28	59.6	
DOUBLE CROSSES					
Inbreds 23, 24, 26, 27			Inbreds 23, 26, 27, 28		
(23 × 24)(26 × 27)	68.8	67.8	(23 × 26)(27 × 28)	68.2	65.0
(23 × 26)(24 × 27)	62.4	60.6	(23 × 27)(26 × 28)	65.0	62.7
(23 × 27)(24 × 26)	62.0	60.2	(23 × 28)(26 × 27)	65.7	63.4
Inbreds 23, 24, 26, 28			Inbreds 24, 26, 27, 28		
(23 × 24)(26 × 28)	65.0	65.5	(24 × 26)(27 × 28)	70.2	66.5
(23 × 26)(24 × 28)	59.8	58.0	(24 × 27)(26 × 28)	62.0	47.7
(23 × 28)(24 × 26)	56.0	58.5	(24 × 28)(26 × 27)	62.7	64.4
Inbreds 23, 24, 27, 28					
(23 × 24)(27 × 28)	71.1	69.2			
(23 × 27)(24 × 28)	58.1	59.4			
(23 × 28)(24 × 27)	58.0	60.4			

sources tend to be more productive than crosses between inbreds from the same variety.^{9,12,16,45} The studies of Eckhardt and Bryan also show that the highest yields are likely to occur between two single crosses, each derived from a different variety.⁹ Furthermore, they found that where early (E) and late (L) lines were used, the combinations of (E × E)(L × L) were more uniform in maturity, ear height, weight, and diameter than were (E × L)(E × L). As a general rule, then, in the production of double-cross varieties, it is advisable to combine the similar or closely related inbreds in the single crosses, and the different or distantly related inbreds in the double crosses.

The most widely grown double cross, U.S. 13, has the pedigree (Wf9 × 38-11)(Hy × L317). Wf9 and 38-11 were selected from Reid Yellow Dent at the Indiana Agricultural Experiment Station; Hy from Illinois High Yield, a selection of Leaming, at the Illinois Agricultural Experiment Station; and L317 from Lancaster Surecrop at the Iowa Agricultural Experiment Station. Undoubtedly the genetic diversity of the inbreds of

this double-cross variety contributed in large measure to its success. Jones and Everett have given the origins and characteristics of several widely used inbreds.¹⁸

Improving inbred lines. Because the number of successful inbred lines of corn has been small in relation to the number developed, and also because the commercially grown hybrids are now difficult to excel, there has been increasing interest in improving the inbred lines now available. Improvements have been made with respect to the inbred itself in yield, disease and insect resistance, stem strength, and quality, and with respect to its specific combining ability. Improvements have used in part the procedures already described for self-pollinated crops.

A version of the pedigree method was used first. A successful single-cross parent was carried into F_2 and succeeding generations, with continuous inbreeding and selection for superior lines.^{16,45} Some inbreds were improved for specific combining ability, and others for date of maturity, resistance to lodging, and resistance to smut. While improvements can be made by using this method, it should be borne in mind that this method can narrow the genetic differences between the parents of single crosses. Hayes described a program of adding resistance to the European corn borer to each of the inbreds used in producing a single cross, which in turn was used as the female parent of a double cross.¹¹ This was done by crossing the inbreds to resistant stocks and selfing and selecting in segregating generations for agronomic type and resistance to the corn borer. The derived inbreds were tested for general and specific combining ability, and the two best ones were used as parents of a new single cross. The new single cross became the female parent of Minhybrid 414, a double-cross variety with considerable resistance to the corn borer and high yield. Hayes has called this the *second cycle of improvement*.

The backcross method is well suited for the improvement of inbreds that have specific weaknesses. Its use in recent years has become a necessity for introducing inbred genotypes into male-sterile cytoplasm and transferring restorer genes to the matching inbred lines.

Richey proposed a method of improving inbred lines, which he called *convergent improvement*²⁹ (page 220). Studies of convergent improvement have indicated a slight but consistent improvement in the inbred lines, but not enough to attract the interest of corn breeders. See Hayes,¹¹ Richey,³² and Sprague³⁸ for reviews.

Other suggestions have been made for developing or improving corn inbreds. These include the use of inversion stocks to identify lines carrying favorable genes on a particular chromosome;⁷ translocation stocks to permit the immediate production of homozygous inbreds;³ and gamete selection, which provides for sampling of the germ plasm of open-

pollinated varieties for gametes rather than zygotes.⁴⁰ These methods have been described in some detail by Sprague³⁸ and Hayes.¹¹ Thus far, they have been of academic interest only.

At the present time, recurrent selection is receiving considerable attention from corn breeders. Its potential is so great that it will be discussed in detail in Chapter 19.

Production of Hybrid Seed

Estimating that 10 lb per acre of hybrid corn seed are used on about 60 million acres of corn in the United States, there is an annual requirement of about 600 million pounds, or 300 thousand tons. It has become such a large business that upwards of 70 seed companies are involved in the production of seed, most of it under contract to farmers. Many companies are developing their own inbred lines and hybrids, most of them with closed pedigrees. The term "closed pedigree" means that the pedigree is not available to the public. Their contribution to the production of hybrid corn has been reviewed by Hayes¹¹ and Crabb.⁵ One additional benefit from this highly organized program of seed production is the provision of sound seed of high quality, in most cases treated against insects and diseases. The methodology of hybrid seed production has been reviewed in some detail by Hayes¹¹ and Poehlman.²⁶

Cytoplasmic male sterility has facilitated the production of hybrid corn because it has eliminated the need for detasseling, which often reduces yield.¹⁹ Cytoplasmic male sterility is incorporated into the inbreds used as the female parents of single crosses. Various combinations of sterile cytoplasm and fertility restorer genes may be used to produce double-cross varieties (Fig. 18.2).

An *advanced generation* of a single cross, such as an F_2 , may be used to produce double-cross varieties. Where only one single cross is an advanced generation, it is advisable to use it as the male parent in the production of the double cross. There are two reasons for this: (1) The F_2 is reduced in yield, and (2), being variable, the F_2 produces its pollen over a longer period. Double crosses from advanced generation single crosses in F_2 and F_3 have not been lower in yield than those made with the F_1 .²⁰

When double crosses were first used commercially, farmers often used seed from the harvest of the double cross to plant the crop the following year. The drop in yield was sometimes above 30 per cent (see Table 18.2). Selfing and sibbing (equivalent to open-pollination in these examples) of the F_1 of the single cross had the same effect, since all plants had the same genotype. The F_1 from a double cross is variable, so that sibbing did not reduce the yields of the F_2 as much as selfing. Because the variety

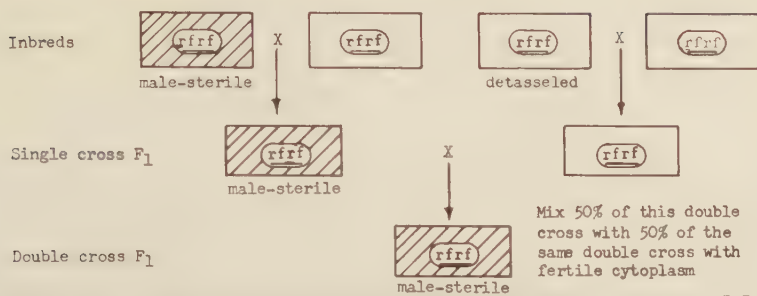
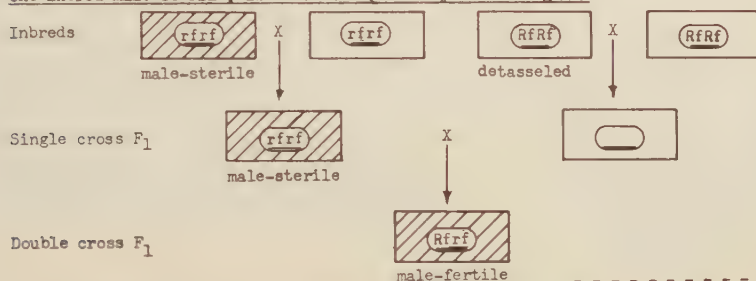
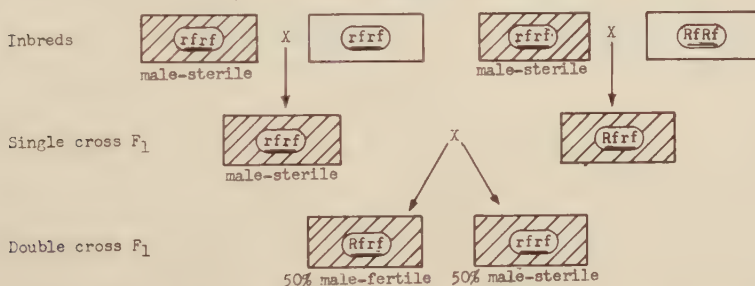
One inbred male-sterile, and no fertility restorersOne inbred male-sterile, and two with fertility restorer genesTwo inbreds male-sterile, and one with fertility restorer gene

Figure 18.2 Alternative methods of using cytoplasmic male sterility to produce double-cross varieties of corn. Male-sterile cytoplasm hatched. Rf is a restorer gene that restores fertility in sterile cytoplasm.

crosses did not drop in yield when sibbed, it may be assumed that the F_1 was not much higher on the average than the parents going into the crosses. Note that the single-cross and double-cross hybrid yields dropped between F_1 and F_2 and not between F_2 and F_3 . Equilibrium is reached in most cases in one generation.

Table 18.2 Comparative grain yields of F_1 , F_2 , and F_3 progenies of single, double, and variety crosses in which the generations are advanced by sib-pollination and selfing. Averages for two years, 1925 and 1927.²²

KINDS OF HYBRIDS	NUMBER OF HYBRIDS, avg.	F ₁ SEED	F ₂ SEED MADE IN TWO WAYS <i>Sibbed^a</i> <i>Selfed</i>	F ₃ SEED MADE IN TWO WAYS <i>Sibbed^a</i> <i>Selfed</i>		
<i>Actual Yields,^b bu per acre</i>						
Single Cross	10	51.4	33.3	33.2	33.5	25.2
Double Cross	8	50.8	41.4	33.9	41.1	26.3
Variety Cross	7	46.9	46.5	29.0	46.3	22.9
<i>Relative Yields, per cent</i>						
Single Cross	10	100	64.8	64.6	65.2	49.0
Double Cross	8	100	81.5	66.7	80.9	51.8
Variety Cross	7	100	99.1	61.8	98.7	48.8

^a Sibbed are brother-sister matings, essentially equivalent to open-pollination in this case.

^b Difference required for significance at 5% = 3.2 bu.

Hybrid Varieties in Other Crops

Hybrid varieties of flowers and vegetables, produced by hand emasculation and pollination, have been available to home gardens and greenhouse production for a number of years. In these cases the high cost of seed has been of little consequence. But the difficulties in, and high cost of, seed production have for many years prohibited the development of hybrid varieties in cross-pollinated crops where hybrid vigor was a well-recognized phenomenon.

Cytoplasmic and genetic systems of pollination control are now making possible hybrid varieties in many crops, even those that are self-pollinated (see Chapter 17). The use of cytoplasmic-genetic male sterility in onions and corn has already been mentioned. After its first report in sorghum in 1954,⁴¹ hybrid sorghum varieties were quickly developed, so that now most of the commercial acreage is sown to hybrid varieties. Although sorghum is a normally self-pollinated crop, pollen dispersal is good. As in corn, seed companies produce and distribute most of the seed. Breeding programs are now under way to develop hybrid wheat, using cytoplasmic male sterility and a fertility restorer factor.²

Genetic male sterility has been more difficult to use, since it requires considerable work to maintain and use in crossing blocks. Suneson has proposed the use of the *ms* gene to produce hybrid barley.^{42,43} While the stimulus from heterosis was as high as 50 per cent above the best



Figure 18.3 The late J. C. Stevens (left), USDA plant breeder, stationed at Chillicothe, Texas, and J. Roy Quinby (right), Superintendent, Texas Branch Experiment Station, Chillicothe, share jointly in the 1955 Hoblitzelle Agricultural Award of \$5,000 for their development of a practical method for producing hybrid sorghum seed. (Courtesy of J. Roy Quinby.)

parent, the difficulties in, and costs of, producing seed have prevented the development of hybrid varieties. Wiebe has suggested that a male-sterile gene in barley be closely linked with the recessive gene for resistance to DDT.⁴⁴ By appropriate management of genotypes and spraying schedules, the female parent in a crossing block would have the genotype *msms*, and all seed produced on it would be crosses to the other variety or line in the crossing block. One problem in barley, and in wheat too, will be pollen transfer. Suneson has found wide differences in seed yield on *msms* plants, due in part to environmental factors.

In castor beans a single gene *f*, when homozygous, prohibits the production of male flowers, so that it functions from a breeding standpoint like genetic male sterility.⁴ This gene has permitted the development and widespread use of hybrid varieties. The female inbred line consists of two genotypes, *Ff* and *ff*, in equal amounts. Only *ff* is harvested, and its progeny will again have equal amounts of *Ff* and *ff*. When used as the female parent in a crossing block, plants of genotype *Ff* are removed

before flowering. All seed on *ff* plants will be crosses with the other inbred line in the crossing block.

Self-incompatibility has been proposed as a means to develop hybrid varieties, whether its genetic basis is understood or not. Odland and Noll²⁵ have suggested a system for its use in cabbage, whereby four homozygous lines are developed with genotypes S_1S_1 , S_2S_2 , S_3S_3 , and S_4S_4 .²⁵ The difficult task is to achieve lines homozygous for incompatibility alleles, though this may be possible by selecting the appropriate environment and time of flowering. It may also be achieved by choosing alleles that will permit selfing under isolation, but not when other pollen is available. Because seed stocks of inbred lines will be limited in amount, the double-cross hybrid will be most feasible.

Hybrid varieties are being developed in alfalfa without knowing the genetic bases of incompatibilities. These have involved both inbred lines and clones. Where inbred lines have been used, they have had at least the following three characteristics: (1) They must be capable of reproducing themselves under isolation; (2) they must produce mostly crossed seed when two of them are grown in adjacent rows or in mixed stands; and (3) they must produce vigorous hybrids. Clones may be used to produce single crosses or double crosses, though commercial varieties will probably be based on double crosses. To produce a single cross, two clones are selected that are self-sterile but which combine to produce a vigorous single cross. The parental clones are propagated by rooted cuttings, an expensive undertaking. The expense has discouraged interest in the single cross. However, if two single crosses could be produced, both of which are self-sterile but cross-compatible, then double-cross hybrid seed would not be too expensive. The problem is to get two pairs of clones with the desired characteristics which will give a vigorous double-cross hybrid.

Recently Ramage has proposed the use of balanced tertiary trisomics to facilitate the production of hybrid seed in barley.²⁷ The nature and management of the trisomics will be described in Chapter 23.

Résumé

The development of hybrid varieties in corn put yields of that crop on a new plateau. It was a revolution in agriculture that continues to change the old order. Cultural methods in corn production have changed to exploit more fully the yield potential provided by hybrid varieties. A new seed industry developed, at first to supply seed of hybrid varieties developed by federal and state researchers, and later to take over almost the entire responsibility of developing both inbred lines and hybrid

varieties. Finally, the revolution spread to other crops, with similar consequences.

It is likely that hybrid varieties of most crops will utilize to some extent the cytoplasmic-genetic forms of male sterility. As a second choice, various manifestations of genetic male sterility will be used. Indeed, in all self-pollinated crops and most cross-pollinated crops, hybrid varieties are not commercially feasible without some form of male sterility.

The use of hybrid varieties has not made the task of plant breeding easier. In corn the number of successful inbreds is very small in relation to the number developed. In part, the problem has been one of finding parental genotypes that nick well together, a large assignment when four inbred lines are needed for a double-cross hybrid. This prompted the following fictitious experience in an article by Dr. F. D. Richey of the U.S. Department of Agriculture, who has made important contributions to the development of hybrid varieties:

The Shattered Dream of a Corn Breeder

Selfed a hundred corn plants,
Put each in a cross;
Selfing without testing,
Means a heavy loss.

Looked around the country,
Found a fertile field,
Used a ten-ten lattice
To find out how they'd yield.

Analyzed the variance,
Wanted just the best;
Planted only thirty,
Threw away the rest.

Thirty, good in hybrids,
That would be a plenty;
Heavy rains, and lodging;
Then there were twenty.

Still had twenty inbreds
Looking mighty keen;
Hot, humid weather;
Smut left thirteen.

Lucky thirteen inbreds,
Glad to be alive;
Wilt, blight, and aphids;
Then there were five.

So passed the summer,
 Full of sweat and tears;
 Came then the harvest—
 Four had rotted ears.

One sturdy inbred,
 All, all alone;
 It has no sex appeal,
 Can't find a home.

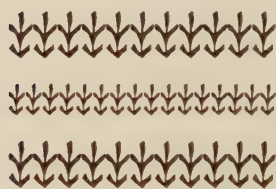
Frederick D. Richey, Knoxville, Tenn.

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RECURRENT SELECTION



Recurrent selection was meant to include reselection generation after generation, with interbreeding of selects to provide for genetic recombination. Thus, selection among isolates, inbred lines, or clones is not recurrent until selects are interbred and a new cycle of selection is initiated.

HULL, 1952 *

RECURRENT selection grew out of a need to increase the number of superior genotypes that could be obtained from breeding stocks of corn. One way to do this was to increase the frequency of superior genes in the gene pool. Another was to increase the chances for genetic recombination to occur. Both may be accomplished by recurrent selection.

* From F. H. Hull, "Recurrent Selection and Overdominance" in *Heterosis*, John W. Gowen (ed.), Ames: Iowa State University Press, 1952, p. 452.

Several features of the standard method of breeding hybrid varieties severely limited the occurrence of superior genotypes. These were (1) the high level of inbreeding, where every generation of selfing reduced heterozygosity by one-half; (2) small population sizes imposed by the limitations of space and time; and (3) linkage.

At the same time, it was realized that early-generation testing would identify superior genotypes, those likely to give superior inbred lines in later generations of inbreeding. Here was a useful screen to keep superior genes for the gene pool.

Out of the need to increase recombinations and the knowledge of the effectiveness of early-generation testing grew the different systems of recurrent selection. Actually, the idea was proposed first by Hayes and Garber⁵ in 1919 and independently by East and Jones⁴ in 1920. They supplied no data to support the idea. Jenkins⁹ was the first to describe the method in detail, and Hull⁷ proposed the name.

All systems of recurrent selection are cyclic in nature. Each cycle requires (1) evaluation and selfing of selections, and (2) crossing of the progenies of superior selfed plants in all combinations and bulking of equal amounts of seed from each cross.

Evaluations may be limited to what can be learned from the selected plants themselves. In corn this would include maturity, height, ear characteristics, kernel characteristics, and disease or insect resistance. Usually, these characteristics will have a high heritability. Obviously, these evaluations will not include yield.

Yield evaluations are obtained from top crosses of selected plants to a tester. Such top crosses will be grown in the following year, during which time the selfed seed is held over until the evaluations for yield have been made. Or the selfed may be grown out as S_1 lines, which in turn are selfed.

After evaluations have been made, the selfed seed of superior plants is grown out for crossing. Progenies of selfed plants are crossed in all possible combinations. This may be done by hand or by growing the progenies together in an isolated block. In the latter case, every effort must be made to get random mating of the progenies. Whether crosses are made by hand or under isolation, equal amounts of seed of each are bulked. The bulked seed is used to start the second cycle of selection, selfing and crossing.

The initial stocks for a recurrent selection program may be an open-pollinated variety, a successful single-cross or double-cross hybrid, or a synthetic variety. Cycles may be continued until no further progress is made.

Four types of recurrent selection are recognized: (1) recurrent selection

for phenotype; (2) recurrent selection for general combining ability; (3) recurrent selection for specific combining ability; and (4) reciprocal recurrent selection.

The term *synthetic* has been generally used for the bulked product of each cycle of recurrent selection, though other terms including composites and populations have also been employed. The symbols and terminology of Lonnquist¹⁴ have been adopted in this discussion. In the first cycle the synthetic is termed syn I; in the second cycle, syn II; and so on. Arabic numerals are used to indicate the generations of the synthetics. The symbol syn-0 indicates the selected selfed lines that are crossed to give the synthetic; syn-1 is the bulked F_1 populations obtained from the crosses (these are often used as the source of selections for the next cycle); and syn-2 is the F_2 generation. For example, syn II-0 would be the progenies of selected selfed plants in the second cycle, which are being crossed together in all combinations by hand or under isolation. The plants grown from these crosses would be syn II-1. It is realized that these symbols lead to confusion when used orally, but no more convenient abbreviation could be found.

Roman numerals as subscripts following a variety name indicate the synthetic developed from that variety. For example, $Krug_I$ identifies the synthetic developed from the first cycle of recurrent selection of the variety Krug; and $Reid_{IV}$, the synthetic from the fourth cycle.

Recurrent Selection for Phenotype

Recurrent selection for phenotype is illustrated in Fig. 19.1. It represents the simplest form of recurrent selection. No tests of combining ability are required, and each cycle may be as short as one year. Obviously, since selection is based on the phenotype of the plant, it is useful only for characters with high heritability. Actually, the method is an extension of mass selection. The refinements of recurrent selection are: (1) The selected plants are selfed, and (2) selected plants are crossed before another round of selection.

An example of this type of recurrent selection is provided by Sprague et al.¹⁵ They compared recurrent selection with conventional inbreeding methods for their effectiveness in raising oil content of kernels of a synthetic variety of corn called Stiff Stalk. The variety was developed in 1936 from a combination of 16 lines with strong stems.

Starting with ten selfed plants (S_1) having an average oil content of 4.97 per cent, two series were carried forward. In the first series selfing and selection for high oil content was practiced for four generations, at which time (S_5) oil content was raised to 5.62 per cent. In the second

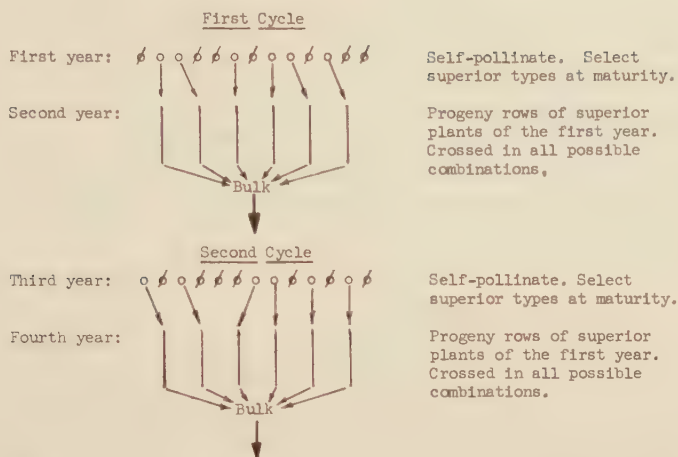


Figure 19.1 Phenotypic recurrent selection. Where it is possible to identify the desired selections before flowering, intercrosses of selections may be made in the first year of each cycle, and the second year and progeny tests eliminated from each cycle.

series there were two cycles of recurrent selection. In the first year of each cycle selfed progenies from ten plants with the highest oil content of the year before were grown and crossed in all combinations, and in the second year the F_1 of these crosses were grown in bulk and the ten plants with the highest oil content were identified. The oil content at the end of the second cycle was 7.00 per cent, a gain of three times that of the selfing series.

After five years, plants in the selfed series were quite uniform, both in plant appearance and oil content. This would be expected if heterozygosity decreased by half with every generation of selfing. The recurrent series was much more variable, and showed no indication of decrease in genetic variability. If all lines saved in the first cycle had contributed equally to selected materials from the second cycle, the percentage inbreeding would be 2.8 per cent. The genetic variability present in the recurrent series would suggest that additional cycles might increase oil content further.

A modification of the preceding program was used by Jenkins et al. in developing stocks with a high concentration of genes for resistance to leaf blight (*Helminthosporium turcicum*).¹⁰ Resistant plants were identified before pollination, and from these plants a bulked lot of pollen was

taken, a mixture of which was used on all selected plants. This meant that selection and crossing was done in the same year. The method was found to be quite effective, particularly during the first two cycles.

Recurrent Selection for General Combining Ability

An illustration of recurrent selection for general combining ability is given in Fig. 19.2. As a three-year cycle, this is its simplest form. One

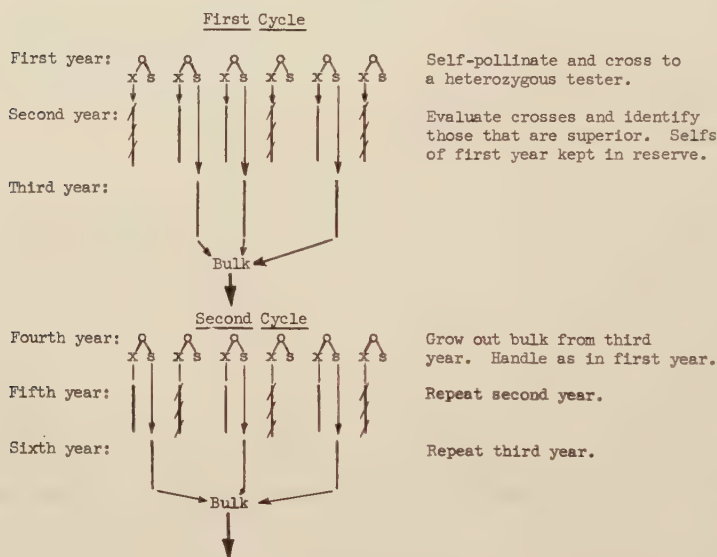


Figure 19.2 Recurrent selection for general combining ability. Often each cycle is extended an extra year, with the first year being used for selfing and the second for increasing the selfed seed and making crosses to the tester. The third year of the diagram above may be extended into a fourth year to permit additional recombinations.

variation is to self single plants in the first year of the cycle, and grow S_1 progenies from these in the second year. These are top-crossed to the tester. In the third year, top crosses are grown in yield tests, and superior plants of the first year are identified. Reserve selfed seed of these plants or their progenies is then grown out in the fourth year, and crosses are made in all combinations. Sometimes syn-2 seed instead of syn-1 is used to start the next cycle.

It is important that the tester have a broad genetic base. Its qualifica-

tions would be similar to those of a good variety for use in top crosses (see Chapter 18).

This method has some similarity to the ear-to-row method of improving corn varieties. In the ear-to-row method, however, the selected plants are not selfed, and germ plasm from the pollen will be obtained at random from the entire population.

Lonnquist used recurrent selection for general combining ability to improve five varieties or populations of corn. The results from each of three cycles are given in Table 19.1. Cycle II gave a mean yield advance

Table 19.1 Yields in bushels per acre of five synthetics and their hybrids to the other four synthetics, Lincoln, Nebraska, 1960.¹⁴

VARIETY	CYCLE I	CYCLE II	CYCLE III
<i>Synthetics</i>			
Krug	88.4	106.6	95.4
Nubold Reid	86.8	102.3	101.0
Synthetic A	115.6	110.6	114.4
Synthetic B	93.6	106.3	109.3
Stiff Strawed Synthetic	86.2	96.7	107.3
Mean	94.1	104.5	105.5
<i>Crosses</i>			
Krug	107.2	116.5	117.4
Nubold Reid	105.6	113.5	115.6
Synthetic A	111.9	115.5	120.6
Synthetic B	104.4	115.2	120.1
Stiff Strawed Synthetic	108.4	117.4	121.2
Mean	107.5	115.6	119.0

NOTE: Yield of U.S. 13, a double-cross hybrid, was 99.4 bu per acre.

of over 10 bu per acre, but cycle III gave a mean advance of only 1 bu per acre. It would appear that recurrent selection had almost exhausted the potential of these populations for further advance as synthetics. If so, this form of recurrent selection would appear to be a powerful means of achieving the full genetic potential of a population. It should be noted also that the synthetics of cycles II and III exceeded U.S. 13, a double-cross hybrid, in yield.

Lonnquist had a second purpose in developing these synthetics; he wished to use them as a source of inbred lines to be used in the production of hybrid varieties. The results of crosses of the synthetics to the other four synthetics (Table 19.1) will give a measure of their potential as sources of genotypes for hybrid combinations. In this respect, each

cycle improved the synthetics, and there appeared to be very little leveling-off in the third cycle. This would suggest that another cycle might improve some of or all these synthetics as sources of superior genotypes for hybrid combinations.

Recurrent Selection for Specific Combining Ability

Hull proposed on theoretical grounds the use of recurrent selection for specific combining ability.⁷ The pattern for this method of selection is the same as that for recurrent selection for general combining ability except that the tester is selected to provide a measure of specific combining ability. The best choice of tester would be a stable, permanent inbred line, particularly if the objective was to develop stocks that would combine well with the inbred tester. Where the search is for stocks that will combine well with a particular single cross, the latter is often considered to be a tester for specific combining ability.

Corn breeders have been hesitant to develop stocks with specific combining ability, particularly if the tester is an inbred line, because the inbred tester may fail for some reason, such as susceptibility to a disease or an insect, or a better inbred may become available, making the inbred tester unattractive. In these events the stocks with high specific combining ability may be of little value. There are, however, some data to show that an inbred line can be effective in a recurrent selection program, not only in improving stocks for specific combining ability, but also for general combining ability.^{6,16}

Reciprocal Recurrent Selection

Reciprocal recurrent selection was proposed by Comstock, Robinson, and Harvey¹ in 1949. Two populations are involved (see Fig. 19.3), usually designated A and B. Each population is handled in the same manner as in recurrent selection for general combining ability except that population A is the tester for B, and B for A. With each cycle the new versions of A and B become the testers. The two populations should be genetically divergent and should be known to combine well together. They may be two varieties, two synthetics, or two single crosses involved in the production of a successful double cross.

This method was proposed and evaluated on theoretical grounds. The authors concluded that (1) under no circumstances would reciprocal selection be more than slightly inferior to recurrent selection for general or specific combining ability; (2) it would be definitely superior to selection for general combining ability for loci at which there is overdominance; and (3) it would be definitely superior to methods proposed by Hull for loci at which there is partial dominance.

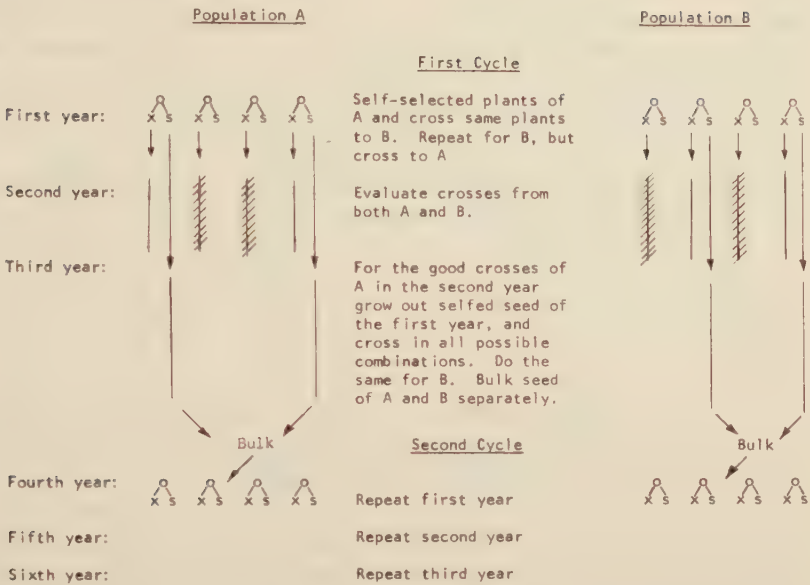


Figure 19.3 Reciprocal recurrent selection. Each cycle may be extended as in Fig. 19.2.

Douglas et al. reported results of three cycles of recurrent selection in corn, in which they followed the procedures outlined by Comstock et al., with only minor exceptions.³ Yellow Surcropper and Ferguson's Yellow Dent, two varieties that were known to combine well, were chosen as source material. Changes in the yields of synthetics of the varieties through two cycles are given in Table 19.2. The yield of the Ferguson's Yellow Dent synthetics were improved in each cycle of selection. No consistent improvement was made in the yield of Yellow Surcropper, although there appears to have been some advance in the first cycle.

Crosses of the synthetics developed from two cycles of selection give

Table 19.2 Yields in bushels per acre of synthetics of Yellow Surcropper and Ferguson's Yellow Dent through two cycles of reciprocal recurrent selection. Average for data from 1957 and 1958.³

COMPOSITE	YELLOW SURCROPPER	FERGUSON'S YELLOW DENT
Open-pollinated Variety	41.5	42.6
First-Cycle Synthetic	47.1	45.1
Second-Cycle Synthetic	43.0	51.1

Table 19.3 Combining ability of synthetics from Yellow Surcropper^a and Ferguson's Yellow Dent from different cycles of reciprocal recurrent selection. Average yields in bushels per acre for 1957 and 1958.³

YELLOW SURCROPPER	FERGUSON'S YELLOW DENT		
	FYD	FYD _I	FYD _{II}
YS	52.4	47.9	
YS _I	57.0	57.2	61.7
YS _{II}		61.3	58.5

^a Subscripts denote cycle of reciprocal recurrent selection.

a measure of the improvement in combining ability (Table 19.3). The base from which to measure advance would be the cross of open-pollinated varieties, namely 52.4 bu per acre. The greatest improvement in combining ability was in the first cycle with Yellow Surcropper; Yellow Surcropper_I combined well with all populations of Ferguson's Yellow Dent. The second cycle did not effect measurable improvement in this variety. Ferguson's Yellow Dent, on the other hand, did not improve in combining ability, with the open-pollinated variety and the syn I giving about the same average yield in crosses to two populations of Yellow Surcropper. Likewise, the syn II of Ferguson's Yellow Dent, when crossed to two synthetics of Yellow Surcropper, gave about the same average yields as the syn I when crossed to the same synthetics of Yellow Surcropper. It should be kept in mind that the combining ability is an average of all materials in each synthetic. The authors felt that reciprocal recurrent selection was a promising method of developing source materials for inbred lines to be used in producing hybrid varieties.

Another report by Thomas and Grissom indicated that two populations of popcorn were simultaneously improved by two cycles of reciprocal recurrent selection for yield, popping volume, and resistance to lodging.¹⁷ Essentially the same improvement, however, was made through selection in a selfing series, where selection was based on results of top crosses to the opposite population. The advantage of the reciprocal recurrent selection was that the synthetics had greater residual variability, which offered an opportunity for further improvement.

Other Crops

Recurrent selection for phenotype appears to be a useful method to improve cross-pollinated species other than corn, as indicated by the successful way in which Johnson changed the growth habit and vigor of Madrid, a variety of sweet clover.^{11,12} Since sweet clover is a biennial,

evaluations as a green-manure crop could be made the first year, and crosses of selected plants the second year. It is widely used as a green-manure crop in the Midwest, being sown with a small grain and plowed under in the fall of the first year or early in the following spring.

A measure of the advance from recurrent selection was obtained by comparing the weights of the open-pollinated progenies in the fifth year of the first two cycles with the weights of open-pollinated progenies from plants of the original population. Green weight was obtained in percentage of Madrid. The results (Fig. 19.4) show a striking response to recurrent selection.

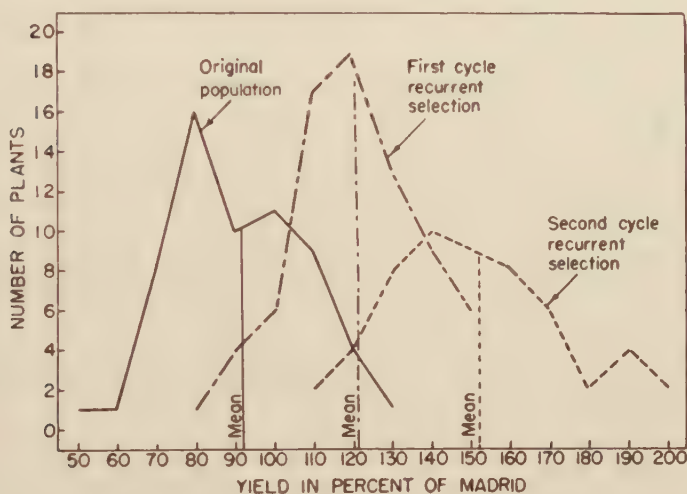


Figure 19.4 Frequency distributions for general combining ability of plants from the original population of Madrid sweet clover and from the first and second cycles of recurrent selection. (Johnson.¹²)

Craig et al. report that they have used reciprocal recurrent selection with some success to improve stocks of strawberries grown from seed.²

Résumé

The merit of recurrent selection procedures to improve populations for use as synthetic varieties or as a source of superior lines has not been fully tested. Certainly, for corn, the evidence does not consistently prove recurrent selection to be more effective than the standard selfing and selection procedures, particularly where plants in the selfing series are evaluated by top crossing. The one distinctive feature, however, of popu-

lations developed from recurrent selection for combining ability is their variability. They would appear to offer opportunities for still further advance. The final test of the value of recurrent selection programs for combining ability will come when synthetic varieties arising out of them are fully evaluated or inbred lines from later cycles of selection are used in hybrid varieties. Variations of recurrent selection are being widely used by corn breeders and are being adapted for use in other crops.

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SYNTHETIC VARIETIES



The synthetic production of an improved variety by inbreeding and cross-breeding seems a reasonable plan.

HAYES AND GARBER, 1919

THE TERM "SYNTHETIC" was used in the preceding chapter to mean the bulked product obtained from each cycle of recurrent selection; synthetics are populations of the breeding nursery. A synthetic variety is basically the same, except that it is a population that is used commercially or intended for commercial use. It is a combination of a number of inbred lines, sibbed lines, clones, or other populations of cross-pollinated crops. Features of the components are: (1) They will have been

tested for their combining ability; (2) they will be preserved for future syntheses of the variety; and (3) they will be combined so as to permit random crossing.

With few exceptions, synthetic varieties have been cross-pollinated forage crops used for hay or grazing, and have been members of the legume or grass family. Forage crop breeders have been working toward synthetic varieties, using methods that are refinements of, or improvements on, mass selection.

In the United States, interest by corn breeders in the commercial use of synthetic varieties followed the development of hybrid varieties. Synthetic varieties of corn have been proposed for areas where corn is a marginal crop and the yields are too low to justify the cost of hybrid corn seed. In other countries, however, where corn improvement is just getting under way or where programs for the production and distribution of hybrid seed in commercial quantities are not feasible, there has been some interest in synthetic varieties.

Producing a Synthetic Variety

Inbred lines are convenient to use as components of a synthetic variety because they are not difficult to maintain. Where selfing is not possible, sibbed lines may be used. Often, in the case of perennials, clones serve as the components. Occasionally, crosses that are entirely or in part derived from inbred lines, sibbed lines, or clones may be combined. It is possible that the components may be combinations of lines, clones, and crosses.

Combining ability may be determined in different ways, depending on the material. Special methods for measuring combining ability have been developed in forage crops where crosses are difficult to make artificially. General combining ability is desired more than specific combining ability because there are several components to a synthetic variety, each being required to combine well with all others. It is necessary also that the combining ability be expressed not only in the syn-1 and syn-2 but also in later generations when the synthetic variety is used commercially.

The breeder of a synthetic variety should be able to reconstitute the variety at any time. For this reason, provision should be made for the maintenance of component parts, whether they be clones, selfed or sibbed lines, or other populations.

Random crossing of the component parts of a synthetic variety is assumed, and each should contribute the same amount of seed to the next generation. Because a synthetic variety is a mixture of many genotypes, it will be subject to change from selection pressures, as was described in Chapter 16.

In the production of both a synthetic and a synthetic variety the component parts are termed syn-0. These would be the inbred or sibbed lines, the clones, or other populations. The F_1 generation from random crosses of the components is termed syn-1, the F_2 generation is syn-2, and so on. A synthetic variety is intended to be used commercially over a number of generations, usually in the syn-2, syn-3, and syn-4. As a consequence, its yield and behavior from generation to generation is an important consideration. It is not sufficient that the syn-1 be superior while succeeding generations become mediocre.

It is readily seen that a single, three-way, or double-cross hybrid could serve as a synthetic variety. In fact, they have been used in this manner as will be seen later in the discussion. It is obvious that the cost of producing seed of a synthetic variety will be much less than a hybrid variety, simply because it can be used for a number of generations. This suggests that synthetic varieties may be substituted for hybrid varieties, even of corn, in those situations where the production of hybrid seed is unfeasible or the costs too high. Or they may serve as substitutes for hybrid varieties until the programs of hybrid-seed production are developed. Actually, in marginal areas of corn production, the wide gene base of a synthetic variety may be more desirable than the more restricted gene base of a hybrid variety.

Predicting the Performance of Synthetic Varieties

Previous discussions of the Hardy-Weinberg law have pointed out that for any one locus, genetic equilibrium is established in one generation under a condition of random mating. It was also pointed out in the discussion of hybrid varieties derived from inbred lines that (1) an F_2 population would show a decrease in vigor compared with the F_1 ; (2) the F_3 and succeeding generations would resemble the F_2 in vigor; (3) crosses of appropriate F_1 hybrids would give double-cross hybrids (these could be called syn-2 populations) about equal in vigor to the F_1 ; and (4) succeeding generations of double-cross hybrids would also be reduced in vigor, but not so much as single-cross hybrids (see Table 18.2). Genetically, synthetic varieties obtained from inbred lines are an extension of double-cross hybrids.

Wright, in 1922, developed a formula to show the performance expected in F_2 from a synthetic formed from any number of inbred lines.²⁵ The formula is

$$F_2 = \bar{F}_1 - \frac{(\bar{F}_1 - \bar{P})}{n}$$

where F_2 is the expected performance of the F_2 , $\bar{F}_1$ is the average performance of the F_1 hybrids from all combinations of the inbred lines, $\bar{P}$

is the average performance of the inbred lines, and n is the number of inbred lines. In effect, this equation says that the yield in F_2 and subsequent generations will be $1/n$ less than that portion of the yield of the F_1 attributed to heterosis. The yield of the F_2 and subsequent generations may be increased, then, by (1) increasing F_1 yields, (2) increasing yields of the parental inbred lines, and (3) increasing the number of lines.

Data provided by Neal will illustrate the use of the formula in estimating yields of single, three-way and double-cross hybrids.¹³ The actual yields in F_2 (Table 20.1) support the theory in that (1) the actual and

Table 20.1 Actual and expected yields of corn obtained in F_2 populations. Adapted from Neal.¹³

NUMBER AND TYPE OF CROSSES	AVERAGE YIELD PER ACRE, bu		$\bar{F}_1 - \bar{P}$, bu per acre	YIELD PER ACRE OF F_2 , bu		PER CENT LOSS IN YIELD	
	$\bar{F}_1$	$\bar{P}$		<i>Actual</i>	<i>Expected</i>	<i>Actual</i>	<i>Expected</i>
Ten, single	62.8	23.7	39.1	44.2	43.3	47.6	50.0
Four, three-way	64.2	23.8	40.4	49.3	50.7	36.8	33.3
Ten, double	64.1	25.0	39.1	54.0	54.3	25.8	25.0

predicted yields of the various hybrid types were not greatly different, and (2) an increase in number of inbred lines from two to four increased the F_2 yields the expected amount. If Neal could have had ten lines that maintained the yields of parents and F_1 of his study, the expected yield of the F_2 would have been 94 per cent of the average of the F_1 . The problem has been to find such lines; it has been a difficult enough task to find four that nick well together to produce a good double-cross hybrid.

Kinman and Sprague obtained the yields of 10 inbred lines and all possible 45 single-cross hybrids between them.⁹ From these data they computed the yields of F_2 populations (syn-2) obtained from different numbers of inbred lines. In all cases, inbred lines were chosen which in all possible combinations gave the highest average yields in F_1 . The significant feature of their data is that the expected yield of the F_2 increased with increasing numbers of inbred lines up to five or six, and decreased as the lines increased from six to ten.

In a similar experiment with ten inbred lines of sunflower, Putt predicted the yields and oil contents of synthetics in F_2 from the yields and oil contents of the 45 possible F_1 hybrids.¹⁵ His data suggest that four inbred lines would perhaps be the optimum number of inbred lines in a synthetic, considering both yield and oil content.

In establishing a synthetic variety the plant breeder has to decide on the number of inbred lines that will give the optimum values of n , $\bar{P}$, and $\bar{F}_1$. The predicted values for F_2 performance facilitate the development of a synthetic variety; they are not a substitute for tests of its actual performance.

When a synthetic variety is produced by random intercrossing of several inbred lines, the syn-1 will be a mixture of single crosses. Any one plant in such a syn-1 will have germ plasm from only two inbred lines. Not until the production of syn-2 will a single plant have germ plasm from more than two inbred lines.

Where heterozygous clones are combined, there is no formula available to predict the performance of the synthetic. As will be seen later, the value of a clone in a synthetic is determined experimentally in a number of ways.

Synthetic Varieties of Corn

On the whole, synthetic varieties of corn have not been competitive with hybrid varieties in the United States, though their development was first proposed by Hayes and Garber in 1919.³ A later report of Hayes in 1926 indicated that five synthetics ranged from 88.3 to 116.6 per cent of the yields of the varieties from which they were developed.² Five synthetic varieties developed by Sprague and Jenkins were not significantly different from the original varieties, and they felt that synthetic varieties of corn were most useful as temporary substitutes for double crosses and as reservoirs for desirable gene combinations.¹⁶ On the other hand, however, Hayes et al. found that a synthetic variety, formed from eight inbred lines with superior combining ability in single crosses, gave yields similar to the double cross hybrid, Minhybrid 403, over a three-year period at 10 locations.⁴

Evidence on the yield of synthetics beyond syn-1 was provided by Lonnquist and McGill.¹² Four first-cycle synthetics were carried through from syn-2 to syn-5 by growing each as an isolated field of 5,000 to 10,000 plants and selecting at harvest 150 to 250 ears from the better appearing plants. The average yields of these synthetics from syn-2 through syn-5, in per cent of syn-2 yields, were 100, 108, 111, and 108, respectively. Some of the increase over syn-2 was due to the higher moisture content, a consequence of a slightly later maturity of the advanced synthetics. Rather strong selection pressure at harvest (less than 5 per cent of the plants were harvested) would also tend to maintain yields at a slightly higher level than the syn-2.

Considerable use of synthetic varieties of corn was made in Mexico, as a result of the cooperative corn improvement program of the Mexican

Government and the Rockefeller Foundation. The first synthetic varieties were made from double top crosses.²⁴ These were produced as follows: (inbred line of Jalisco 35 $\times$ Gto. 59A) $\times$ (inbred line of Gto. 59A $\times$ Jalisco 35). The variety Gto. 59A was used as the top-cross parent to evaluate inbred lines of Jalisco 35; and the variety Jalisco 35 was used as the top-cross parent for inbred lines of Gto. 59A.

A later group of synthetic varieties developed in Mexico included advanced generations of double crosses made from S_1 and S_2 inbred lines.²³ The F_1 generations were used as hybrid varieties by the more progressive farmers. The subsequent generations served as synthetic varieties to provide an improved source of seed for those farmers who were unable or unwilling to plant newly crossed seed each year.

Synthetics of Sunflowers

In producing single-cross hybrid varieties of sunflower, it has been necessary to use two inbred lines that will each produce some seed under isolation, but which will hybridize when grown together in a crossing block. Advance is a hybrid variety produced in this manner in Canada. Unfortunately, the level of crossing has been variable, and sometimes has been below 50 per cent. As an alternative to hybrid varieties, Putt has evaluated synthetics produced from four inbred lines.¹⁴ He concluded that synthetic varieties were a desirable alternative to single-cross hybrids in sunflowers where the percentage of hybrid seed produced in a crossing block is low and variable. The cost of seed of a synthetic variety was estimated to be 10 cents per pound, as compared to 25 to 40 cents per pound for seed of a single-cross hybrid.

Synthetic Varieties of Forage Crops

Sustained interest in forage crop breeding got under way after breeding techniques were well developed for self-pollinated crops, and corn breeders were turning their attention to the production of hybrid varieties. The first serious work was undertaken at Aberystwyth in Wales under the direction of R. G. Stapleton¹⁷ and, later, T. J. Jenkin,⁵ and at Saskatoon, Saskatchewan, Canada, by L. E. Kirk.¹⁰ The earliest breeding efforts, as might be expected, involved some form of mass selection, and considerable progress was made. Continued improvement required more efficient and refined methods.

Because the botanical features of forage crops are so different from those of corn, it was necessary to develop special breeding techniques and procedures. Pollination control is much more difficult in forage species because the sexes are not separated and the flowers are small. Self-incompatibility or severe inbreeding depression are often present,

which prevent or restrict the development of inbred lines. On the other hand, most forage crops are perennial, and may be adapted to clonal propagation. This has permitted breeding procedures not possible for corn.

Forage crops, particularly those used for grazing purposes, are usually grown as mixtures in dense stands, where competition is intense between and within species. With different years, locations, and at the same location in the same year, the competitive abilities of different components of a forage mixture will change. The primary consideration is forage production, but components of a forage variety must in most cases be able to produce some seed. In other words, an improved variety of a forage species has to fit into a complex and shifting situation. It is in large part for this reason that breeders of forage crops have worked toward varieties with a broad genetic base.

Strain building. This method of improving forage crops was first proposed by Jenkin.⁵ As he used the term, it meant almost any method of developing a superior strain. In its more complicated forms, as he and others employed them, it was similar in many respects to present methods of developing synthetic varieties.^{10,18}

Combining ability. Forage breeders quickly realized when they composited several lines or clones together that the combining ability of these materials would be important. The problem was how to measure it. The following tests, most of them similar in principle to those of corn, have been used:

1. *Open-pollination progeny test.* Progenies are grown from promising open-pollinated plants in the nursery without too much regard for the source of the pollen. Actually, a plant would probably be pollinated by nearby plants flowering at the same time. This would be similar to the ear-to-row method used in corn (Fig. 16.4), and is a test of general combining ability.

2. *Top-cross test.* As in corn (Chapter 18), top crosses are made by growing clones or lines in rows alternating with a tester. If top crosses are made to an inbred line or a clone, they will determine specific combining ability. On the other hand, if the top-cross parent is an open-pollinated variety or some other mixed population, general combining ability will be measured.

3. *Single-cross test.* Any one single cross will measure specific combining ability. The average combining ability of several different single crosses of one line or clone will reflect its general combining ability. Often, where plants or clones are self-incompatible, crosses are made without controlled emasculation and pollination, by growing the parents

under isolation or by growing them close together in the field and including inflorescences of both parents in the same selfing bag. The advantage of single-cross tests is that they clearly show which particular components combine well. Where single crosses are made in all possible combinations of lines or clones, they are called *diallel crosses*.

4. *Polycross test*. This test requires that all selected materials be grown together under conditions of random pollination. It was developed by forage breeders to measure general combining ability, and is described in more detail below.

In all these tests, excepting in some cases the top-cross and single-cross tests, crosses are made without emasculation. For this reason, selfing instead of crossing can occur, and this has complicated evaluations of the plant selections.

In all cases the measure of combining ability is obtained from the progeny. It must be emphasized that the progeny should be grown under conditions approximating those in commercial use. Usually, this will mean the use of close plant spacings, several replications, and enough locations to adequately sample the environment. If the new variety is to be grown in mixtures, progenies from the tests are sometimes evaluated as the component of a mixture.

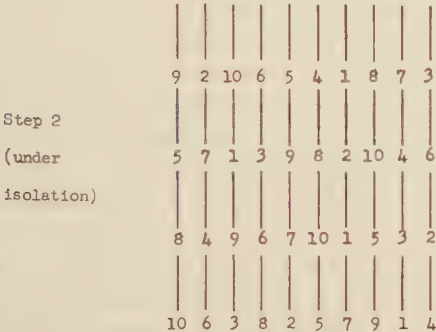
Polycross test. The polycross test was proposed independently by H. N. Frandsen in Denmark,¹ H. M. Tysdal et al. in the United States,²¹ and S. J. Wellensiek in Holland.²² It was suggested as a substitute for the top-cross test and was first referred to as the "polycross" by Tysdal et al. This test is carried out on clones, inbred lines, or other populations after they have been evaluated phenotypically (see Fig. 20.1). The test consists essentially of a *polycross nursery*, where the crosses are made, and the *polycross progeny test*, where the progenies of the crosses are evaluated.

A polycross nursery is grown under isolation and is designed to permit random pollination. To achieve this, the nursery is made up of several replicates, each one with the entries randomized, and the whole nursery is confined to as small an area as possible. Theoretically, if random pollination occurs, the seed produced on a line or clone in a polycross nursery should be the same as a composite of all single crosses of the same line or clone.

Random pollination may be prevented by different characteristics of the lines or clones with respect to (1) volume of pollen produced; (2) timing of pollen shedding; (3) differential cross compatibilities; (4) level of selfing; (5) plant height; and (6) lodging. If pollen travels only a short distance, the location of the lines or clones in the polycross nursery will affect the degree of random crossing.



Between 100 and 300 clones or inbred lines are established, evaluated over one or two years, and the best 25 to 50 selected for a polycross test.



The 25 to 50 selected clones or inbred lines are planted in a four- to ten-replicate polycross replicate randomized. The object of this planting is to obtain completely random pollination. Seed from all replicates of each clone or inbred line is composited separately and it is called polycrossed seed.



The bulked seed from each entry of the polycross nursery is included with one or more check varieties in a replicated polycross progeny test, at one or more locations. Superior polycrosses are assumed to be 2, 5, 6, 8, and 9.



Clones or inbred lines numbered 2, 5, 6, 8, and 9 from step 2 are established in a replicated crossing block. This is called syn-0. The seed from the block is harvested in bulk.



The crossed seed is increased as syn-1.

Figure 20.1 Steps in the development of a synthetic variety, using a polycross test. With perennial crops, each step may take more than one year. The synthetic variety is usually evaluated as syn-2 and syn-3 with other commercial varieties.

For each clone or line equal amounts of seed are taken from each replication and composited for the polycross progeny test. Where an entry in the polycross nursery is undesirable in appearance and pattern of development or shows susceptibility to a disease or an insect, it should not be harvested.

The polycross seed of each entry is then used to plant a replicated

yield test, often referred to as a polycross progeny test. The test may be at more than one location. Check varieties usually are included in the test also. Distinctly superior polycrosses are identified, having in mind not only yield but also disease resistance, stem strength, flowering behavior, and other characteristics important in a forage crop.

Sometimes the decision about superiority cannot be made for all materials, and perhaps half the clones or lines with the best combining ability will be included in another restricted polycross nursery and polycross progeny test. In any case, some four to ten lines or clones will be picked as superior in combining ability and will be used to constitute a synthetic variety.

Clones or inbred lines for the synthetic variety are then established in a crossing block, which is designed and handled to permit random and equal crossing of all components. Often this is achieved by several replications of each entry and by managing the development of the entries so that they all flower at the same time with the same number of flowers. Two problems present themselves immediately. First, as mentioned above for the polycross nursery, it is extremely difficult to achieve random pollination in a crossing block. Second, the combining ability of a selected clone or line may not be the same with other selected components, as it was with a large number of other entries in the polycross nursery. The materials growing in the crossing block are termed syn-0. Equal amounts of seed are harvested from each replicate of each component and bulked. Some forage breeders choose to grow the syn-0 in a greenhouse and make all possible single crosses by hand.

The next generation is called syn-1. It is grown under isolation to provide seed for extensive testing and perhaps for distribution. If sufficient seed is available, the syn-1 will be grown in yield tests in comparison with established varieties. Frequently the syn-1 shows some heterosis, particularly if inbred lines are composited to make the synthetic variety. Better evaluations of the synthetic variety will be obtained from the syn-2, which should be included in tests over a number of years under different environments. Tests will, of course, simulate as closely as possible the conditions of commercial production. If superior to other commercial varieties, the new synthetic variety will be released for commercial production.

The polycross test has been compared with other tests of combining ability by a great many forage breeders working with a great variety of crops. Opinions on its merits vary a great deal. Tysdal and Crandall felt that the polycross test not only identified lines or clones with superior general combining ability, but also provided populations that were a source of superior genotypes.²⁰ By comparing polycross, top-cross, and

single-cross tests, these authors found that out of seven clones being evaluated, the same two were identified as superior in all tests. On the average, the polycross test would be equal to other measures of general combining ability.

Components of a synthetic variety. The number of clones or inbred lines that have been composited to form a synthetic variety have usually varied between four and ten. The optimum number would be closer to ten, if all were to combine well together.

It is the responsibility of the breeder to maintain the components of a synthetic variety so that it can be resynthesized at any time. This often presents a problem, particularly where clones are involved. Sometimes the clones are maintained in a greenhouse, where there is less danger of loss.

Once a synthetic variety has been named and released, the same components are always used in subsequent releases. At least this has been the practice in the United States and Canada. Swedish and Russian plant breeders, on the other hand, add or subtract components if it will improve the performance of the variety. Opinion varies about this whole question of the merit of modification of a synthetic variety following its first release.

Comparison of syn-1 with later generations. The relationship of syn-1, syn-2, and later generations in corn is rather clear, and can be predicted to a large extent. This is not the case with forage crops, at least where synthetics have been developed from highly heterozygous clones. However, the relative performance of the different generations of a synthetic have been of concern to the forage-crop breeder because the commercial crop of a synthetic variety is mostly syn-3 and syn-4. Newly developed synthetic varieties are usually tested into syn-2 at least, and perhaps into later generations also. As a general rule, in the absence of inbreeding, yields have not dropped after the syn-2.

Kehr et al. studied forage yields of alfalfa synthetics from syn-1 through syn-4.⁸ In their studies they included 14 multiple-clone synthetics, each made up of 4 to 6 clones, and 5 two-clone synthetics. The results (Table 20.2) showed that the syn-1 had the highest yield; the greatest drop in yield occurred between the syn-1 and the syn-2; and the syn-2, syn-3, and syn-4 were not greatly different.

Inbreeding, however, can occur where a small number of clones or inbred lines are combined to form a synthetic. Knowles reported that a small degree of inbreeding reduced the vigor of syn-2 and syn-3 generations of crested wheatgrass.¹¹ He pointed out that inbreeding would be magnified if some components of the syn-0 produced more pollen than others, or if the components failed to flower simultaneously.

Table 20.2 Average adjusted three-year forage yields of multiple- and two-clone synthetics compared with check varieties.^a Yields in tons of 12 per cent moisture hay, at Lincoln, Nebraska, 1952-1954.⁸

SYNTHETIC OR VARIETY	GENERATION				AVERAGE
	<i>Syn-1</i>	<i>Syn-2</i>	<i>Syn-3</i>	<i>Syn-4</i>	
Multiple-clone ^b	4.43	4.30	4.21	4.18	4.28
Two-clone ^b	4.46	4.08	4.14	4.14	4.20
Ave. 6 check varieties	4.16	4.16	4.15	4.11	4.15

^a Yields adjusted according to the method of statistical analysis.

^b There were 14 multiple-clone and 5 two-clone synthetics.

Synthetic varieties of alfalfa. Through 1965, 21 varieties of alfalfa have been registered by the Crop Science Society of America (see page 381). Seventeen of these were released as synthetic varieties. Prominent among these are Ranger, Vernal, and Lahonton. Resistance to bacterial wilt, a widespread and destructive disease, is an important feature of most of these synthetics, as well as production and adaptation.

The development of Ranger, a synthetic variety of alfalfa, is shown in Fig. 20.2.^{7,19} Ranger was developed and released as a variety resistant to bacterial wilt. The five components of Ranger were selected, not only because of their resistance to wilt, but also because they had diverse origins and were similar in appearance. Ranger was a very successful variety for several years in the Great Plains and is still widely grown.

The Origin of the New Alfalfa Strain, A136

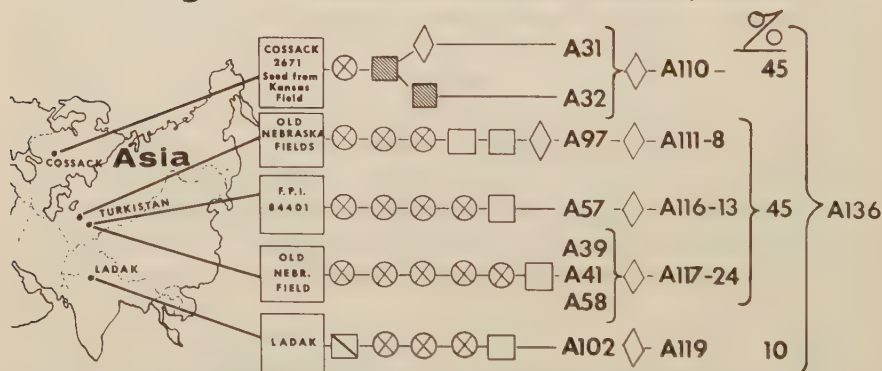


Figure 20.2 Origin, history, and breeding methods used in developing Ranger, a variety of alfalfa; ⊗ = selfed plants, susceptible eliminated; □ = strain outcrossed with many wilt-resistant lines; ▨ = strain outcrossed with one wilt-resistant line; ◇ = isolated increase. (Prior to being named Ranger, the strain was called A136.) (Tysdal.¹⁷)

Synthetic varieties of other forage crops. While mass selection still predominates in forage grass improvement, synthetic varieties have been successfully produced. Among these are Vinall Russian Wildrye, Homestead bromegrass, Lancaster smooth bromegrass, Nordan crested wheatgrass, and Ithaca timothy. Improved characters include forage yield, leafy erect plants, palatability, and seed yield.

Swedish plant breeders have developed Primus and Gloria timothy, Skandia II, and Barge orchardgrass, Viking red fescue, and Victoria perennial ryegrass.

Several synthetic varieties of both red and white clovers have been developed. The methods used are essentially those used in alfalfa and forage grasses. Lakeland and Kenland red clovers and Louisiana 51 white clover are examples of synthetic varieties.

Résumé

The term "synthetic variety" has been used in various ways. At one extreme it is nothing more than the product of mass selection, and at the other, the advanced generations of a successful double cross. It would seem desirable to limit the term to mean a variety of a cross-pollinated crop that is the product of the combination of clones, lines, or other populations which have been tested for their combining ability. Using this definition, products of mass selection, to which progeny tests are not applied would be excluded.

Synthetic varieties represent a refinement in breeding cross-pollinated crops, which logically should follow mass selection in crops where it is applicable. This has been true for forage crops, but not for corn. In the United States, synthetic varieties of corn have followed the development of hybrid varieties and have been used in or considered for areas where corn yields are too low to justify the extra cost of seed of hybrid varieties. However, where corn improvement programs are just being initiated in some countries, synthetic varieties look promising as a first step.

The polycross test plays an important role in evaluating the components of synthetic varieties of forage crops. For these crops it is performing the same role as the top cross does in measuring the general combining ability of corn.

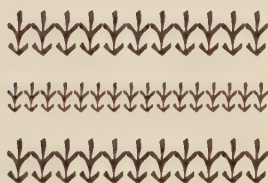
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21

AUTOPLOIDY IN PLANT BREEDING



Agriculture in its historical perspective shows a parallelism between the introduction of new crops and the greater prevalence of species with higher and higher numbers of chromosomes.

EIGSTI, 1957*

OPINIONS vary a great deal with respect to the value of autopolyploidy as a means of improving plants. On the one hand are the pessimists who point to its minor role in evolution and reason that its performance for plant breeders will be similar. Others refer to the successes that have been achieved and suggest that this is only the beginning.

* From O. J. Eigsti, *Amer. J. Botany*, 44 (1957), 272.

Polyploids

Species that have their chromosomes as multiples of a basic number (x) are polyploids. Stebbins has estimated that 30 to 35 per cent of the angiosperm species are polyploid, with the amount rising to about 70 per cent for the grasses.⁴⁹ Many of the crop species which have been developed most recently are polyploid. This includes such crops as bread wheat, tobacco, upland cotton, and many of the leading varieties of ornamental bulbs. There is voluminous literature on polyploidy in relation to the improvement of crop plants, including several reviews.^{11,12,31,35,42,49,50}

The basic number of chromosomes is often referred to as a *genome*, and where genomes differ they are often assigned a letter designation, such as the A, B, and D genomes of common wheat. Genomes are usually identified by their pairing relationships with one another. Where they differ only slightly, they may be identified by the same letter with a subscript added, as B and B₁. In the same genus, genomes may have different numbers of chromosomes; for example, safflower genomes have ten or twelve chromosomes.

Multiples of the same genome are *autoploids* or *autopolyploids*. Mostly, these occur as autotetraploids, though the chromosome number may vary from 3 to 12 times the basic number. Species with multiples of different genomes are termed *allopolyploids*, *allopolyploids*, *amphiploids*, or *amphidiploids*. Intermediates between these types have been called *segmental allopolyploids* by Stebbins.⁴⁹ Several criteria are available for deciding the type of ploidy involved. Morphologically, an autoploid will resemble the type from which it arose, whereas an allopolyploid will be intermediate between its parental species, and will resemble the hybrid between these species. The chromosomes of an autoploid will often occur in meiosis as associations of more than two, whereas in an allopolyploid they will usually be in pairs. The plant giving rise to an autoploid is fertile and that giving rise to an allopolyploid is sterile. These criteria, particularly as they distinguish between allopolyploids and segmental allopolyploids, will be discussed in more detail in the next chapter.

Induction of Polyploids

Until a satisfactory technique was available to induce polyploids easily and at will, interest in polyploidy by plant breeders was not very great. One of the earliest methods was that involving decapitation and generation of the callus tissue, either with or without the use of indoleacetic acid to stimulate regrowth. This was successfully used with only a few genera such as *Solanum* and *Tabacum*. Müntzing found heteropolyploid

plants among germinating seedlings, but at a very low frequency.³⁴ Heat treatments were used to produce tetraploids in corn.

The use of colchicine permitted the production of the vast number of polyploids that have been developed in the past 20 years. Colchicine is an alkaloid obtained from the crocus (*Colchicum autumnale*) and has been used for many years as a medicine, primarily to give relief in attacks of acute gout. For the induction of polyploids it has been used in a multitude of ways, from solutions in water or glycerine, through lanolin pastes, to agar preparations. Colchicine has been most successful when it has been applied to rapidly developing somatic tissues, which have included germinating seeds, young seedlings, and developing buds. Many ingenious methods have been devised to expose these meristematic tissues to the optimum concentration of colchicine. Roots have been susceptible to injury, so seedlings have often been inverted and only the growing point submerged in a colchicine solution while provisions have been made to keep the roots moist. For seed and seedling treatments, the concentration of colchicine in aqueous solution has usually varied from 0.1 to 0.4 per cent and the time from 30 minutes to 3 hours, though concentrations as low as 0.05 per cent have been used for 24 hours or more. After treatments, seeds or seedlings are thoroughly washed. For buds, concentrations have usually ranged from 0.2 to 0.5 per cent, and treatments have been made intermittently for 2 to 6 days. Experimentation will determine the best treatment for a particular species. Extreme care should be used in handling colchicine; it should be kept out of the eyes, and hands should be washed after contact with it, to avoid skin irritation.

The effect of colchicine has been to paralyze the spindle mechanism in mitosis, which prevents the migration of the divided chromosomes to the poles at anaphase. In other words, a restitution nucleus is formed with twice the normal number of chromosomes. Without chromosome migration to the poles, cell division does not take place. There are many opportunities for chimeras to occur, where only portions of the growing tissue are doubled. Indeed, in such chimeras, if the diploid ($2n$) tissue is more aggressive in development, the doubled tissue may never develop sufficiently to be apparent. Polyploidy may be confined only to the epidermis or only to the internal tissues¹⁰ (see Fig. 8.2). Where polyploids are identified by the appearance of the epidermal cells, it is readily apparent that the actual chromosome numbers of the progeny of treated plants may not agree with those predicted, since sporophytic cells develop from internal tissue.

Other chemicals have been used to induce polyploids, but not so successfully as colchicine. These include chloral hydrate, ether, chloroform,

acenaphthene, phenylurethane, nitrous oxide, and several other compounds.

Some attention among corn geneticists has been given to the genetic induction of autopoloidy. Beadle found that plants homozygous for the recessive asynaptic gene, *as*, produced eggs with the diploid number of chromosomes, which resulted in the production of autopoloids.³ The recessive elongate gene *el*, when homozygous, was found by Rhoades to produce varying numbers of diploid eggs.⁴⁴ Alexander has proposed a method for producing autotetraploid synthetic varieties by genetic means, using the elongate gene and established tetraploid stocks.²

Effects of Autopoloidy

Inasmuch as autopoloidy has not had a profound effect upon the evolution of a species, it is perhaps not surprising to learn that it often does not have a striking effect on the morphology and physiology of a plant. Almost without exception, autopoloidy increases cell sizes, at least of meristematic tissues. Reliable indications of polyploidy among plants treated with colchicine is the size of the stomatal cells and pollen grains (Fig. 21.1). Larger cell sizes, however, have not necessarily led to larger plants. Where cell size is affected, the proportion of different substances such as water, protein, chlorophyll, cellulose, auxins, and vitamins in the cell will also be affected.

Leaf thickness has usually been greater in autopoloids because of its direct relationship to cell size. Leaves have often been shorter and broader also. Plant organs may show some increase in size, termed *gigas* characteristics. These organs usually have a determinate type of growth, and may include sepals, petals, anthers, fruits, and seeds.

Chemical composition of plant parts has been affected by polyploidy. Vitamin A activity in tetraploid corn is approximately 40 per cent more than in the diploid.⁴³ Vitamin C has been higher in many vegetables and fruits following chromosome doubling. Tetraploid tobacco has contained 18 to 33 per cent more nicotine than the diploid. In perennial ryegrass (*Lolium perenne*) the tetraploid has had more sugar and dry matter.⁵¹ Peto and Boyes found sugar content of sugar beets to decrease more with increases in root size in the diploid than in the triploid.⁴⁰ Chlorophyll content of a number of genera was not changed consistently by doubling the number of chromosomes.⁴⁷

In general, the growth rate of polyploids is less than that of diploids. Where comparisons have been made, tetraploids have had a lower auxin content, and this may explain their slower growth. They flower later and over a longer period of time. Branching, or tillering in grasses, has often been reduced by autopoloidy.

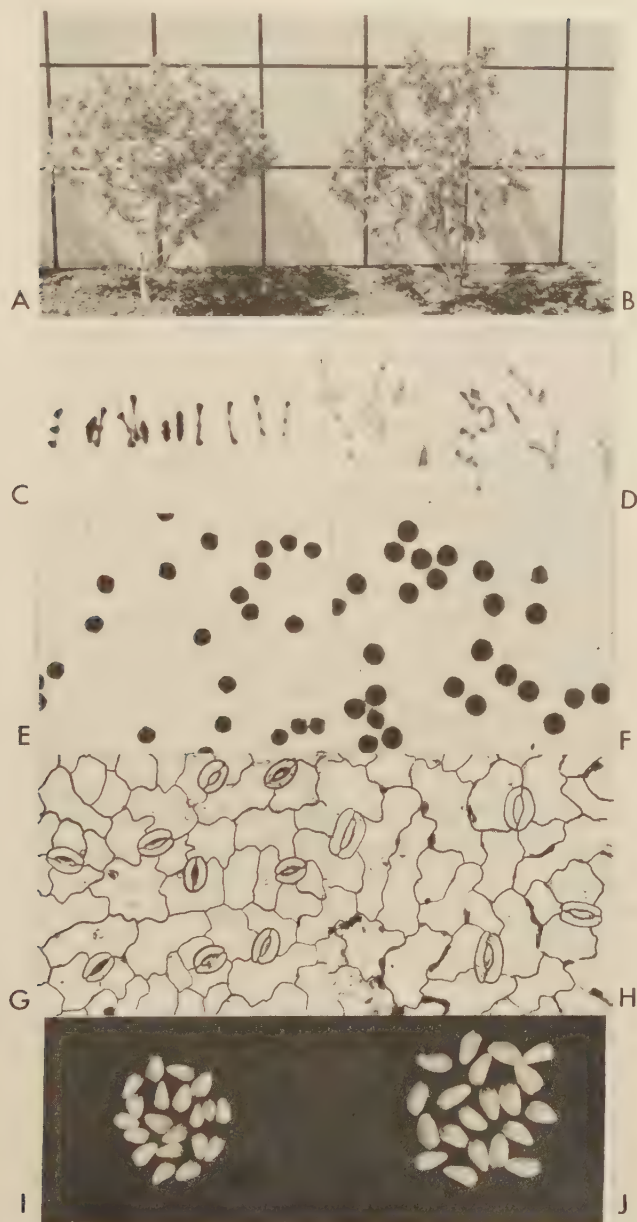


Figure 21.1 Comparison of diploid, left, and tetraploid, right, in safflower (*Carthamus tinctorius*). *A* and *B*—plants near maturity (the lines of the grid are 12 in. apart); *C* and *D*—PMCs in late meiosis, diploid with 12 pairs of chromosomes, tetraploid with six quadrivalents and 12 bivalents ($\times 630$); *E* and *F*—pollen ($\times 55$); *G* and *H*—epidermal peels, with outlines of cells inked in ($\times 210$); *I* and *J*—seeds (achenes). (Schank and Knowles.⁴⁵)

Autoploids may have ecological requirements different from those of the diploid. Seed of the triploid watermelon has a higher heat requirement for germination than does the diploid. Doubling of the chromosome number of rye changed the photoperiod requirement so that tetraploid plants were female-sterile and set no seed when grown under natural day lengths in the winter.³⁷ When given supplemental illumination to provide a 17-hour day, seed was produced. The diploid produced seed at both day lengths. Usually, comparisons of diploids and tetraploids have been made under the environment and cultural conditions of the diploid, and this has favored the diploid.

One general feature of autoploids, and one that has seriously limited their utilization in agriculture, has been their poor pollen production and their sterility. Fertility may be reduced very slightly to 80 to 95 per cent of the diploid, as in some tetraploid varieties of maize,⁴² or to almost complete sterility, as in cotton.⁴ At one time this sterility was attributed to irregularities in chromosome behavior at meiosis, but genic imbalance following chromosome doubling is now believed to be the major factor. This imbalance leads to disharmonies in development. In lettuce, as an example, it was found that part of the sterility was due to the failure of the pollen tube to complete development.^{13,14} Parthasarathy reported that in *Brassica campestris* var. *toria*, prefertilization abnormalities in the embryo sac and failure of fertilization accounted for much of the sterility.³⁸ In autotetraploid rye, degeneration of the endosperm (and less often, of the embryo) is largely responsible for sterility.¹⁹

Self-incompatibility systems in diploid species are often weakened, or may disappear, at the polyploid level. This is particularly true of the gametophytic type. Where the incompatibility system continues to function, it will jeopardize the survival of rare polyploids that do occur. This is discussed in more detail in Chapter 15.

It is important to realize that one cannot always predict the effects of autopolyploidy on plant appearance or behavior. The response may be different, not only with different species but with different varieties within a species.

Success with Autoploids in Plant Breeding

The first artificially induced autotetraploid in crop plants was achieved in 1916 when Winkler doubled the chromosome number of various species of *Solanum*.⁵² It is something of a contradiction to note that whereas allopolyploidy has been much more important than autopolyploidy in the development of domesticated crops, the latter has been more important than the former in their improvement over the past 30 years. Eigsti and

Dunstin¹² have given details of the use of autopolyploids in agriculture, and Stebbins^{49,50} has summarized their role in plant breeding. Examples given here are some, but by no means all, of the autopolyploids that have been successful. Details may be obtained from reviews.^{33,38,41}

In crops grown for seed. Since autopolyploidy has reduced fertility, it might be expected that it would not have an important role in the improvement of crops grown for seed. In general, this has been true, for of the vast number of autopolyploids produced, very few have been successful. In wheat, barley, corn, and flax, autopolyploids have been disappointing in spite of many years of work. Low fertility has been the main problem.

Tetraploid rye is the best example of success through autopolyploidy in a seed crop. After several years of work in Sweden and in other European countries, tetraploid varieties of rye have been released for commercial production. One of these varieties, Fourex, has been successfully grown in California, but primarily as a cover crop. While the sterility of the tetraploid varieties appears to be more than in the diploids, the larger size of the seed more than compensates for this shortcoming. With the larger size of seed has been associated superior sprouting ability and higher protein content. Tillering has been reduced, and straw length increased.

In crops grown for forage. Orchard grass and alfalfa are examples of natural autopolyploids that are successful crops. Timothy (*Phleum pratense*) appears to be an autohexaploid, originating from *P. nodosum*. In Sweden, greatest success with artificial autotetraploids has been achieved with alsike clover (*Trifolium hybridum*) and with red clover (*T. pratense*). The tetraploids have been more productive vegetatively, and have grown more rapidly in the second year. Their great disadvantage has been reduced seed production.²³ One reason for the reduced seed production in tetraploid red clover is the large flowers, which are unattractive to honeybees.¹⁸ While bumblebees are effective pollinators, they are often scarce.

In crops grown for roots. After a great deal of research, it is now apparent that triploid sugar beets are a commercial success. In both Europe and Japan^{24,30} they are becoming increasingly more important. Triploids have a larger root than the diploid, have maintained the sugar content of the diploid, and hence have yielded more sugar per acre. They are produced by interplanting diploids and tetraploids, and harvesting seed from the tetraploids. An alternative method is to plant the seed field to a mixture of 75 per cent tetraploid and 25 per cent diploid seed. The harvested seed, which is used for commercial production, is 40 per cent diploid, 50 per cent triploid, and 10 per cent tetraploid; such varieties are called "polybeets".¹⁸ Autotetraploids have smaller roots and yield

less than the diploids. Autotetraploid strains of turnips (*Brassica campestris rapifera*) have been outstanding in Sweden.

In fruit crops. Striking success has been achieved with autopolyploids of fruit crops. Gros Michel, at one time an important banana variety, is a triploid with 33 chromosomes. Its vigor and seedless characteristics have been distinct commercial assets. The same would appear in part to be true of tetraploid grapes;³⁶ they have a larger berry with fewer seeds, but the clusters have less berries, and the canes are brittle and less abundant (Fig. 2.3). Triploid watermelons have been grown commercially in Japan,²⁴ and it appears that their culture will develop further in other countries. Their production is similar to that of triploids in sugar beets, with the production of the triploid seed on tetraploid plants. Experience in Japan and in the United States indicates that the performance of the triploid is unpredictable and not related to either the diploid or tetraploid.¹⁷ The main advantage of the triploid has been the absence of developed seed; the undeveloped seeds are no more objectionable than the seeds of cucumber. Though most varieties of apples are diploid ($2n = 34$), triploid varieties have better keeping quality and on the average have a higher content of ascorbic acid.

In ornamental plants. By far the greatest success with autopolyploidy has been achieved with ornamental plants, particularly where they are propagated vegetatively. Spontaneous autopolyploids, valuable because of their gigas characteristics, may be found among narcissus, tulips, hyacinths, gladiolus, dahlia, cyclamen, and chrysanthemum. By way of example, most narcissus varieties until 1885 were small diploid types. These gave way to the larger triploid forms, and in turn these yielded to the tetraploid types, which are still larger. Experimental tetraploid Easter lilies look promising.¹⁵ Among ornamentals reproduced by seed, snapdragons and marigolds have been successful as tetraploids.

Cytological Behavior of Autopolyploids

The introduction of more than two homologous chromosomes into the meiotic process means that during prophase and metaphase there will be multivalents instead of only bivalents present. In an autotriploid, most of the chromosomes will appear as trivalents, with some bivalents and univalents. Similarly in autotetraploids, the associations will be quadrivalents or bivalents, with some trivalents and univalents. The proportions of the different types will depend upon the species and varieties involved. Among tetraploids there does not appear to be a consistent or important relationship between the number of multivalents and fertility. Randolph estimated that, in corn, irregularities in chromosome behavior during meiosis accounted for only 5 to 15 per cent of the

sterility.⁴² Among triploids, however, much of the sterility appears to have a cytological basis. In triploids the chromosome numbers of micro- and megaspores will vary from x to $2x$, with most of those with intermediate numbers being inviable. Micro- and megaspores that are viable will usually have one or two complete genomes. Occasionally those with $x + 1$ and $2x - 1$, or even $x + 2$ and $2x - 2$, may be viable.

The amount of pairing will have a direct bearing upon the breeding behavior of an autopoloid. If the chromosomes of a hybrid between two diploid varieties are doubled, there may be a tendency for the doubled set obtained from one variety to pair independently of the doubled set from the other variety. This is called *preferential*, or *selective*, *pairing*. With complete preferential pairing, no new recombinations of chromosomes or genes could occur; the progeny would all resemble the doubled F_1 . With bivalent pairing prevailing at meiosis, no sterility should arise from this source. If the intervarietal diploid hybrid showed heterosis, and it was maintained in the tetraploid, complete preferential pairing should preserve the heterosis indefinitely. Actually, the effect of a high level of preferential pairing is to make the autopoloid behave like an allopoloid.

Geneticists and plant breeders are now giving some attention to a technique of increasing preferential pairing, called *diploidization*. The possible role of mutagens for this purpose is described in Chapter 24.

Genetics of Autopoloids

The genetics of autopoloids is complicated by each allele being present more than twice and by the presence of multivalent associations of chromosomes at meiosis. In the diploid, there are three genotypes possible when two alleles are present, but with the same two alleles in an autotetraploid, five genotypes may be obtained. Considering the alleles A and a , these genotypes are

$AAAA$	quadruplex
$AAAa$	triplex
$AAaa$	duplex
$Aaaa$	simplex
$aaaa$	nulliplex

The proportion of dominant to recessive genes is altered in two of these genotypes, the triplex and simplex, from the situation in the diploid. The effect of this on the phenotypes will, of course, depend upon the dominance relationships of A and a . If A is completely dominant, only two phenotypes will be possible, but if it is incompletely dominant or the effect of A is cumulative, there may be up to five phenotypes. In

diploid *Primula* the gene *B* for magenta flower color is completely dominant to the gene *b* for red color, but in the tetraploid the genotypes *BBBB*, *BBBb*, and *BBbb*, as expected, are red, but the genotype *Bbbb* has colors varying from magenta to red.⁴⁸

Theoretical genetic ratios expected from autopolids have been computed for the tetraploid (Table 21.1). Assuming that a gene is close to

Table 21.1 Frequency of phenotypes obtained in selfed and test-cross progenies of tetraploids with chromosome and chromatid segregation (assuming a dominant gene *A* and its recessive allele *a*).

GENOTYPE	RANDOM CHROMOSOME SEGREGATION		RANDOM CHROMATID SEGREGATION	
	<i>Selfed</i>	<i>Test Cross</i>	<i>Selfed</i>	<i>Test Cross</i>
<i>AAAA</i>	1:0	1:0	1:0	1:0
<i>AAAa</i>	1:0	1:0	783:1	27:1
<i>AAaa</i>	35:1	5:1	21:1	3.7:1
<i>Aaaa</i>	3:1	1:1	2.48:1	13:15
<i>aaaa</i>	0:1	0:1	0:1	0:1

the centromere, so close that no chiasma occurs between them, Muller has computed the ratios that would be expected from different genotypes;³² this is called the *chromosome theory of inheritance*. This theory assumes that each chromosome has an equal chance of pairing with the three other homologous chromosomes, and it makes no difference whether they associate as bivalents or quadrivalents. If *A* is assumed to be completely dominant to *a*, the gametes of the hybrid *AAaa* between genotypes *AAAA* and *aaaa* will be in the following ratio: *1AA:4Aa:1aa*. Their combinations will be as follows:

	<i>1AA</i>	<i>4Aa</i>	<i>1aa</i>
<i>1AA</i>	<i>1AAAA</i>	<i>4AAAa</i>	<i>1AAaa</i>
<i>4Aa</i>	<i>4AAAa</i>	<i>16AAaa</i>	<i>4Aaaa</i>
<i>1aa</i>	<i>1AAaa</i>	<i>4Aaaa</i>	<i>1aaaa</i>

The frequency of the genotypes in the F_2 will be *1AAAA:8AAAa:18AAaa:8Aaaa:1aaaa*, and the frequency of the phenotypes *35A—:1aaaa*. Considering the example in Fig. 21.2, only the gametes *AA* and *Aa* can occur, and only one phenotype in the progeny, if *A* is completely dominant to *a*.

The *chromatid theory of inheritance* assumes that a chiasma occurs between the locus of the gene in question and the centromere, so that each chromatid in the region of the gene has the same chance as any other of being present in a microspore or megaspore.²⁰ It further assumes in autotetraploids that the chromosomes associate as quadrivalents in meiosis. If the genotype of a duplex hybrid is assumed to be $BBBBbbbb$, the genotypes of the gametes will be in the following proportions: $6BB:16Bb:6bb$ (or $3BB:8Bb:3bb$). Their combinations will be as follows:

	$3BB$	$8Bb$	$3bb$
$3BB$	$9BBBB$	$24BBBBb$	$9BBbb$
$8Bb$	$24BBBb$	$64BBbb$	$24Bbbb$
$3bb$	$9BBbb$	$24Bbbb$	$9bbbb$

The frequency of the genotypes will be $9BBBB:48BBBBb:82BBbb:48Bbbb:9bbbb$, and of the phenotypes $187B\text{---}:9bbbb$ (often expressed as $21B\text{---}:1bbbb$). With chromatid inheritance, a triplex plant (Fig. 21.2 and Table 21.1) will yield a small number of recessive plants, which will not be possible with chromosome inheritance. In contrast to the tetraploid, chiasmata have no effect on the ratios obtained for a single locus in a diploid.

Mather has pointed out that chromosome segregation and chromatid segregation are limiting types and that the expected segregations should

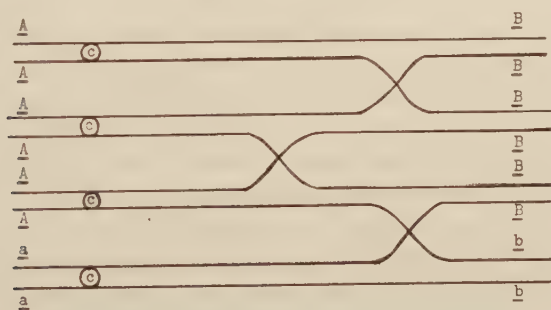


Figure 21.2 Quadrivalent at first metaphase in a triplex plant. The alleles A and a , close to the centromere ©, move with it at first anaphase, and as a result, they exhibit chromosome inheritance. Because of the chiasmata between the centromere and the locus of B and b , the latter show chromatid inheritance.

be intermediate, their values depending upon the frequency of quadrivalent formation and the amount of equational separation of the chromatids.²⁹ Little has reviewed the theories of gene segregation in some detail.^{26,27} His examinations of the data on segregations in tetraploids indicate that they supported a chromatid type of inheritance, provided quadrivalents were formed in meiosis. Fasoulas has given simplified formulas for gene segregations expected in autotetraploids.¹⁶

From Table 21.1 it is obvious that a triplex plant cannot be distinguished from a quadruplex on the basis of a progeny test (S_1), assuming random chromosome segregation; both will breed true for the dominant condition. To identify a triplex plant, it will be necessary to carry the progeny one additional generation (S_2) to identify duplex plants of the S_1 . Even assuming chromatid inheritance, the frequency of recessives is so low that it is doubtful they would occur in the progenies of triplex plants that would be normally grown.

The problem of identifying a triplex plant indicates the difficulty in achieving genetic purity in tetraploid stocks, if a gene is completely dominant. Deleterious recessive genes could persist in a tetraploid and manifest themselves only rarely in the homozygous condition. Progeny tests would have to be carried two generations to identify the homozygous dominant genotype with confidence.

Reversion to the Diploid Condition

Diploid types may appear in populations of induced tetraploids. In corn, this has been a consequence of the parthenogenic development of the unfertilized female gamete, and appears in 1 out of about 1,000 plants.⁴² These plants are completely self-fertile but cross-sterile, like other diploids, with the tetraploid forms. In sorghum, Chen and Ross found 5 in 1,088 plants.⁹ Paradoxically, they found that colchicine treatments of tetraploid seedlings increased the incidence of diploids, presumably due to somatic chromosome reduction.

What is the fate of diploids that appear in tetraploid populations spontaneously or through mixing? In self-pollinating crops, their survival, as expected, is related to their competitive vigor. In most raw polyploids the diploid would be more productive, and would replace the tetraploid rather rapidly. In cross-pollinating species the situation is quite different. Experience in Sweden with tetraploid rye and other cross-pollinated crops, where the triploids die at an embryonic stage, indicates that there is a threshold value for diploids, below which the diploids will disappear and above which they will eliminate the tetraploids.¹⁸ This threshold value for diploids is different in each crop. In rye, it is between 30 and 40 per cent, and in red clover, between 2 and 3 per cent.

Breeding Procedures with Autoploids

Breeding procedures with induced autoploids are directed toward two important objectives: (1) the achievement of a genotype or combination of genotypes superior to the best diploid in some important character; and (2) the reduction of sterility, at least in seed crops. Tetraploids have been of most interest to plant breeders, and thus will get major attention here.

Doubling of a diploid species will be more successful than the doubling of a species that is already a polyploid. In cultivated wheat and oats, with 21 pairs of chromosomes, doubling of the chromosome number has led to dwarfed, nonvigorous types that are sterile. In rye and barley, with seven pairs of chromosomes, the tetraploid has compared favorably with the diploid. In potatoes, Johnstone found that the cultivated form, with 24 pairs of chromosomes, was depressed greatly in vigor, size, and fertility by doubling, whereas doubled wild diploid species were as vigorous or more vigorous than the corresponding diploid.²² The upper limit to ploidy appears to vary with different genera.

Since the value of a polyploid cannot be predicted from the behavior of a diploid, a large number of different diploid genotypes, or varieties, should be used in any program of doubling chromosome numbers. This will permit the doubling of the largest number of different alleles. This is particularly important where the diploid is self-incompatible due to *S* alleles, since this will increase the number of these alleles in the tetraploid population.

The first generation, or "raw" polyploid, is likely to be inferior to the diploid, at least under the environment of the diploid. This raw polyploid is just the beginning of the breeding program, not the end. Because the environmental requirements of the autoploid may be different from those for the diploid, its full value may not be realized until it is tested under a variety of growing conditions.

Hybridization and selection at the polyploid level will usually increase fertility and improve the performance of polyploids. Hybridization permits new combinations of genes, and rigid selection will preserve only the more desirable genotypes. Both should be practiced over several generations. This improvement as a consequence of hybridization is one reason that cross-pollinated species have responded more successfully to autoploidy than have self-pollinated species. It has been self-pollinated tetraploid plants, such as chili, sesame, gram, and tomatoes, which have not responded to selection. In other self-pollinated plants, however, the situation has been better; five to six generations of selection have raised fertility in some lines of flax to 80 per cent of the diploid.⁴⁶ The fer-

tility of a tetraploid population of *Brassica campestris* var. *toria* has been improved by the mass-pedigree system of breeding.³⁹

Where autopoloids reproduce sexually, their propagation may require isolation from the diploid. Crosses of tetraploids to the diploid may yield triploids, and these may have economic value, as in sugar beets and watermelons. More often, the cross will yield an aborted zygote. Tetraploid rye under isolation may have a seed set up to 60 per cent, but when exposed to diploid pollen, the seed set is severely reduced. In comparing diploid and tetraploid rye for yielding ability, it has been necessary to grow them in separate tests, but with a variety of another crop such as wheat common to both tests.

Haploidy

A haploid, or monoploid, has only one genome present, so that each gene is present once; that is, all genes are *hemizygous*. In the lower plant organisms, haploidy may be the normal state; such is the case in the gametophyte of mosses and the mycelium and spores of fungi. In crop plants, however, haploidy is an abnormal state, except in the inconspicuous and parasitic gametophytes—the pollen grain, the pollen tube, and the embryo sac. Kimber and Riley have reviewed haploids in angiosperms.²⁵

The haploid of an autopoloid is often called an *autohaploid* (or *autopolyhaploid*) and is similar to a diploid. For an allopoloid, it is called an *allohaploid* (or *allopolyhaploid*). Remarks here apply primarily to a haploid with one genome.

A haploid plant is often smaller than the diploid (Fig. 21.3). It is usually sterile, a consequence of a disturbed meiosis. Where all chromosomes migrate to one pole in the first meiotic division, haploid gametes are produced, and all such gametes have exactly the same genic composition. Their union gives rise to a plant homozygous for all its genes.

This ability of haploids to produce homozygous and pure-breeding plants suggests their value in plant breeding.⁷ If it were possible to produce haploids at will, and the haploid chromosome number doubled with colchicine, it would be possible to achieve pure-breeding lines without several years of inbreeding.

Résumé

An excellent summary of the role of autopoloidy in plant breeding is given by Hagberg and Åkerberg;¹⁸ it is repeated here.

In summarizing the possibilities of polyploidy breeding—only autopolyploids are concerned here—we can state that a favourable view of their pros-

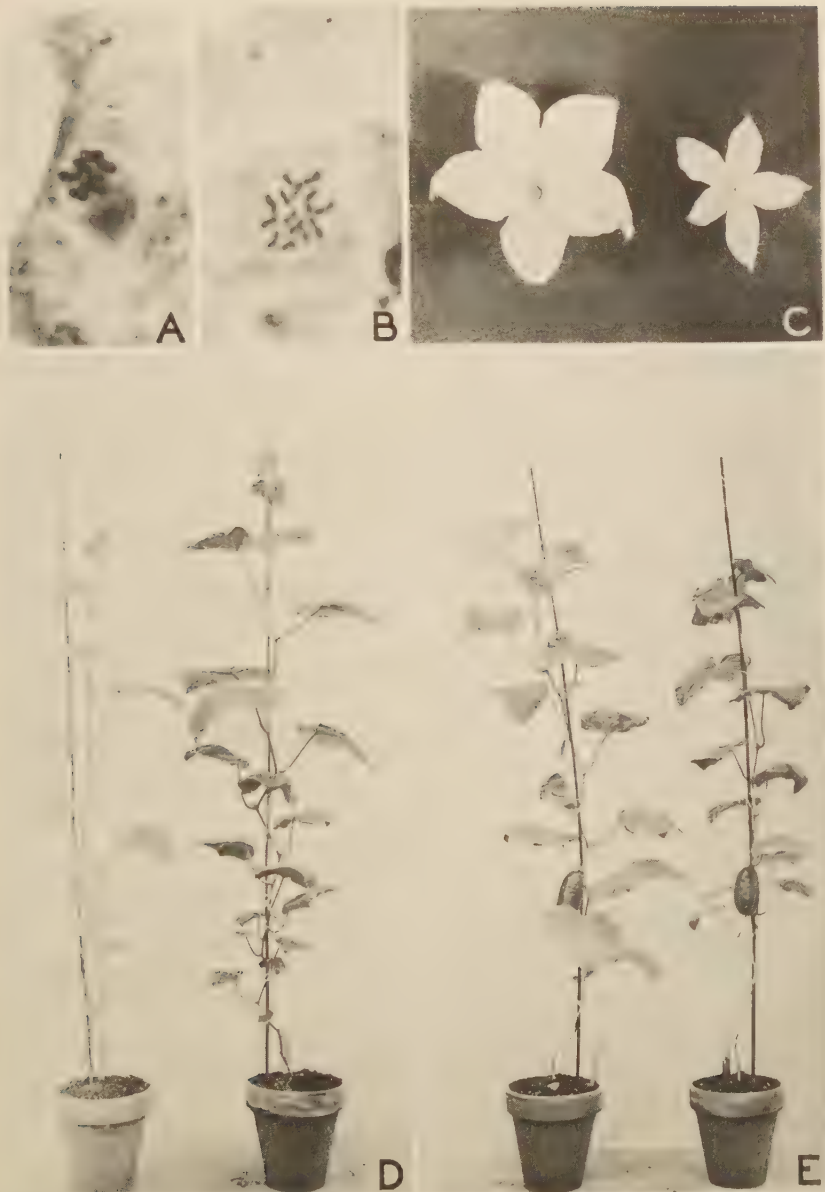


Figure 21.3 *A*, metaphase of monoploid plant of cucumber (*Cucumis sativus*), showing seven chromosomes. *B*, metaphase of diploid plant showing 14 chromosomes. *C*, male flower of diploid plant (left) and monoploid plant (right). *D*, monoploid plants. *E*, diploid plants. (From L. E. Aalders, *J. Hered.*, 49 (1958), 40.)

pects may be taken if, among other things, the following three conditions are fulfilled:

a) The original material must have a relatively low chromosome number—the doubled number must not exceed the so-called optimum chromosome number for the genus.

b) The original material must be a cross-fertilizing population so that new combinations at tetraploid level can be easily realized and heterozygosity effects be utilized.

c) The development of the vegetative parts must be the essential factor in our assessment of the plant's yielding ability as, for instance, in root crops and grassland plants in which seed production is of course not unessential but of comparatively small importance. An exception to this is tetraploid rye, the grain yield of which can well compete with that of the diploid in spite of the fertility being somewhat lower.

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ALLOPLOIDY



Ployploidy will help us the most if our objective is to produce a crop plant with entirely new properties or to extend the area of cultivation of a species into a region where it previously has not been successful. In general, we cannot expect by ployploidy to make marked improvements in yield or quality of old, established, highly selected crops, without accompanying disadvantages.

STEBBINS, 1956

THE MOST IMPORTANT contribution of alloploidy, or amphiploidy, to the improvement of crop plants is in the past—and in the future. This is simply because time, often a great deal of time, is necessary to mold

the "raw" allopolyploids into something better than the parental species. Much of the research endeavor on allopolyploids has been in terms of re-synthesis of allopolyploids, efforts at enlightenment into the origins of many of our crop plants. These studies have provided guide lines for the synthesis of new types in the future. They have also indicated procedures to follow into the systematic transfer of genes from one species to another.

By definition, an allopolyploid is the plant derived from the combination and doubling of genomes that are different. The difference, however, will be one of degree; different genomes can show nearly perfect homology, or they can fail to pair. Müntzing¹⁵ has preferred to make no classification based on these differences, but Stebbins²² has narrowed the term to include only those cases where the combined genomes are so different that they fail to pair or form only a small number of loosely paired bivalents. For those allopolyploids ranging between autopolyploids and allopolyploids with very different genomes, Stebbins has used the term *segmental allopolyploid*, implying that only segments of the chromosomes in the component genomes are different. Unless otherwise stated, the discussion here will refer to allopolyploids as defined by Stebbins.

Production of Allopolyploids

Allopolyploids have been produced most often by doubling the chromosome number of F_1 hybrids between species. They have also been obtained by crossing autotetraploids developed from the parental species. The two methods yield essentially the same type of allopolyploid. Doubling the chromosomes of an F_1 , of course, will yield an allopolyploid that has two identical homologs of each chromosome; with normal chromosome behavior the allopolyploid will breed true. This will be the case also if the parents are homozygous autopolyploids and gametes have the polyploid chromosome number. However, if the autopolyploid parents are not homozygous and produce heterozygous gametes, the resulting allopolyploid will be heterozygous and will not breed true. If the autopolyploid parents produce gametes deficient in, or with extra, chromosomes, this will lead to variability among allopolyploids and their progeny.

Because the chromosome number of a sterile F_1 hybrid has been doubled, there is no guarantee that the resulting allopolyploid will be fertile. Greenleaf⁵ and Clausen³ reported that allopolyploids developed from *Nicotiana glauca* and four strains of *N. tomentosus* varied in female fertility; two were completely sterile, and fertility in the other two was 1 per cent or less. The pollen of all four allopolyploids was functional on *N. glauca*. In both microsporocytes and megasporocytes of the allopolyploids the chromosome pairing was normal, but the embryo sac rarely advanced beyond

the 2- or 4-nucleate stage. But the cross of *N. tabacum* to the *N. sylvestris-tomentosa* hybrid gave an F_1 with 15 per cent of the ovules fertile. It was concluded that complementary genes for sterility were involved and that the chemical-physiological processes of each species were different and conflicting in their allopolyploid. The variability in the morphology and fertility of an allopolyploid, depending on the choice of varieties of the component species, suggests that several races or varieties of each species should be used.

The value of an allopolyploid is not necessarily related to the value of the species entering into its combination. That is, genomes may interact in combination to yield a product more valuable than either genome alone. This has been true in wheat, tobacco, cotton, and other crop plants.

The value of an allopolyploid will be measured fairly accurately by the appearance of the undoubled hybrid between the component species. If it is vigorous and adjusted to its environment, the allopolyploid produced from it will be similar. Greater success may be expected if the hybrid is completely sterile and if the parental genomes do not pair to any extent. Failure to pair in meiosis may not necessarily be due to great differences between chromosomes; it may be a consequence of physiological disturbances due to genes.

Most allopolyploids are tetraploids or hexaploids and combine the genomes of two or three diploid species. As many as seven different genomes have been present in one allopolyploid species.²¹ The latter was obtained from doubling the chromosome number of the hybrid *Bromus carinatus* $\times$ *B. tritii*, the former with 56 chromosomes ($2n$) and the latter with 42. The allopolyploid formed 49 bivalents or 47 bivalents and one quadrivalent at meiosis, and was fertile and vigorous.

Examples of Allopolyploids

Cereal crops. The classical example of allopolyploidy in crop plants is provided by wheat. While the details and locations will never be known definitely, cultivated wheat appears to have evolved in the area from the southern Caucasus through eastern Turkey to northern Syria and northern Iraq.^{12,17,19}

Einkorn (*Triticum monococcum*) developed from *T. aegilopoides* by selection of types better adapted to cultivation. The date is unknown, but it probably occurred about 7,000 B.C., when the first crops were domesticated. Einkorn is a diploid with 7 pairs of chromosomes; it has the genomic formula AA.

The tetraploid emmer wheat (*Triticum dicoccum*) was formed from a cross of einkorn to a wild grass species, also with 7 pairs of chromosomes, which probably was *Aegilops speltoides*,^{17,18} but may have been an

Agropyron species.^{13,14} It is also quite possible that the wild allotetraploid, *T. dicoccoides*, was formed prior to domestication from a cross of *T. aegilopoides* and *A. speltoides*, and that *T. dicoccoides* gave rise to *T. dicoccum* by Mendelian variation.¹⁶ By 4,000 B.C., emmer was grown from southern Iraq through Asia Minor to the Rhine River of Europe. The other tetraploid wheat species developed from emmer. All have been given the genomic formula AABB.

Wheat species with 21 pairs of chromosomes originated from crosses of cultivated tetraploid species to *Aegilops squarrosa*, a wild diploid species with the genomic formula DD, probably about 2,000 to 3,000 B.C. Chromosome numbers doubled following the cross to give allohexaploids with the genomic formula AABBDD. Important species with 21 pairs of chromosomes are common wheat (*Triticum aestivum*) and club wheat (*T. compactum*).

The ploidy of oats resembles that of wheat, with seven chromosomes in each genome, and with species at the diploid, tetraploid and hexaploid levels. Most of the species of economic importance are hexaploid; they include common oats (*Avena sativa*), red oats (*A. byzantina*), and naked oats (*A. nuda*). Two wild species, *A. fatua* and *A. sterilis*, are hexaploid, a contrast to wheat, in which no hexaploid species is wild.

In contrast to wheat and oats, the cultivated species of barley are diploid. There are several wild species at both the diploid and tetraploid levels.

Fruit crops. There are numerous examples of successful allopolyploids among the small fruits.⁴ The genus *Rubus* includes a bewildering array of species, all with chromosomes in multiples of 7, and ploidy up to 12 times the basic number. It contains the raspberries, mostly diploid; blackberries and dewberries, mostly diploid or with ploidy at six or above; nespberries, loganberries, and boysenberries. The nespberry (4x) arose from a cross of *Rubus trivialis* (2x) and *R. strigosus* (2x), the former the southern dewberry and the latter the American red raspberry. It resembles a strong growing raspberry, but the drupelets adhere to the core as in the dewberry, and the core to the calyx as in the raspberry; it is difficult to pick. The loganberry (*R. loganbaccus*) (6x) arose from a hybrid between the native dewberry (*R. ursinus*) (8x) and the European raspberry (*R. idaeus*) (2x), where the unreduced gamete of the latter combined with the reduced gamete of the former. The loganberry was crossed in turn to the eastern dewberry, to produce the youngberry with the same chromosome number as the loganberry but not fertile with it in crosses. The boysenberry appears to be related to the loganberry, but its origin is unknown. Strawberries are in multiples of seven, with wild species as diploids, hexaploids, and octaploids, and the domestic species an octa-

ploid. Polyploidy has been involved in the development of blueberry (*Vaccinium*) species, there being seven or eight diploid species, seven or eight tetraploid species, and three hexaploid species. Of the three recognized species of cranberry, two are diploid and one tetraploid.

Vegetable crops. A hybrid between radish (*Raphanus sativus*) and cabbage (*Brassica oleracea*), both diploids ($n = 9$), was first produced by Sageret in 1826, but the first allopoloid from the hybrid was achieved by Karpechenko.^{8,9} It resulted from the fusion of diploid gametes in the F_1 hybrid. Nagaharu U showed that *Brassica napus* (kohlrabi, swede, or rape), with 19 pairs of chromosomes, is an allopoloid developed from a cross between *B. campestris* ($n = 10$), a species of rape, and *B. oleracea* ($n = 9$).²⁴ It probably arose repeatedly, since the distributions of the diploid species overlap. The relationships of other polyploid species of *Brassica* are shown in Fig. 22.1.²⁴

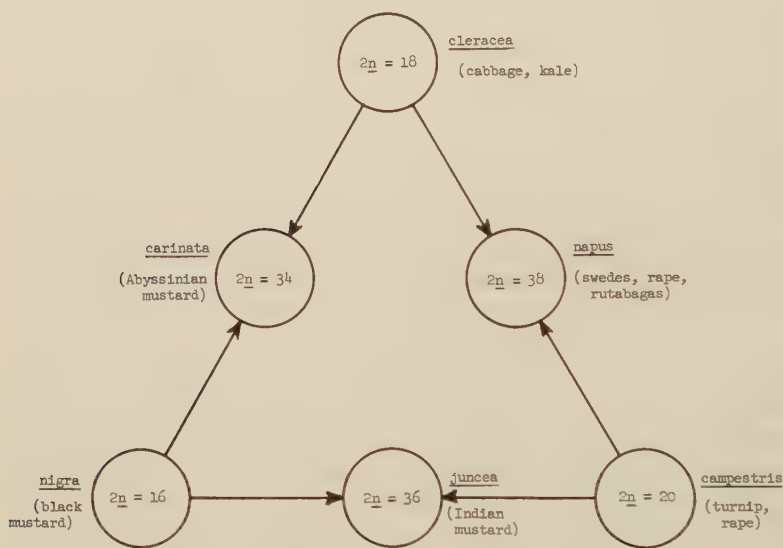


Figure 22.1 Origins of three *Brassica* species by allopoloidy, often termed the Triangle of U. (From U.²⁴)

In Sweden, these relationships between *Brassica* species are being utilized in the improvement of *B. napus* as an oil crop, particularly with respect to its winter hardiness.⁶ In both kale (*B. oleracea*) and turnip rape (*B. campestris*), there are genes for increased winter hardiness. Tetra-

ploids of the latter diploid species have been crossed to give allopolyploids similar to, and easily crossed with, winter rape (*B. napus*). Some disturbances were present in the first generations, but later generations were stable. A selection of the synthesized allopolyploid was higher yielding and more winter hardy than the natural allopolyploid. It was obvious that the variability of the diploid ancestral species could be used to improve not only rape but rutabagas (swedes).

New allopolyploids. Except for a few examples among small fruits (see above), very little has been accomplished by plant breeders in terms of developing successful new allopolyploids. In part, this is simply because nature in her laboratory has been exploring combinations of species for a long period of time, and has already accomplished many of the successful ones.

An allopolyploid from wheat (*Triticum aestivum*, $n = 21$) and rye (*Secale cereale*, $n = 7$) has been known for almost a century. It has been called *Triticale*. One problem in the allopolyploid is that it is self-pollinated, as is wheat, while rye is cross-pollinated. Normally, rye shows inbreeding depression when it is selfed, and it is supposed that the rye chromosomes cause some inbreeding depression on the self-pollinated allopolyploid.⁶ Experience in Sweden indicates that the most successful allopolyploids are those where the rye parent has been self-fertile and vigorous.

Attempts are being made in a number of laboratories to synthesize new species of cereals from combinations of the genomes of wheat, rye, *Aegilops*, and *Agropyron*. It is hoped that some of these will yield (1) superior versions of allopolyploids already available; (2) new food crops, perhaps some of them perennial; and (3) superior feed or forage crops.

Weeds. These unwelcome companions of man's crops have exploited allopolyploidy also. A good example is Johnson grass (*Sorghum halepense*). Recent research has shown that this weed ($n = 20$) was derived from a cross of domesticated sorghum (*S. vulgare*) ($n = 10$) and *S. virgatum* ($n = 10$), followed by doubling of the chromosome number.¹ *S. virgatum* is native to North Africa, and it is assumed that the allopolyploid originated there. *S. virgatum* has very weak rhizomes, and *S. vulgare* has none, but some allopolyploid derivatives are strongly rhizomatous. This illustrates that allopolyploids may sometimes have unpredictable characteristics.

Genetics of Allopolyploids

The chromosome behavior of allopolyploids in meiosis will give a clue to their genetic behavior. Where the chromosomes pair as bivalents, disomic ratios should prevail. If the different genomes have genes in common, duplicate-factor ratios would be expected. In fact the occurrence of duplicate-factor ratios is sometimes considered to be evidence of allo-

ploidy. Where multivalent associations occur in an allotetraploid, tetrasomic ratios occur for some loci.

Whether it is a segmental or true allopolyploid, a good deal of the genetic material is present more than twice. The work with wheat has indicated that some of the chromosomes of one genome may have a function somewhat like that of a chromosome in another genome. Where a haploid is produced in common wheat, it will have 21 chromosomes, all expected to occur as univalents in meiosis. Sears has pointed out that up to four bivalents are known to occur in such haploids.²⁰ It is assumed that members of each pair come from separate genomes. Where chromosomes of two genomes are similar, but not homologous, they are termed *homoeologous*. Sears also showed that similar genetic material could be present in two chromosomes incapable of pairing with one another. The amount of duplicated genetic material will vary with the allopolyploid. In contrast to wheat, upland cotton appears to have very little duplication in the different genomes.

Where duplication exists, it tends to make the species less flexible and less sensitive to loss of genetic material. In common wheat, Sears has developed a series of 21 different nullisomics, each deficient for a different pair of chromosomes. Such nullisomics are viable because the functions of the missing chromosome have been taken over by other chromosomes. Nullisomics have never been achieved in diploid species. Radiation of common wheat ($n = 21$) will produce only a small number of chlorophyll mutants; the same treatment of durum wheat ($n = 14$) will yield considerably more; and when applied to einkorn wheat ($n = 7$), the number is very large. In other words, where more than one genome is present, gene mutations (or losses) often fail to manifest themselves because genes in other genomes will carry on their functions.

The presence of hidden gene mutations in an allopolyploid may have some interesting possibilities. The allopolyploid may become loaded with mutated, and perhaps deleterious, genes that may be a disadvantage. The other possibility is that the mutated gene may take on new functions which work to the advantage of the allopolyploid. In any case there is evidence that the self-sufficient genomes going into an allopolyploid may lose their self-sufficiency in the allopolyploid combination. Clausen cites the example of the recessive gene *m* for the mammoth growth habit in tobacco.³ The dominant gene *M* for normal growth is present in both *Nicotiana sylvestris* and *N. tomentosiformis*, the components of cultivated tobacco, and might be expected to be present at two loci in the latter. But it is present only once, in the *N. tomentosiformis* genome, which would suggest that the same gene in the *N. sylvestris* genome has been lost or has changed in function.

This process of divergence within the component genomes of an allopolyploid has been termed *diploidization*. It involves chromosomal as well as genic changes. Chromosomal changes, such as inversions and translocations, involving large or small sections of the chromosomes, reduce the opportunity for pairing of homoeologous chromosomes, sometimes called *heterogenetic pairing*.

The powerful role of a gene or a few genes in controlling chromosome pairing in an allopolyploid has been demonstrated by Riley in his studies of diploidization of common wheat (*Triticum aestivum*).¹⁶ As pointed out above, when a polyploid is formed, there is very little pairing of chromosomes in meiosis, which suggests that the three genomes A, B, and D are quite different. There is an average of less than one bivalent, most of them open, and an occasional trivalent. However, when chromosome V is absent in the polyploid, there is a striking increase in chromosome pairing at meiosis; in each cell, averages of 2.5 closed bivalents, 1.2 open bivalents, 1.6 trivalents, and occasionally a quadrivalent are present. It is evident that chromosome V reduces the pairing of homoeologous chromosomes.

Riley then compared normal hexaploid wheat ($2n = 42$) with nullisomic V, which has both chromosome V's missing ($2n = 40$). The normal hexaploid, as expected, had 20.9 bivalents per cell and occasional univalents. But without chromosome V, there were averages of 17.2 bivalents, 0.5 trivalents, 0.6 quadrivalents, 1.4 univalents, and occasional associations of 5 and 6 chromosomes. In the absence of chromosome V, an allopolyploid has tended to take on the chromosome behavior of an autopolyploid. Further work showed that the genetic material responsible for diploidization is in the long arm of chromosome V.

It is obvious that the management of chromosome V extends the operations of the wheat breeder. By removing it in crosses of wheat to other species, chromosome pairing of homoeologous chromosomes from different species can be increased. With chromosome pairing will come recombination and the development of unusual combinations of genes. Using appropriate techniques, chromosome V can be reintroduced into the revised combination of chromosomes, to produce diploid chromosome behavior in meiosis.

Résumé

When an allopolyploid crop species has been resynthesized, it has rarely if ever been as good as the long-established product. The "raw" allopolyploid has been embellished by nature and the hand of man, though its superstructure is still present. This should encourage plant breeders who are attempting new combinations of genomes. If a new allopolyploid is not so

valuable as an established one, but better than the resynthesis of the latter, then it may have agricultural value after a period of several years of selection and hybridization. The greatest success will follow incorporation of as much variability as possible into the gene pool of the new allopolyploid. It is very probable that the new allopolyploid may have its greatest success in environments and in uses different to some degree from those that prevail for the component species.

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ANEUPLOIDS



The pure-breeding types (with one extra pair of chromosomes) are more distinct from the original form from which they arose than some of the species of Datura which have been founded on single factor differences. Our types we have ventured to call artificial or synthesized "new species." They differ from the ancestral form not by a single factor but by a whole group of factors. Their greatest difference from species in nature appears to lie chiefly in the fact that we know their method of origin, having made them up to specifications, as it were, from a knowledge of the different parts of the chromosomes involved.¹

BLAKESLEE, 1934

IN THE EVOLUTION of natural species, changes in chromosome number have occurred without polyploidy being involved. Such changes, involv-

ing the loss or gain of one or a few chromosomes, have been termed *aneuploidy*. Aneuploidy has usually involved changes in structure, inasmuch as a reduction in chromosome number has required the transfer of essential chromosome material from the lost chromosome to one or more of those remaining.

These changes in chromosome number through aneuploidy have required a great deal more time than changes through polyploidy, apparently because such changes have had to be nicely synchronized with structural changes. It is no surprise, then, to learn that aneuploidy has been of little or no consequence in the domestication of crop plants.

In recent years, however, aneuploids have been used with some success by plant breeders for specific purposes. It is likely that their use will increase in the future. They are examined here with respect to the breeding consequences of (1) chromosome additions, (2) chromosome deletions, and (3) chromosome substitutions.

Chromosome Additions

By appropriate techniques the amount of chromosome material added to the normal chromosome complement of a species may vary from a small block of a few genes to one or more entire chromosomes. Of most interest here are additions of whole chromosomes.

Primary trisomics. The addition of one extra chromosome of the normal complement is termed a *primary trisomic*. There are as many primary trisomics possible as there are chromosome pairs, and each is often distinct in appearance. Where a particular chromosome is present four times, it is called a *tetrasomic*. Trisomics are symbolized as $2n + 1$; and a gamete with an extra chromosome, as $n + 1$.

Primary trisomics have been used to assign genes to chromosomes, as indicated in the following example. If a is a recessive allele not yet assigned to a chromosome, a plant homozygous for this allele is crossed to all trisomic stocks. In F_2 from crosses to all but one stock, assuming that all stocks are homozygous for A , the segregation will be $3A_-:1aa$. Where the cross is made to a plant trisomic for the chromosome carrying allele A , two F_1 types will be produced (Fig. 23.1). One, from the union of n gametes from each parent, will give an F_2 segregating in a 3:1 ratio, as above. The other, from a trisomic plant resulting from the union of n and $n + 1$ gametes, will give a disturbed ratio. In Fig. 23.1 it is assumed that the three chromosomes of the trisomic distribute at random and that gametes with and without the extra chromosome are equally viable. In this case, the F_2 segregation would give only one homozygous recessive plant in 36. However, meiosis in a trisomic usually gives more microspores and megaspores with n chromosomes than with $n + 1$, and pollen

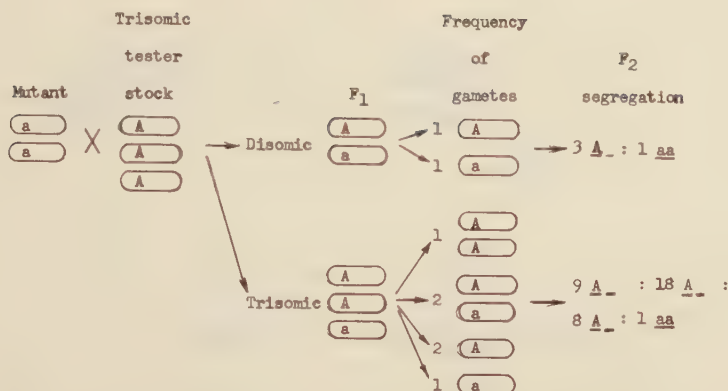


Figure 23.1 Segregations in F₂ from disomic and trisomic F₁ plants obtained from the cross of a plant homozygous for a recessive allele and a trisomic tester homozygous for the dominant allele. Only the chromosomes of the trisomic are shown, and it is assumed that chromosome segregation in meiosis is normal and all gametes are equally viable.

with $n + 1$ chromosomes often is either nonfunctional or less competitive. As a result, a trisomic may yield mostly disomic types. Even in this case the proportion of homozygous *aa* plants in the F₂ will be less than expected.

The presence of one or more extra chromosomes with the normal chromosome complement usually leads to chromosome imbalance and reduction in vigor. Multivalent chromosome configurations occur in meiosis, with a consequent loss in fertility. Because gametes with extra chromosomes are nonfunctional, completely or in part, trisomics revert to the normal diploid condition unless maintained by strong selection pressures.

Balanced tertiary trisomics and the production of hybrid seed. In addition to the primary trisomics mentioned above, there are secondary and tertiary trisomics. A *secondary trisomic* has one extra chromosome present with two identical arms; such a chromosome is termed an *isochromosome*. This means that the chromosome material of that particular arm is present four times in each cell instead of twice. A *tertiary trisomic* has an extra chromosome present, which is constituted of parts of two different chromosomes. It is obvious that chromosome breaks and fusions must occur before development of the tertiary trisomic.

Ramage has described a method that he has developed and is using to produce hybrid barley and which involves balanced tertiary trisomics.⁸

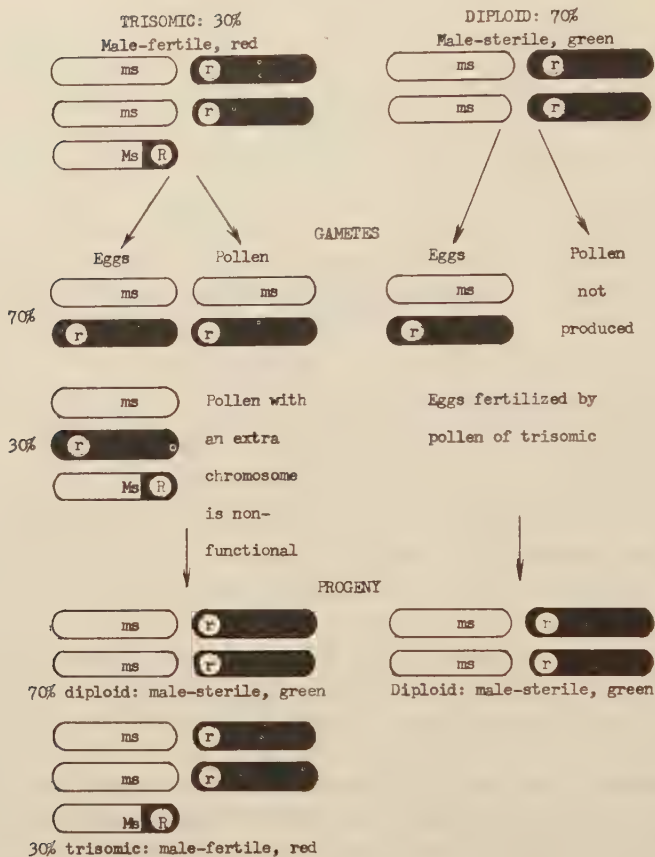


Figure 23.2 Use of a balanced tertiary trisomic in the production of hybrid barley. The tertiary trisomic has a recessive allele for genetic male sterility (ms) on the normal chromosomes, and the dominant allele for male fertility (Ms) on the extra chromosome and closely linked with the interchange break point. The extra chromosome is marked with a dominant gene for red plant color (R). Seed from green plants (diploids) is sown as the female parent in a crossing block; it will give only male-sterile plants. Seed from red plants (trisomics) will serve as a continuing source of balanced tertiary trisomics and male-sterile diploids. (Adapted from Ramage.⁸)

His method is illustrated in Fig. 23.2. The tertiary trisomic is balanced, since it has a recessive gene for male sterility (ms) present on one pair of the normal chromosomes and the dominant allele of this gene (Ms) on the extra chromosome. This means that diploid progeny of the

trisomic will always be male-sterile and trisomic products male-fertile; there are approximately 70 and 30 per cent, respectively, of each. All progeny of diploid plants will be male-sterile also. Actually 1 per cent or less of the progeny of the tertiary trisomic will be primary trisomics, but these will be male sterile because all chromosomes carry the *ms* allele. As in most trisomics, at least in diploids, the extra chromosome is not transmitted through the pollen.

If a second pair of normal chromosomes carry the recessive gene *r* for green color, and the extra chromosome carries the corresponding allele *R* for red color, then all red plants in the population will be trisomics and all green plants diploids.

The trisomics in the mixture of tertiary trisomics and diploids are hand-harvested and used as a source of trisomics and diploids in the following year. The remaining diploids are harvested with a combine and used as a female parent in a crossing block. The use of balanced tertiary trisomics in hybrid barley seed production requires that the trisomic produce abundant pollen and that the environment be such that the pollen is transported by wind to male-sterile diploids.

Chromosome addition lines. Chromosome addition lines are lines with one extra pair of chromosomes. If they involve chromosomes of the normal complement, they are called *tetrasomics*. As in trisomics, meiosis is disturbed, and *n* gametes are more successful than *n* + 1 gametes, with the result that tetrasomics revert to the disomic condition.

However, following chromosome breaks and fusions, it has been possible to increase chromosome number and duplicate at the same time a part of a chromosome. Blakeslee has described two examples in *Datura*, the Jimson weed, in which he has increased the chromosome number from 12 pairs to 13 and duplicated one arm of one chromosome.¹ Chromosomes in *Datura* are numbered 1,2, 3,4, and so on to 23,24. True-breeding types with chromosome arm 2 duplicated and 13 pairs of chromosomes are shown in Fig. 23.3.

Of more interest in plant breeding has been the addition of single chromosome pairs of a different species. These have been called *alien addition lines*, or *alien addition races*, a term proposed by Clausen.³ They have been of interest because they present an opportunity of adding a few of the characteristics of the second species. It was reasoned that the extra chromosome pair should behave normally at meiosis because, being nonhomologous with the other chromosomes, it would not enter into multivalent chromosome associations. In general the addition of an extra foreign chromosome has been very disturbing to a plant's metabolism and extremely so if the added chromosome makes up a larger proportion of the total chromosome material in the addition line.

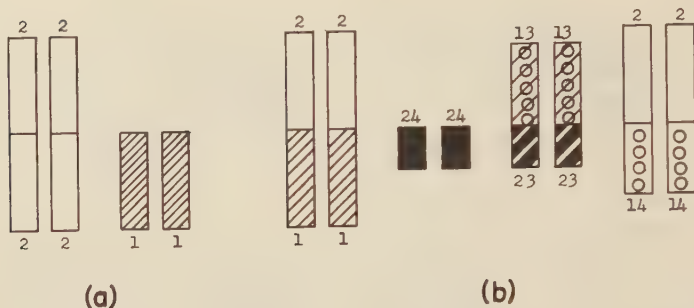


Figure 23.3 Extra-chromosomal types in *Datura*, each with chromosome arm 2 duplicated, and one extra chromosome pair present. In A, arm 2 is duplicated in an isochromosome 2.2 and 1 is present as a telocentric chromosome. In B, arm 2 has been attached to arm 14 of chromosome 13.14, and arm 13 of the latter to arm 23 of chromosome 23.24. Arm 24 of the latter is present as an isochromosome. Both A and B give plants that are similar in appearance. (From A. F. Blakeslee, *J. Hered.*, 25 (1934), 80.)

Considerable interest has centered on the addition of rye chromosomes to those of common wheat ($2n = 42$). In particular, it was hoped that some addition line might have greater winter hardiness. Though rye and wheat cross quite readily, the chromosomes do not pair, and it has not been possible to recombine characteristics of the two genera. Addition lines have been obtained from one or more backcrosses to wheat of the allopolyploid produced from a cross of the two species.

Addition lines of rye chromosomes to wheat have not been promising.⁹ Wheat chromosomes usually behaved normally or almost normally in meiosis, but the regulation of their behavior failed to extend to the pair of rye chromosomes. The latter often failed to pair or the pairing was out of phase with that of the wheat. Addition lines were less fertile, and the level of fertility was not related to the meiotic behavior. Because the alien chromosome was frequently lost in meiosis, many gametes had the normal haploid chromosome number. Pollen with the haploid chromosome number was much more competitive than pollen with an added chromosome. As a consequence, several of the progeny of addition lines were normal wheat ($2n = 42$). If continued over a few generations, the population would have no addition types present.

Alien addition lines in tobacco (*Nicotiana tabacum*), with one or two pairs of chromosomes from *N. glutinosa*, reproduced themselves reasonably well.³ Such types were resistant to tobacco mosaic, where an alien chromosome carried the gene for resistance. On the whole, however,

alien addition lines have not been of much interest to breeders of crops reproducing by seed, mostly because of their tendency to lose the alien chromosomes and revert to the diploid condition. For crops propagated by vegetative means, addition lines would have more promise.

Duplications. Where only a portion of a chromosome of the same species is added to the chromosome complement, it is called a *duplication*. Duplications may be produced in a variety of ways, which need not be considered here. However, duplications usually involve the translocation of a segment of a chromosome from one chromosome to its homolog or to another chromosome. Usually, the added portion is an insertion. It is obvious that chromosome breaks are involved in such duplications.

The development and identification of duplications require an intimate knowledge of the appearance of chromosomes and some knowledge of the location of genes on the separate chromosomes. Without this knowledge it is impossible to know whether a duplication is present or not. For this reason the study of duplications is limited to only a few species. One of these is barley.

Hagberg, working with barley, is optimistic about the role of translocation as a means of improvement.⁴ He has been particularly interested in chromosome 5, because it carries 13 out of the 14 reported genes for mildew resistance, and chromosome 6, which has a gene for high α -amylase activity. High α -amylase activity is required in a good malting barley. By appropriate crosses of translocation stocks, types were developed with the short arm of chromosome 6 duplicated, though no report was available on their performance.

Accessory, or B, chromosomes. In some species small chromosomes present in varying numbers are additions to the normal chromosome complement. In corn and a few other species they have been called *B chromosomes*, but more often they have been termed *accessory*, or *supernumerary*, *chromosomes*. They appear to have no genes present, and in most cases are entirely or largely heterochromatic. They show unstable behavior, both within and between species. When present in small numbers they usually have little or no effect on a plant's appearance or behavior. By appropriate breeding procedures the number of accessory chromosomes may be increased. In rye, one or two accessory chromosomes adversely affect fertility, whereas ten or more are necessary to reduce fertility in corn. There are conflicting reports regarding beneficial effects of small numbers of accessory chromosomes. Müntzing has reviewed much of the literature on accessory chromosomes.⁶

Peterson and Wernsman have described a technique where translocation stocks involving B chromosomes and normal (or A) chromosomes

are used to transfer portions of a chromosome from one variety to another.⁷ Roman and Ullstrup have used such stocks to locate genes in corn.¹⁰ Accessory chromosomes appear useful only as a means to evaluate and manage chromosomes; in themselves, they have no proven value in the improvement of crop plants.

Some of the earliest duplications were achieved by Blakeslee¹ in *Datura* which has been mentioned previously. One true-breeding type was developed with the 2 arm of chromosome 1.2 attached to chromosome 11.12. This meant that the 2 arm was present four times instead of twice, as in the normal diploid. It was similar in appearance to a secondary trisomic with the extra chromosome an isochromosome 2.2. It was also similar to the extra-chromosomal types of Fig. 23.3.

Chromosome Deletions

Chromosome deletions have not been promising as a means of crop improvement. Deletions of a chromosome pair have been possible only in polyploid plants where the functions of the missing chromosome pair are taken over by other chromosomes, which may be homologous if an autopolyploid is involved or homoeologous if an allopolyploid. Invariably, types with a deleted chromosome pair have been reduced in vigor and fertility. Plants deficient in one chromosome of a pair are called *monosomics*, and those deficient in both members of a pair are *nullisomics*. They are symbolized as $2n - 1$ and $2n - 2$, respectively. Gametes with a deficient chromosome are symbolized as $n - 1$.

Monosomics. Monosomics of wheat have been developed and studied in great detail by Sears, using the variety Chinese Spring.^{11,12} Monosomics have been used successfully in both wheat and tobacco as a means to assign genes to chromosomes, in much the same manner as trisomics.^{11,12,13}

Nullisomics. Nullisomic lines, where they are available, may also be used to assign genes to chromosomes. In contrast to monosomics, nullisomics of wheat, where they are fertile, are capable of breeding relatively true for the nullisomic condition. Nullisomic lines of wheat have not been promising agronomically; they have lacked vigor and have often been male- or female-sterile. Their greatest value in breeding has been, and will be, in facilitating chromosome substitutions and assigning genes to chromosomes.

Chromosome Substitutions

By appropriate backcrossing procedures, using monosomic or nullisomic stocks, chromosomes may be substituted one for another.^{2,11,12,15} The substituted chromosomes may be from the same or another species

or genus. If from another species, the product is sometimes called an *alien substitution line* (or *race*).³

Substitutions within a species. As a breeding technique, chromosome substitutions have looked most promising if made within a species. Using Sear's monosomics and nullisomics of the Chinese Spring variety of wheat, it has been possible to develop Chinese Spring types identical to the original type except for the substitution of individual chromosomes from other varieties. Using chromosome substitution and monosomic and nullisomic analyses, a great many genes have been assigned to chromosomes. By 1958, 19 genes governing resistance to stem rust in eight varieties of wheat had been associated with nine chromosomes by various workers.¹⁴ Unrau and his associates extended these studies to include quantitative characters, and found that individual chromosome substitutions in Chinese Spring modified this variety significantly with respect to yield, earliness, lodging resistance, height, and other characters.^{5,14} There are many cytological examinations required to identify the appropriate parents in making a substitution, and this to a large extent has discouraged chromosome substitution as a breeding technique for general use.

Using chromosome substitutions, Sears was able to assign the chromosomes of wheat to seven homoeologous groups.^{11,12} In each group there are three chromosomes, one from each of the A, B, and D genomes.

Alien substitution lines. Alien substitution lines may be developed by using alien addition lines as the donor parent and monosomic or nullisomic lines as the recurrent parent in a backcrossing program.⁹ Such lines have been developed in wheat, using substituted single rye chromosome pairs. With 21 pairs of wheat chromosomes and 7 pairs of rye chromosomes, 147 different alien substitution lines are possible. It is readily seen that a great deal of work is involved in a full assessment of the value of substituting rye chromosomes for those of wheat.

Résumé

Aneuploids that have been useful directly as improved varieties are extremely rare or nonexistent. The gain or loss of a chromosome or a large portion of a chromosome causes such chromosomal imbalance that vigor and fertility are severely reduced. Aneuploid types often revert to the euploid condition in sexual reproduction. It is possible that aneuploid types may be of direct value in species reproducing asexually.

This does not mean that aneuploids do not have a function in plant breeding—quite to the contrary. Through the use of monosomic, trisomic, and nullisomic types, it is possible to assign genes to chromosomes in species where other methods do not work because of the large number of

chromosomes with similar sizes and shapes. Chromosome substitutions are made possible, with precise measurements of the contribution of each to plant performance. The first steps are being made in the use of balanced tertiary trisomics in the production of hybrid varieties.

Aneuploidy will be even more useful in plant breeding as techniques improve in the breakage and reconstitution of chromosomes. Such advances will come in part with improved mutagens. Most important, however, will be a knowledge of the chromosomes in the species under study, skill in manipulating them, and frequent use of a microscope.

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24

MUTAGENS AND CROP IMPROVEMENT



Knowledge of the laws of mutation will probably lead to the artificial production of mutations at will, and thus the creation of completely new properties in plants and animals.

DE VRIES, 1901

THE STORY of the domestication of crops and their improvement over the centuries is largely one of man's exploitation of gene mutations. It is a well-established fact that mutations are still occurring, and it is reasonable to believe that they will serve to improve crops even further. It is understandable that plant breeders should be concerned with

methods that will either increase mutation rates or yield mutants unavailable from natural sources.

The earliest attempts to modify plants by using X-rays and ultraviolet rays were reported by Alberto Pirovano in Italy in 1922.³⁵ His report followed some ten years of study of plants exposed not only to radiation but also to various magnetic devices. His work remained largely unknown in America.

When it was reported in 1927 by Muller²⁸ that mutation rate in *Drosophila* could be increased markedly by the use of X-rays, and a year later Stadler³⁶ found the same to be true for barley and maize, there was considerable interest in the possibilities of exploiting induced mutations to improve varieties of numerous crops. Most plant breeders abandoned their interest in X-rays during the first decade after the appearance of Muller's and Stadler's reports.

The sustained and thorough studies of the effects of X-radiation by Gustafsson and others associated with him in Sweden stimulated an interest in radiation genetics and mutation breeding the world over. He began this work in 1928 and focused his interest mainly on barley. In addition, a great many mutagenic agents, both ionizing and chemical, became available to plant breeders after World War II. In some instances, both in Sweden and other countries, success has followed the use of mutagens, and a few varieties from mutation-breeding programs are now in commercial production. The availability of mutagens and limited successes have encouraged most plant breeders in recent years to inquire into, or actually to investigate, the use of mutagens in the improvement of their crops. Several publications deal in detail with the role of induced mutations in plant breeding.^{10,11,15,16,18,33,39}

Mutagenic Agents

With the advent of the Atomic Age, several ionizing mutagenic agents besides X-rays were used. In recent years, however, there has been increasing interest in, and use of, chemical mutagens. While mutagenic agents have had different effects, and some seem to affect certain regions of chromosomes more than others, none has been found that will produce specific gene changes at will.

Ionizing radiation. The type of ionizing radiations most commonly used, and their characteristics, are listed in Table 24.1. Ionizing radiations produce their effects by (1) ionization (production of ion pairs) when they interact with matter; and (2) excitation, and with it a transfer of energy. The effect is in general proportional to the energy absorbed by a particular tissue, so it is important to use an ionizing agent that will penetrate to the proper depth. Energy transfer is higher with increases

Table 24.1 Types of radiation and their sources. *

TYPES OF RADIATION	SOURCE	DESCRIPTION	HAZARD	NECESSARY SHIELDING	PENETRATION INTO TISSUE ^a
X-rays	X-ray machine	Electromagnetic radiation	Dangerous, penetrating	A few mm of lead except for very high-energy installations	A few mm to many cm
Gamma rays	Radioisotopes and nuclear reactors	Electromagnetic radiation similar to X-rays	Dangerous, very penetrating	Several inches of lead or several feet of concrete	Many cm
Neutrons (fast, slow, and thermal)	Nuclear reactors (piles) or accelerators	Uncharged particles slightly heavier than proton (hydrogen atom); not observable except through its interaction with nuclei in the material that it traverses	Very hazardous	Thick shielding composed of light elements, such as concrete	Many cm
Beta particles, fast electrons, or cathode rays	Radioactive isotopes or accelerators	Electrons (+ or -) ionize much less densely than alpha particles	May be dangerous	Thick sheet of cardboard	Up to several mm
Alpha particles	Radioisotopes	Helium nuclei, ionize very heavily	Very dangerous internally	Thin sheet of paper	Small fraction of a mm
Protons or deuterons ^b	Nuclear reactors or accelerators	Nuclei of hydrogen ^b	Very hazardous	Many cm of water or paraffin	Up to many cm

* From National Academy of Sciences-National Research Council Publication 891. *Mutation and Plant Breeding*, A. H. Sparrow, p. 58.

^a It is assumed that penetration is into an ordinary plant tissue of average density.

^b A proton is the nucleus of the common isotope of hydrogen, and a deuteron is the nucleus of the heavy isotope of hydrogen.

in the density of ionization; this will vary from eight ionizations per micron in high-energy beta and gamma radiation, through X-rays, neutron radiation, and alpha radiation, to atomic rays with 130,000 per micron.

The dose of ionizing radiation has been measured in various units. The oldest unit is a *roentgen* (abbreviated as r). It is a unit of exposure and not a unit of absorption. The *rad* (radiation-absorbed dose) is a unit of absorbed dose, and equals 100 erg/g. For X-rays, 1 rad is equal in energy to 1.08 r in water.

X-ray machines are still the most widely used source of ionizing radiation, and they are popular because they are available, easily operated, and versatile. Although radioisotopes as sources of gamma and beta radiation are not so convenient as X-ray machines, they are better for deep penetration of vegetative tissue. Radioisotopes are sometimes applied internally, the most common being phosphorus-32, sulfur-35, carbon-14, and tritium. Tritiated thymidine has been of particular interest because it is selectively concentrated in chromosomes.

Ionizing radiation can cause: gene mutation; chromosome, chromatid, or subchromatid aberrations; changes in chromosome number; inhibition of cell division; induction of mitotic activity; death of nuclei or cells; partial or complete sterility; retardation or stimulation in growth rate; and the induction of abnormal growth. Needless to say, great care must be exercised in the use of ionizing radiation.

Ultraviolet radiation. Ultraviolet radiation, except for the shortest wavelengths, does not cause ionization. Its effect is obtained through a process of excitation. One problem in using ultraviolet radiation is its low penetrating power, which limits its use on plant material almost entirely to pollen grains. Radiation is applied with an ultraviolet lamp.

Compared to X-rays, ultraviolet radiation produces more gene mutations relative to chromosome aberrations. Deficiencies induced by ultraviolet radiation are terminal, but deficiencies induced by X-rays are interstitial and associated with chromosome breaks.

Chemicals. The following chemical mutagens have been used:^{2,3} alkylating agents (among these are the mustards, epoxides, ethylene imines, methane sulfonates, and beta-propiolactone), urethane, the alkaloids, the peroxides, formaldehyde, and substances related to nucleic acid and nitrous acid. Even vegetable oils have produced chromosomal aberrations and gene mutations.⁴⁰

Chemicals produce effects similar to those of ionizing radiation, but the proportion of chromosome aberrations to gene mutations varies considerably with the different mutagens. As a group, compared to ionizing

radiation, chemical mutagens produce more gene than chromosome changes.

Of most interest recently is ethyl methane sulfonate (EMS), which is relatively nontoxic. EMS treatments are accomplished by soaking seed or the roots of young seedlings in an aqueous solution. Rao and Sears feel that all mutations produced in wheat by ionizing radiation may be explained as due to deficiencies or duplications.²⁷ On the other hand, in their research, EMS produced many mutant types that behaved as if they were due to changes in single genes. Among these were plants that were lethal lutescent, pale yellow-green, and compacted (like the *compactum* subspecies). Neuffer and Ficsor describe a method of successfully treating corn pollen with EMS which induced breaks and gene changes in a small chromosome segment.²⁴

Procedures

Procedures in the use of mutagens vary with the species, with the objectives, and with the part of the plant under treatment. Most often, radiation treatments have been applied to seeds, and for many reasons. Seeds can be treated under rather rigidly controlled conditions, which include water content, temperature, oxygen level, and atmospheric pressures. Many seeds can be treated at one time. The effect of radiation on one cell of a seed may be magnified through plant development into a large part of the plant. In discussing these procedures it is assumed that the objective is the achievement of a desirable gene mutation, either directly in or for later incorporation into an acceptable commercial variety.

Seeds. For most crop species the optimum treatment levels have already been reported, at least for X-rays. If such information is not available, it must be obtained by trial runs. Usually, treatments are preferred which kill 50 to 70 per cent of the seeds or developing seedlings. Experience has indicated that different varieties react differently to mutagens, so it is advisable to treat several varieties, particularly if a new allele is desired. X-radiations used for the seeds of some crops are:¹⁶

Peas	5,000-15,000 r
Barley	10,000-15,000 r
Wheat	15,000-20,000 r
Flax	40,000-50,000 r
Mustard	>100,000 r

In X-ray treatments of seeds, chromosome aberrations increase exponentially (often as the square) with the dose, whereas gene mutations

increase linearly.⁶ As a result, the chromosome aberrations impose an upper limit on the dose, and this limits the number of gene mutations. Seeds of cereals appear to be most sensitive to radiation damage at moisture contents of 3 to 4 per cent.

Plants grown from treated seed are referred to as the M_1 generation (M = mutation), and succeeding generations as M_2 , M_3 , and so on. In M_1 it is recommended that treated seeds, at least of cereal crops, be grown in dense stands so that only one branch (tiller) grows from each seed. This is advised because the first tiller in cereals has shown the maximum number of mutations. At the same time, the stand should not be so dense that many of the plants fail to develop, since plants carrying mutations frequently are less competitive.

M_1 plants will rarely exhibit gene mutations, since mutations are mostly recessive. However, they will exhibit deformities, many of which will not be carried over into the next generation. Usually, M_1 plants with reduced fertility are discarded because sterility almost always is a consequence of chromosome changes. Such plants do not show a higher rate of gene mutation in succeeding generations.

In both self- and cross-pollinated plants, at least where the latter are self-compatible, M_1 plants are bagged to prevent outcrossing. If self-incompatible, several M_1 plants should be bagged together, or the entire M_1 should be grown under isolation. This isolation is important, even in self-pollinated crops, because there have been reports of mutations being obtained which were later proved to be a result of outcrossing to other materials in the nursery. Treated plants are often rendered pollen-sterile, which will magnify levels of outcrossing. Bagging is less necessary if there is no source of the desired character in the nursery or in existence.

The M_2 generation will consist of single rows, each from a single M_1 plant, and usually of 10 to 20 plants. On theoretical grounds, Yoshida has found that nine plants from each M_1 head of cereal crops will be most efficient in the use of space and time.⁴⁴ Most conspicuous will be the recessive mutations affecting chlorophyll production. These will grade from albinos to types just a shade lighter green in color than normal plants. If a single locus is involved, and the entire stem and inflorescence of the M_1 plant carries the mutation, then one-fourth of the M_2 row would be expected to be homozygous for the recessive mutant allele. Such monohybrid ratios are rare because M_1 plants develop only in part from mutated cells. Mostly they are mutant mosaics, with the mutation appearing in only a portion of the inflorescence. In such cases the proportion of homozygous recessives in M_2 rows will be less than one-fourth.

Where the M_2 , following radiation, is grown in bulk, Yoshida calculated that one seed per M_1 plant is the best number to plant, if the number of

heads on an M_1 plant is three or less.⁴⁵ Where the number of heads per M_1 plant is four or more, two seeds per plant is sufficient for planting the M_2 . In no situation would it be desirable to use more than one seed per M_1 head. This means that the number of M_2 plants in most situations need not be more than twice that of the M_1 .

Selection will begin in M_2 . For this reason the M_2 should be grown under conditions that will permit the best expression of the character desired. For example, if resistance to stem rust is desired, the nursery should be inoculated with the rust. Every tenth or twentieth row should be sown to untreated seed. Plants with the desired characteristic should be taken from as many rows as possible, though two or three plants from any one row should be sufficient. Selected plants should be selfed, particularly if the crop is cross-pollinated. In only rare cases will normal plants be harvested and carried forward into M_3 , even though mutations are sometimes not expressed until M_3 . The reason is obvious: Too many F_3 rows would have to be grown.

Where the treated variety is an adapted type, and it is desired to change one gene, it will be necessary to grow a very large number of M_2 plants, perhaps as many as 10,000 or more. The reason is that a desirable mutant, without other undesirable characteristics, is a very rare event.

In the M_3 it will be possible to have many rows breeding true for the character selected in M_2 . At this stage, or even in M_2 , the combination of a mutant allele with other characteristics will begin. Most likely the backcross method will be used, but other breeding methods may be used as alternatives. Where crossing or backcrossing is not contemplated, the M_3 and succeeding generations in the case of self-pollinated crops will be handled as in the pedigree method. In the case of cross-pollinated crops, M_3 materials homozygous for the desired character could be bulked and improved in succeeding generations by mass or recurrent selection.

Recently, mutants produced by mutagens, or populations from seeds or plants that were treated by a mutagen, have been treated again, or even several times, with the same mutagen.¹⁰ The term *mutation cycle* has been used to cover the period from the beginning of one mutagenic treatment to the beginning of the next.

Vegetatively propagated species. Again, general rules for treatment levels will not always apply, and preliminary tests will be necessary unless there is published information. Vegetative tissue will not endure high doses of ionizing radiation, the rates usually being between 2,000 and 4,000 r. For sensitive herbaceous materials the rate is reduced about one-half, and is doubled for lignified or dormant tissue.²⁶ When deep penetration of tissue is desired, radioisotopes will be more useful than X-rays, and cobalt-60 has been widely used.

Where the presence of a virus is a possibility, great care will be necessary, either in differentiating between the effects of the virus and the mutagen or in eliminating the virus from the treated materials.

The plants grown from treated material frequently show *primary effects* of the treatment, manifested as increased fleshiness of leaves and abnormal venation or leaf shape. If propagated vegetatively to a second generation (the terms M_2 , M_3 , and so on for successive propagations will be used, though biologically they are quite different from the same terms used for plants propagated by seed), the permanent mutations become divorced from the primary effects. If not apparent in M_2 they are unlikely to appear in succeeding propagations. However, in the M_2 , the cuttings from a treated M_1 are unlikely to be uniform for the mutant character, and it will be necessary to go to M_3 to achieve it.

Often mutants are periclinal chimeras (see Fig. 8.1), with a mutation present only in the outer layer. In other cases, periclinal chimeras have been treated and the outer layers have been suppressed, with further development taking place from inner layers having a different genotype. Though sometimes strikingly different from the original, these latter types cannot be considered as mutations. There is some interest in the use of radiation to stabilize chimeras by making all layers of the same genetic constitution.

Dormant scion wood of fruit trees is radiated during the winter and spring, and grafted onto parent stock. Usually, the primary shoots from radiated buds are normal and are pruned back to the original radiated stock. New shoots are more likely to be mutants. By repeated pruning back, the treated wood may yield several generations of mutant shoots.

Radiation of pollen. There has been considerable interest in the radiation of pollen because any genetic change transmitted through the generative nucleus to the fertilized embryo will be incorporated in all cells of the plant developing from the embryo and there will not be competition from unaffected cells, as occurs when seeds are radiated.

Konzak used X-rays, thermal neutrons, and ultraviolet radiation on barley pollen, and found that the frequency of mutations was low compared with results obtained when X-rays were used on seeds.²⁵ In a study of the sensitivity of corn pollen to gamma rays at different stages of development, Singleton found that mutation rate increased by a factor of 15 from a low just before meiosis to a high about one week before pollen dehiscence.³³ Sensitivity then dropped suddenly and remained low in the mature pollen. Pollen was most sensitive to damage during meiosis, but a week later showed scarcely any damage. A radiation of 1,500 r in one day gave higher mutation rates than similar or larger amounts spread out over a period of time.

Use of adequate control material. It must be emphasized that all experiments evaluating the use of mutagens should have adequate control material. Without a control, which is subjected to the same scrutiny and the same treatments exclusive of the mutagen, the investigator will never be absolutely sure that the mutations are spontaneous or induced. Certainly, without a control, the differences in rate between spontaneous and induced mutations cannot be put in quantitative terms. The use of a control is even more important where a search is being made for genes with small effects on quantitative characters.

Effects of Mutagens

Mutagens may affect plants or plant parts by inducing (1) changes in genes, (2) structural changes in chromosomes, and (3) physiological disturbances. These effects are not mutually exclusive. For example, breaks in chromosomes involved in structural changes may cause gene mutations; or very small structural changes, such as inversions or deletions, may be interpreted as gene changes. Physiological disturbances are meant to include those effects on plants that follow directly from treatment by mutagens, but which disappear later in the development of the plant or which do not carry over into the next generation or next propagation.

Gene mutations. Gene mutations induced by ionizing radiation were considered by Stadler in many cases to be small deletions.^{37,38} Some of the more recent evidence supports his view, and other research does not. For example, Giles has found that induced mutations have mutated back to the original form in *Neurospora*, which could not occur if the gene had been deleted.¹² If the gene mutations are partly or mostly deletions, it will be difficult to accept them as a long-time approach to plant improvement.

With very few exceptions gene mutations induced by mutagens have been recessive in diploid species. For example, out of 70 mutations with dense heads and stiff stems (*erectoides* types) in barley, only two were almost completely dominant for ear density.¹⁷ Where the same mutation affects more than one character, it may behave as a recessive for one character and dominant for another.

Frequently in studies of the inheritance of the mutant recessive allele, the homozygous recessive genotype occurs at a reduced frequency, but rarely below 20 per cent instead of 25 per cent. The reduction cannot be attributed to reduced viability of mutant seeds, and presumably is due to reduced competitive ability of gametes carrying the mutant allele.¹¹

In polyploid plants such as wheat, many mutations are dominant or partially dominant. Gaul feels that this probably happens because they

involve changes in chromosomes.¹¹ These often survive in polyploids, but rarely do in diploids.

Almost all induced mutations have been deleterious. In this respect they have been similar to mutations occurring spontaneously. Gustafsson has estimated that the chance of a favorable mutation is, at the most, 1 in 1,000.¹⁶

Often the mutated genes have been pleiotropic in expression or have been linked to other mutated genes. As pointed out above, this has made it difficult in many cases to use a desirable mutant gene, at least directly.

Gene mutations appear in many cases to be the same as spontaneous mutations. In barley, mutagens have caused changes in genes which result in erect stems, naked kernels, and smooth awns, all such changed genes being allelic to those already produced spontaneously and having similar effects.³⁹ However, though allelic and similar, they have not been identical. This has also been true of two or more induced alleles at the same locus; though having similar effects, they have not been identical.¹⁷

Like spontaneous mutations, the induced gene mutations may range in their effects from those equivalent to that of a polygene to those almost equivalent to a change in species (Fig. 24.1). Usually, viability is reduced in mutations with large effects. Careful studies have indicated that the frequency of mutations is inversely proportional to their effects.

In many cases mutants have different environmental requirements. For example, some of the barley mutants produced in Sweden have had a higher nitrogen requirement than untreated barley, while other mutants have suffered from too much nitrogen. Mutations changing the time to flower and mature will, of course, affect adaptation.

Chromosome changes. Chromosome changes have included duplications, deletions, translocations, inversions, isochromosomes, and breakage-fusion-bridge cycles. It is obvious that this provides a means of chromosome engineering whereby the plant breeder can change the structure of chromosomes to suit his purposes.

Aging of seed appears to have the same effects on chromosome aberrations and seed viability as X-radiation.³¹ It also increases the sensitivity of chromosomes to damage from radiation.

Physiological effects. The direct effects of a mutagen will vary greatly, depending on the mutagen, the part of the plant treated, the level of treatment, the post-treatment environment, and many other circumstances. Frequently, at certain levels of radiation of seeds, there is a stimulation to the early development of seedlings. As already mentioned, it is impossible to separate most physiological disturbances in M_1 from effects resulting from changes in the chromosomal material. Such separations are made in later generations or propagations.



Figure 24.1 Induced mutations of spike form in the two-rowed spring barley Haisa II. From left to right: spike branching, dense spike, lax spike, Haisa II, "gigas mutant," enlarged glumes, and short awn. (Courtesy Dr. Horst Gaul, Max-Planck-Institute for Plant Breeding, Cologne, Germany. *Radiation Botany*, 4 (1964), 155.)

Objectives and Achievements in Mutation Breeding

The description of the effects of mutagens will have indicated some of the objectives in mutation breeding. In most situations this method will complement other methods, but in some situations it may be the only recourse to the plant breeder.

Changes of a single gene or a small block of genes. This use of mutation breeding is intended to leave the genotype of the variety intact except for a change of a gene or a few genes that will improve the variety. It is obvious that this would be a procedure to use where a variety is already very good, or widely grown, but deficient in perhaps one respect, such as resistance to disease. Some plant breeders would even restrict the term "mutation breeding" to this objective alone.¹¹ In sexually reproducing plants it has been a substitute for the backcross method of breeding.

When considering vegetatively propagated crops, where a successful variety consists of only one heterozygous genotype, it will be realized that neither backcrossing nor any other conventional breeding method will

reconstitute the variety with or without an added character. This is the primary reason why mutation breeding has been of interest to breeders of such crops. To effect changes of one or a few genes, the breeder can use only spontaneous or induced mutations.

Table 24.2 Some examples of barley mutants with well-established higher yielding capacity.^{a, b}

CHARACTER AND SERIAL NUMBER	NUMBER OF YEARS TESTED	RELATIVE YIELD
Spring Barley		
Early, M ₈ 90	4	108
Large-kerneled, 44/7	10	107
Early, less straw-stiff, 44/4	9	109
Large-kerneled, early, 3978	6	115
Erectoides, 2660	5	105
Erectoides, 2654	8	103
Smooth-awned, 4033	7	107
Semi-smooth-awned, 3945	6	113
Early, W 3	4	107
Winter Barley		
Early, 506	5	114
Early, 481	8	107

^a Kernel yield of the mother line is taken as 100.

^b Adapted from National Academy of Sciences-National Research Council Publication 891, *Mutation and Plant Breeding*. H. Gaul, p. 206.

Gaul has listed 11 examples in spring and winter barley (Table 24.2) where mutants obtained directly from mutated varieties have exceeded the original variety in yield.¹⁰ He points out that Jutta, a variety of winter barley, exceeded the parent variety Kleinwanzlebener Mittelfruhe in winter hardiness, straw strength, and yield, and was grown on 10 per cent of the winter barley acreage in Middle Germany. Pallas, a barley variety released in Sweden because of its stiff straw, was developed by ionizing radiation.⁵

The first cereal variety released in the United States which resulted from the use of thermal neutron radiation was the oat variety Florad, developed from the variety Floriland, in Florida.^{7,8} Compared to Floriland, Florad is three weeks earlier and has higher yield. It has good quality and is resistant to crown rust, an important disease in Florida. Since there was sterility and chromosome aberrations in the material from which Florad was selected, there is a possibility that it did not develop directly from the radiation but from outcrossing to other oat materials in the nursery.

A peanut variety, NC4X, was developed by Gregory^{13,14} from a mutant obtained by X-radiation of a bushel of seed of a superior selection. From the nursery, planted to the bushel of seed, 84,213 plants were obtained, of which 11,000 were rated as mutants. From normal-appearing plants taken at random from the field, a selection was obtained which was later named NC4X. Compared to the original strain, it was consistently higher in yield.

Varieties of cross-pollinated crops developed by X-ray treatments are Regina II summer oil rape and Primex mustard, both in Sweden.¹ The evidence is not clear whether these varieties are products of recombination of variability already present or of radiation.²¹

Lapins used ionizing radiation to produce a compact dwarf type of growth in Lambert, a variety of sweet cherry.²⁰ The fruit was normal but the fruit stems were shorter. A dark-red version of the apple variety Cortland has been under test in Canada for a number of years.⁴ It was produced by thermal neutron treatments of dormant scions in early spring.

Using gamma radiation from a cobalt-60 source, carnations were mutated from white to red flowers, from red to variegated, and from standard-double to single-flowered types.²⁸ Also in carnations, a mostly male-sterile type was rendered male-fertile by X-radiation.^{29,30} Reference has already been made in Chapter 15 to the induction of self-compatibility by X-radiation.

Development of new alleles for transfer. Usually, the desired character induced by a mutagen will be combined with other desirable characters by conventional breeding methods. For example, the bush bean variety Sanilac was developed from a cross between an early bush-type mutant, induced by X-rays in the vine-type variety Michelite, and other strains resistant to anthracnose (*Colletotrichum lindemuthianum*).⁹ This was followed by four generations of backcrossing to the bush-type mutant and five generations of selection. The best selection was named Sanilac.

It is obvious that all these are macromutations, mutations that are qualitative in nature. Thus far, mutation breeding has had its greatest success with such mutants, and for two reasons: First, the mutation can be easily identified where suitable screening techniques are used, and vast amounts of material can be treated. Second, the plant breeder focuses his attention on one objective, and does not permit other variability in the treated population to complicate the issue.

Mutations affecting polygenes (they have been termed "micromutations") have been difficult to identify and incorporate into an improved variety. This, of course, would be expected, since variability based on naturally occurring polygenes has been difficult to manage by conven-

tional methods. Attention has been given to this use of mutation breeding only in recent years.

Changes in chromosome structure. Using X-rays, Sears was able to transfer a portion of an *Aegilops umbellatum* chromosome to a chromosome of common wheat (see Chapter 26). The translocated segment carried resistance to leaf rust.

In barley, reference has been made in Chapter 23 to interest in duplicating a segment of chromosome 6 which is known to carry genes for high α -amylase activity that could improve malting quality.²² The allele *V* for two-row heads in barley interacts favorably with the allele *v* for six-row in heterozygotes. By combining *V* and *v* on the same chromosome, it would be possible to have a pure line of genotype *VVvv*, which presumably would exhibit the vigor of the genotype *Vv*. Such additions to the chromosomes of barley will be greatly facilitated by current knowledge of gene locations on the linkage groups.

Some progress has been made in the diploidization of tetraploid barley.⁶ Two diploid varieties have been chosen, which combine to form a vigorous F_1 hybrid at the tetraploid level. In one variety the chromosome structure is altered by radiation treatments over several generations. Then the varieties are crossed, and chromosomes of crossed seeds are doubled by colchicine treatments. Doubled chromosomes from each variety should pair separately, and multivalent associations should not occur. If the tetraploid behaves as a diploid in meiosis, sterility will be reduced and the tetraploid will breed true. It will breed true because the paired chromosomes are exactly the same.

Gaul has suggested a method, which he calls "recurrent mutation induction."¹¹ By repeated mutagenic treatment, at least once per generation, gene mutations and chromosome aberrations are induced in autopolyploid populations from widely different sources. Then the treated autopolyploids, and the F_1 plants also, are crossed in as many combinations as possible, the purpose being to induce as much structural and genic variation as possible. Finally, selection is made for fertile, desirable types.

Increases in crossing-over. There is evidence that the use of X-rays or gamma rays with high temperature will increase the frequency of crossing-over in some regions of the chromosome, and particularly those adjacent to the centromere.⁴³ Crossing-over in a region where it normally does not occur will release variability in heterozygous plants. Such released variability would be very difficult to separate from variability induced by mutation of genes.

Résumé

Mutagens have been employed for some 40 years in the improvement of crop plants. In recent years a great many plant breeders have explored their use on crop plants in a superficial way, and some have intensively investigated their possibilities. Some success has been achieved in producing directly or indirectly a number of better varieties in both self-pollinated and vegetatively propagated crops. But this progress has not threatened to displace other breeding methods. In sexually reproducing crops it appears to be primarily a means of developing variability, which can then be utilized by conventional breeding methods.

When used to develop variability, it has been most effective where rather specific objectives were established. Where abundant variability already exists, as in cross-pollinated populations, it has not been easy to separate induced from natural variability.

Rapid advances are being made toward a full understanding of the process of gene mutation, and with this understanding will come control of the process. Where control is achieved, mutation breeding would replace many other methods of breeding.

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25

INTERSPECIFIC HYBRIDIZATION



Recombinations of the F_2 , however manifold they may seem, are in reality but a narrow segment of the total imaginable recombinations of the parental species. There are in other words, powerful restrictions to character recombination in the F_2 .²

ANDERSON, 1939

AS PLANT BREEDERS have exhausted the variability in a species, or as they have sought some character not present in it, they have turned to crosses between species. Where the parental species have been widely different, the achievement of a hybrid has been an exciting event. In many cases, however, the excitement has given way to disappointment, for

the hybrid has been sterile, or hybrid populations have not given the desired combination of the characters in the parental species. Man attempted and achieved hybrids between animal species at a very early date, but only one sterile hybrid, the mule, became of value to him. It was not until he recognized sex in plants that he made any planned and significant progress in exploring the possibilities of interspecific hybrids. In fact it was during his investigations of sex in plants that Koelreuter, in 1760, made the first reported hybrid between plant species, using *Nicotiana rustica* and *N. paniculata*.

It is difficult to make a neat classification of the populations obtained from interspecific crosses. They will range from those similar to populations from intervarietal crosses to those in which F_2 plants vary in chromosome number and a large number of genes. Two types of F_2 populations are considered in some detail: (1) those from parental species differing in several genes, and (2) those from parental species differing in genomic constitution.

Reasons for Making Interspecific Hybrids

Perhaps the main objective of interspecific hybridizations in field and vegetable crops is to improve a species by transferring to it one or a few characters from another species, cultivated or wild. Since the transfer of one or a few genes is of such importance economically, and since special techniques are sometimes involved, it is dealt with separately in the following chapter.

A second reason for making interspecific hybrids is to achieve new character expressions not present in either parent. New character expressions have been important among ornamental crops, where there has been a premium on novelty. With a host of differences in the genes in the parental species, unusual types may not be unexpected. Even a few complementary genes could produce types beyond the ranges of either parents in such quantitative characters as earliness, height, and yield.

Interspecific hybrids may be made for a third purpose. The hybrids, particularly when they are sterile, may be treated with colchicine to double the chromosome number, and thus achieve a new allopolyploid species. This has been discussed in detail in Chapter 22.

The fourth reason has its basis in curiosity and aesthetics. Plant breeders and geneticists have been curious to see what would happen, or they have been interested in exploring the relationship of one species with another, often of the domestic species with related wild species. Plant breeders need not apologize for this curiosity or this desire to explore the order of things, for these have been part of the advance of science and

human civilization. One practical value of such studies is that they may identify species that will be useful to the plant breeder.

Overcoming Difficulties in Obtaining F_1 Hybrids

Stebbins^{33,34} and Dobzhansky⁸ have discussed in detail the barriers that prevent the achievement of a cross between two species. These will be listed here to provide a background for discussions of the ingenious means employed by plant breeders and geneticists to overcome these barriers. These barriers are:

1. Failure of pollen to reach the stigmas of the other species.
2. Failure of pollen to germinate, the pollen tube to grow, and fertilization to take place.
3. Failure of the zygote to develop into a seed, and the seed into a mature plant.

Aware of these obstacles in the attainment of interspecific hybrids, what have plant breeders done to circumvent them? Chances of success have been improved by several techniques.

Cross using different varieties of two species. Certain varieties will "nick" better in crosses between species. For example, Hollingshead found that some crosses of *Crepis tectorum* to *C. capillaris* produced zygotes that died in the seedling stage, whereas other crosses gave hybrid plants that developed normally, except that they were sterile.¹⁴ A single gene present in some plants of *C. tectorum* was responsible for the lethal effect.

Failure of hybrids to grow normally may be a consequence of complementary genes coming from separate parents. It has been observed by Hermesen that some intraspecific hybrids of wheat varieties suffer variable degrees of necrosis, depending on the parents.¹³ Necrosis is manifested as gradual death of leaves and leaf sheaths. Two complementary loci, Ne_1 and Ne_2 , were found to be responsible, with several alleles at each. Various combinations of the alleles at the two loci produced a wide array of necrotic F_1 plants, from those that failed to produce seed to those that were essentially normal.

Cross in both directions. Often a cross is highly successful in one direction and will fail in the other. Usually a cross is more successful if the female parent has the larger chromosome number. From crosses of wheat species Thompson concluded that this is the result of chromosome balance in the endosperm.³⁸ Lewis points out that if one species has a self-incompatibility system, this species will inhibit the development of pollen

from a species without such a system.¹⁹ The reciprocal cross is not inhibited.

Pollinate at different stages in development of style and stigma. Often, early pollinations will succeed better than those made when the stigma is normally receptive. In cabbage (*Brassica oleracea* var. *capitata*) Pearson overcame self-incompatibility by bud pollination made from one to five days before anthesis, and this was attributed to the longer period of time available for the pollen tube to grow before degeneration of the ovules.²⁷ The same situation will hold for interspecific crosses in which the growth of the pollen tubes is slow.

Shorten length of style. This will shorten the distance that the pollen tube of the other species, often proceeding with difficulty, has to travel to effect fertilization. When pollen from various species of *Tripsacum* was placed on the silks of corn, the pollen tubes were unable to grow the great distance to the ovules.²² Fertilization was achieved simply by removing most of the silk.

Remove stigma before pollination and substitute a small block of agar with sugar and gelatin. This technique, in some unexplained way, has permitted interspecific hybridization in potatoes (*Solanum*) where pollen tube growth was inhibited.³⁷

Apply growth-promoting substances to pistils of female parent. Crosses of diploid and tetraploid pear varieties were facilitated by treatments of the ovaries with β -naphthoxyacetic acid.⁷ A concentration of 1 per cent naphthalene acetamide in lanolin, applied to the base of the style of the Easter lily, permitted several interspecific hybridizations that were previously impossible.⁹ Gibberellic acid applications to the culms of auto-tetraploid barley ($2n = 28$) increased pollen tube growth when pollinated by rye ($2n = 14$).¹⁸

Graft female parent to male species. There are several reports of interspecific hybrids being achieved more easily if the species to be used as the female is grafted onto the species used as the male.^{11,28}

Double chromosome number of one or both parents. Crosses of narrow-leaf trefoil, *Lotus tenuis* ($2n = 12$) and broadleaf bird's-foot trefoil, *L. corniculatus* ($2n = 24$), were not achieved until the chromosome number of *L. tenuis* was doubled.⁴¹

Use mixed pollinations. In cotton, Beasley found that certain interspecific hybridizations failed because only a few seed were produced in each boll, too few to prevent its abscission.⁴ To induce boll retention, he pollinated first with 6 to 12 grains of the female species. Then an excess amount of pollen of the male species was applied. The bolls adhered, and among the normal seeds were some small ones that produced hybrids.

Excise developing embryo and grow it on a nutrient medium. Laibach was unable to get crosses in the normal manner between the wild flax species *Linum perenne* and *L. austriacum*.¹⁷ He then removed the developing seeds from the unripe bolls about 14 days after pollination. These were placed in a vial on cotton wool moistened with a 10 to 15 per cent solution of cane sugar, the vial and its contents being sterilized previously. After another 14 days they became white and hard like normal ripe embryos. On removal from the vials they were washed in running water and laid on moist blotting paper for germination. Development was normal but slower than for seeds of parents. Since then, the excised embryo technique has been used in many ways to achieve interspecific hybrids. Examples are: the tetraploid cotton species, *Gossypium hirsutum*, crossed with various diploid species;⁴ crosses of *Solanum* species;¹⁵ interspecific hybrids of *Prunus*³⁰; *Hordeum jubatum* $\times$ *Secale cereale*⁵; and *Hordeum sativum* $\times$ *H. bulbosum*.¹⁶ Frequently the embryo is inhibited in its development by some incompatibility with the endosperm. Maheshwari and Rangaswamy have provided an excellent review of embryo development in plants.²¹

Graft hybrid to stems of either parent or another species in the genus. From crosses between the sweet clover species *Melilotus alba* and *M. dentata*, it is possible to obtain hybrid seedlings, but these are deficient in chlorophyll and die eventually. By grafting these seedlings to vigorously growing plants of *M. alba* and *M. officinalis*, Smith was able to carry three hybrids through to flowering.³²

Sterility of F₁ Hybrids

Even when the interspecific hybrid will develop to the flowering stage, it is usually found that it is partially or completely sterile. Indeed, where the fertility is normal, it usually indicates that the parental species differ in a few or many genes, and not in chromosome number and structure. Sterility is most pronounced in the F₁, though there are instances where the F₂ is less fertile than the F₁.

Chromosomal sterility. Hybrid sterility has been found to have either a chromosomal or a genic basis. Chromosomal sterility occurs as a result of differences in number or structure of chromosomes in the parents, and is manifested in meiosis of the hybrid by complete or partial failure of the chromosomes to pair, by associations of more than two chromosomes, by inversion loops in pachytene stages, by bridges and fragments in anaphase, and by micro- and megaspores having variable numbers of chromosomes. In general, but not always, the degree of chromosomal regularity in meiosis is directly proportional to the fertility of the hybrid.

There are examples of F_1 hybrids having complete, though perhaps loose, pairing of chromosomes at first metaphase of meiosis, but showing complete sterility. When crossed, *Primula verticillata* and *P. floribunda*, both with nine pairs of chromosomes, give an F_1 plant having nine loose bivalents. But the hybrid is completely sterile. It was suspected that some sort of complementary gene action was involved, until the chromosome number of the F_1 was doubled, when the hybrid was completely fertile.⁴⁰ It is assumed that in spite of the gross similarity of the chromosomes of the two species, there are important though small structural differences, so that the gametes lack some important genic materials necessary for viability. Stebbins has termed this situation *cryptic structural hybridity*.³³ Such structural hybridity arising as a result of two successive inversions is shown in Fig. 25.1. In a diploid species such cryptic structural hybridity would probably render 50 per cent of the gametes sterile, without affecting chromosome pairing. A similar situation in several chromosomes would make most of the gametes sterile, but again might not reduce pairing appreciably.

The converse does not occur. Where the F_1 has few or no chromosomes paired, the hybrid is never fertile, unless, of course, the chromosome number is unreduced, and gametes include all the chromosomes of the F_1 . Such gametes, if they meet, will produce an allopolyploid. Where the fertility of a sterile F_1 is restored by chromosome doubling, it may be assumed that the sterility has a chromosomal and not a genic basis.

Genic sterility. Hybrid sterility due to genic causes will include those cases where the chromosomes are not involved. It may be manifested by the failure of the plant to produce flowers, failure of meiosis to take place in either the anther or the ovule, or both, and failure of the meiotic process itself through asynapsis or desynapsis.

Overcoming sterility in the F_1 . Two procedures are possible if the F_1 hybrid is sterile. The first is to backcross to one or both parents. The female gametes in a hybrid frequently have a higher survival rate than the male. Where pollen has minute chromosome deficiencies or duplications, or where it carries an extra chromosome, it usually fails at some point in the development of the gametophyte: The pollen may abort, it may fail to germinate, or the pollen tube will not reach the egg. The egg, apparently because it is protected and sustained by maternal tissue, may survive with minor irregularities in its chromosomal and genic constitution. The hybrid from the cross of *Triticum aestivum* ($2n = 42$) $\times$ *T. timopheevi* ($2n = 28$) is self-sterile, but will set seed if crossed to either parent.¹

The second recourse is to double the chromosome number of the F_1 hybrid. Often the resulting allopolyploid is fully fertile (Chapter 22).

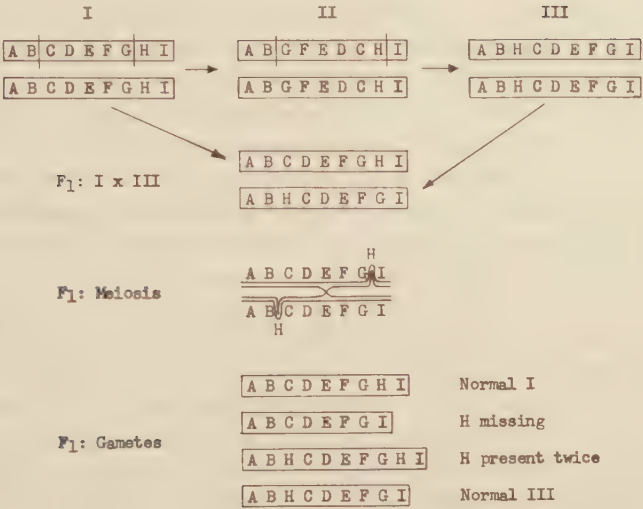


Figure 25.1 Diagram showing the development and consequences of cryptic structural hybridity, considering only one pair of chromosomes. I is the original chromosome pair with the positions shown of breaks involved in an inversion. II is the chromosome pair resulting from the inversion in I, with breaks shown of a second inversion. III is the chromosome pair resulting from the inversion in II. The F_1 of the cross $I \times III$ is normal because both chromosomes have a full complement of genes, though arranged differently. In meiosis of the F_1 the H segments of the chromosomes fail to pair, and form loops. A chiasma between the loops gives rise to two gametes with a normal complement of genes, one deficient in the genes of region H, and one duplicated for the genes of region H. Gametes deficient or duplicated for the genes of even a small region often are nonfunctional. If several chromosome pairs had similar differences in an F_1 , practically all the gametes would have deficiencies, duplications, or both. (G. L. Stebbins, Jr., *Variation and Evolution in Plants*. 1950. Adapted from Fig. 23 by permission of Columbia University Press, New York.)

Degeneration in F₂

From some interspecific crosses the F_1 may show normal development and normal or near-normal fertility, but its progeny will be less vigorous and fertile on the average, and some F_2 plants may be quite abnormal or sterile. Muller attributes this to the results of segregation of complementary gene systems of parental species.²³ He considers each species to be homozygous for certain genes that complement one another, with different genes or alleles being involved in each species. In F_1 the complement-

ing genes are present in the genomes from each parent, and development and fertility are not greatly affected. But in F_2 , some loci become homozygous without the complementing alleles at another locus being present. As illustrated by Stebbins,³⁴ one species may have the genotype $AABB$, with AA complementing BB , and the other species the genotype $aabb$, with aa complementing bb . It is assumed that aa is not complementary to BB nor AA to bb . A cross of the two species would give an F_1 with genotype $AaBb$, which is balanced and viable, but in F_2 , genotypes such as A_bb and $aaB_$ would show degeneracy and sterility.

On the other hand, from studies of crosses of cotton species, Stephens feels that degeneration is a consequence of deficiencies and duplications of chromosome material, each so small that chromosome pairing in meiosis is not affected.^{35,36} In other words, this would be a consequence of cryptic structural hybridity (Fig. 25.1) in which the gametes with deficiencies or duplications are functional but yield plants that show the adverse effects of the deficiencies or duplications. In cotton, this has resulted in hybrid populations which, on selfing, revert to types like the parental species. The most viable gametes are those having the chromosomes of one or the other parent.

In this situation large populations should be grown in the hope that a balanced assembly of genes and chromosomes will be achieved which will show the characteristics desired. In cotton, a few backcrosses to one or the other parent have usually resulted in the full recovery of the recurrent parent.³⁶

Interspecific Hybrids from Parents Differing in Many Genes

Perhaps the best examples of species differing in several genes are the wild and cultivated versions of many field and vegetable crops. Crosses of such species release a vast array of types, many of them representing steps in domestication. It is assumed here that the parental species have the same chromosome number and structure and that the chromosomes pair in a regular manner in meiosis of the F_1 hybrid. Obviously, differences between the parental species will be based on gene differences throughout the length of all the chromosomes. The effects of the individual gene differences may be large or small.

Among the populations of wide crosses usually grown by a plant breeder, every plant looks different. As a consequence, such populations are difficult to classify and manage. Generally, parental extremes are rare or fail to appear. Certain combinations of the characteristics of the parents may not be present. Probably because they are difficult to classify neatly, there are not many detailed analyses of hybrid populations from parents differing in many genes.

One of the best studies has been provided by Anderson.² He crossed two wild species of tobacco that differ greatly in floral morphology (Fig. 25.2). *Nicotiana alata* has a long floral tube, a wide limb (floral disc), and deep floral lobes, whereas *N. Langsdorffii* has a short floral tube, narrow limb, and shallow lobes. Other differences between the parents were not included in studies of hybrid populations.

The extremes expected from recombination of the floral characters of the parents are compared in Fig. 25.2 with the extremes actually ob-

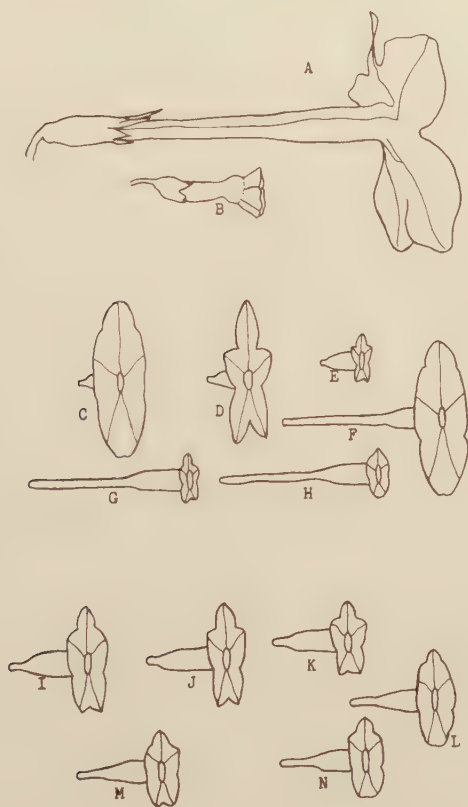


Figure 25.2 A, typical flowers of *Nicotiana alata*. B, *N. Langsdorffii*. C-H, extreme recombinations to be expected with complete recombination of tube length, limb width, and lobing of parental species. I-N, actual extremes of recombination found in 147 plants of the F_2 generation. (Combined from three figures in Anderson.²)

tained. It was obvious that there were severe restrictions on the character combinations that could be achieved.

The absence of parental extremes, considering most quantitative characters under study by Anderson, is due in large part to the small populations he used and the large number of genes involved. This has been considered in Chapters 7 and 10.

Classifications of parental species, F_1 and F_2 , for tube length and limb width are shown in Fig. 25.3. Had there been free combination of the

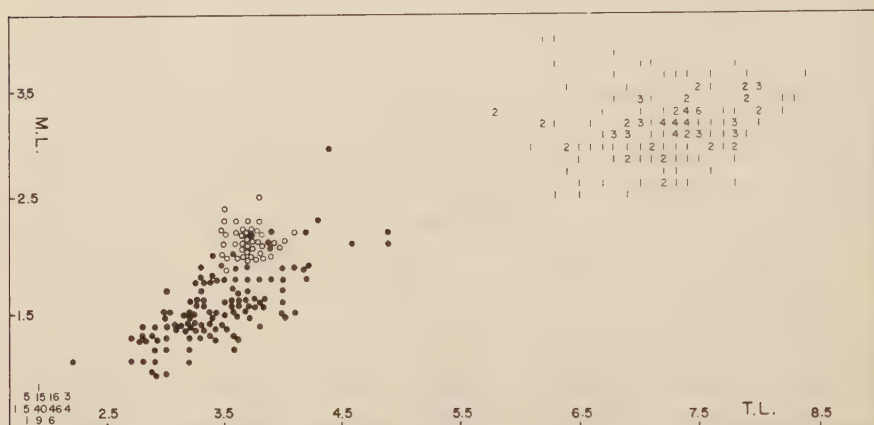


Figure 25.3 Correlation between limb width (maximum lobe) and tube length in *Nicotiana Langsdorffii*, *N. alata*, one F_1 family of 41 plants and one F_2 family of 118 plants. Open circles represent the F_1 , solid circles the F_2 numbers. *N. Langsdorffii* (lower left) and *N. alata* (upper right). (From Anderson.²)

tube length and limb width of the parental species, the F_2 population would have been distributed over the entire diagram. Instead, it was confined to an ellipse clustered near the F_1 , with the ends of the ellipse pointing in the direction of the parents. None of the F_2 could be considered a parental type. Classifications of other floral characters gave a similar pattern. This led Anderson to say, "It is therefore certain that the recombinations which we have observed are only an insignificant fraction of the recombinations possible under free assortment."²

Anderson lists four factors that restrict character recombinations in F_2 : (1) gametic elimination, (2) zygotic elimination, (3) pleiotropy, and (4) linkage. His studies did not permit precise measurements of the first two factors, and probably neither was important in restricting variability in F_2 .²

Undoubtedly, pleiotropy had an important effect in restricting unusual

combinations such as long tube length and narrow limbs, and short tube length and wide limbs. Genes were considered to have primary effects, which were manifested in a somewhat similar way on the development of two characters. For example, the genes that govern tube length have similar effects on limb width, with the result that tube length and limb width increase or decrease together. Pleiotropy results in positive correlations in plant characters.

Linkage is also heavily involved in restrictions on free recombination in F_2 . Assuming that perhaps 50 to 100 genes are involved in the inheritance of each floral character, this means that each arm of each chromosome has one or more genes affecting each character. This also means that an assembly of genes modifying a character toward one parent in F_2 will include with it other assemblies of linked genes that modify other characters in the direction of the same parent. Strong selection for flowers with long tubes, like *N. alata*, results in selection for the wider limb and deeper floral lobes of this species. As with pleiotropy, linkage gives correlations of character dimensions in F_2 . However, the restrictions imposed by linkage can be overcome to some extent, given time and large populations. The restrictions due to pleiotropy cannot.

The question, then, is how to proceed in managing F_2 populations from crosses involving parental species differing in many genes. It is assumed that the cross is between a wild and domestic species. The answer is governed by the objectives.

If the purpose of the cross is to achieve the extremes of two quantitative characters in the parents, say, long floral tubes and narrow limbs as in Fig. 25.2, it would be advisable to cross the segregate having the longest tubes with the segregate having the shortest limb width. This would be expected to shift the ellipse to the right. Succeeding generations of similar crosses should shift the ellipse still farther in the same direction. Another procedure is to intercross plants having tube lengths about 3.9 to 4.1 cm and limb widths of 1.4 to 1.6 cm. Since these probably have slightly different genotypes, their progeny may also shift the ellipse to the right.

More likely, the objective in crosses of wild and domestic species will be the reconstitution of the domestic species, with the addition of one or a few characteristics of the wild species. The obvious breeding procedure is to backcross to the domestic species and select for the character of interest in the wild species.

Interspecific Hybrids Where the Parents Differ in Genomic Content

It has already been pointed out that species must have several chromosomes in common for a hybrid between them to be fertile. For species

hybrids at the diploid level, chromosomes cannot differ in content or structure without giving rise to gametes deficient in genes necessary for their proper function and survival. As a consequence, hybridizations of distinctly different diploid species are usually sterile.

Among polyploid species, fertile hybrids are often obtained between species at different levels of polyploidy. However, fertile F_1 hybrids are obtained only if the two species have genomes in common. *Triticum aestivum* ($2n = 42$) will cross with *T. durum* ($2n = 28$) because both have the genomes A and B, or 14 chromosomes in common. The results from this cross will be considered in some detail because it is typical of many similar crosses of polyploid species.

At first metaphase in meiosis (Fig. 25.4) there are typically 14 pairs of chromosomes (14_{II}) and 7 single chromosomes (7_I). The paired chromosomes belong to genomes A and B, and the single chromosomes to genome D. At first anaphase, the paired chromosomes separate, and members of each pair go to opposite poles. At the same time, the single chromosomes divide equationally, and one group of 7 goes to each pole. At second anaphase, the 14 chromosomes of the A and B genomes divide equationally, and the 7 chromosomes of genome D migrate at random to either pole. In actual fact this represents the ideal situation: The number of pairs may be somewhat less than 14; multiple associations of chromosomes may occur; and the single chromosomes may lag at first anaphase and be lost.

In the distribution of D chromosomes at second anaphase, it is expected that, most often, 3 will go to one pole and 4 to another, followed by a 5:2 distribution. (The expected numbers may be determined from the expansion of the binomial $(\frac{1}{2} + \frac{1}{2})^7$, where the chances of each chromosome going to one or the other pole is $\frac{1}{2}$.) As a consequence, gametes would be expected to have intermediate numbers (16, 17, 18 and 19) of chromosomes.

If gametes with intermediate numbers of chromosomes were in greatest abundance, it would be expected that (1) most of the progeny of the F_1 hybrid would also have intermediate numbers of chromosomes, and (2) progeny with the chromosome numbers of the parental species would be rare. In actual fact, the opposite is the case; plants with 28, 29, and 30 chromosomes, on the one hand, and 40, 41, and 42 chromosomes, on the other, are most abundant. In succeeding generations the hybrid population will be mostly two types, one with 28 chromosomes, and the other with 42. One reason for this situation is that the gametes with the low and high numbers of chromosomes are more balanced than those with intermediate numbers; they have two or three genomes, or approach this condition. The second reason for only two types developing in the

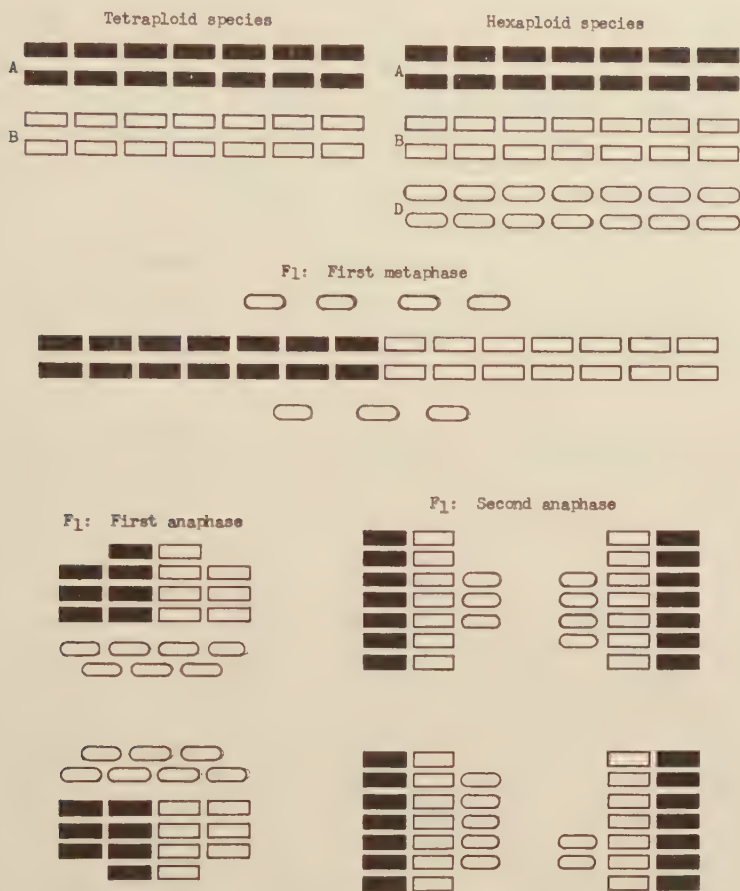


Figure 25.4 Schematic representation of chromosome behavior at first metaphase, first anaphase, and second anaphase of an F_1 hybrid from a tetraploid wheat species (with genomes A and B) crossed to a hexaploid species (with genomes A, B, and D).

hybrid population is that combinations of gametes with intermediate numbers of chromosomes often give rise to zygotes that fail to develop or are sterile.

In the hybrid population from the cross of *T. aestivum* with *T. durum*, the types with 28 chromosomes will resemble *T. durum* (since it has the genomic constitution AABB) and the types with 42 chromosomes (or genomic formula AABBDD) will look like *T. aestivum*. This means that it is difficult or impossible to develop an intermediate type that breeds true.

This tendency for two types to develop is hastened by the fact that wheat is self-fertile. Where the population is cross-pollinated, the effect is similar except that cross-pollination will permit the occurrence of combinations of gametes with different chromosome numbers. In other words, even if two types like the parental species develop in the population, cross-pollination will permit F_1 plants to be produced. The frequency of the F_1 types will depend upon the competitive ability of the male gametes with different chromosome numbers in pollination and fertilization. If a plant tends to be fertilized with gametes having its own chromosome number, the effect again will be for two types to predominate in the population, these having the chromosome numbers of the parents. In any case, it will be impossible to fix a type intermediate between the parental species.

This tendency for parental-type gametes to be produced in greater abundance than expected will occur when parental species have the same chromosome numbers, but have different genomes. Studies of F_1 and later generations of crosses of species of brome grass with 56 chromosomes (*Bromus inermis*, *B. tyttholepis*, and *B. pumpeilianus*) indicated that unless gametes were constituted of multiples of genomes (of 7 chromosomes) they were largely nonfunctional.²⁴⁻²⁶ Further, most of the functional gametes had genomic combinations like those of the parental species. This meant that under a system of selfing, hybrid populations took on the characteristics of the parental species. Under outcrossing, as expected, there occurred many combinations of gametes with different genomic constitutions. Hybrid populations, when backcrossed to a parental species, gave more progeny like the parental species than expected. As Nielsen et al.²⁶ say, "The polyploid grasses are visualized here as composed of a series of genome systems that form the chromosome complement. Each component, though very similar, is characterized by a specific synthesis system that tends to function as a cohesive unit."

Asexual or Long-lived Perennials

All the discussion to this point has been directed toward types that are short-lived and reproduce sexually. Where a crop is reproduced asexually and not grown for its seed, fertility is of less importance. Once a desirable type is achieved, whether it is an F_1 or a selection from a segregating generation, it may be perpetuated indefinitely. Examples are numerous among ornamental and fruit crops. Ornamentals such as narcissus, daffodils, tulips, iris, roses, primroses, delphiniums, and rhododendrons have been improved by interspecific hybridizations, as have apples, cherries, plums, peaches, grapes, many berries, citrus, and bananas. The same

is true of certain trees used for wood, such as species of poplar (*Populus*) which are reproduced by cuttings.

With long-lived trees, even if they cannot be reproduced asexually, a great deal of effort may justifiably be expended in the production of vigorous F_1 hybrids. The effort is justified simply because of the high yield of the individual tree. Many interspecific hybrids of pines (*Pinus*) are more vigorous than the parents, and the seed can be produced cheaply.²⁹ Even among forage grasses, the value of sterile interspecific hybrids has been apparent. Love found that two species of needle grass (*Stipa*) in the same plot produced hybrids that were more vigorous than the parents.²⁰ He felt that they were numerous enough to increase forage yield materially. They remained green for a longer period than the parents.

Among vegetables, the potato and sweet potato, which are reproduced as tubers, any desirable product of interspecific hybridization can be exploited immediately and indefinitely. The same is true of such field crops as sugarcane, ramie, hops, agave, abaca, and the mints.

Unexpected Results of Interspecific Hybridization

Unpredicted types. Studies of the products of hybridization of some natural species have shown that unpredicted types can occur. Some of these, as summarized by Stebbins, are unusual leaf forms in violet (*Viola*), "new" types of iris (*Iris*) in the Mississippi Delta area, "species" of berry (*Rubus*) in the eastern United States, and unusual carpel and sepal numbers in snapdragon (*Antirrhinum*) and peony (*Paeonia*).³³

Increase in mutation rate. The gene for petal spot in upland cotton (*Gossypium hirsutum*) became more mutable¹² when transferred to the genetic background of Sea Island cotton (*G. barbadense*). A hybrid between *Tradescantia canaliculata* and *T. humilis* showed three times as many gross structural changes as had occurred in either parent.¹⁰ Even if this were proved to be generally true, it would be difficult to exploit. Increased variability from mutation would be masked by the greater variability occurring as a result of many new combinations of genes.

Résumé

Interspecific hybridization has been complicated by the barriers that surround most species and prohibit or reduce crossing with related species. These barriers manifest themselves in many ways, from failure of the germination of the pollen of the male parental species to degeneration of the F_2 . One problem of the plant breeder has been to overcome these

barriers. A second has been to manage the great variability of segregating populations to achieve the objectives of the cross.

Where species differ in terms of a few genes, hybrid populations are similar to those from crosses of varieties. However, if widely different species will cross to give F_2 populations, the populations will fall between two extremes. Where differences are based on a large number of genes, the hybrids fall into an ellipse intermediate between the parents, mostly clustered around the F_1 , with the ends of the ellipse pointing toward, but not reaching, the parental types. Conversely, where the species differ in chromosome structure or number, segregating populations tend to resemble the parents, and intermediate types are scarcer than expected.

As a tool of the plant breeder, interspecific hybridization has been utilized most in the improvement of plants reproduced asexually. This has included the ornamentals, forest trees, and tuberous vegetables. Where interspecific hybridization has been used to improve crops reproducing sexually, it has been applied in the transfer of one or a few genes from one species to another.

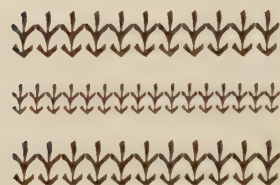
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26

INTERSPECIFIC TRANSFER OF CHARACTERS



The transfer of characters from one species or genus to another is not only of great potential practical importance, but is of considerable genetic interest as well. In general, the wider the transfer, the more interesting it is genetically. From a practical standpoint, too, the greater the distance over which transfers can be made, the greater the possibility of introducing useful characters not present in the host species. It is therefore important to extend the limits of transfer as far as possible.

SEARS, 1956

EXCEPT FOR HYBRID CORN, no plant-breeding accomplishment has had more impact on agricultural production in the United States than the

transfer of genes for stem-rust resistance to bread wheat from other species. During years of rust epidemics—usually when moisture supplies were ample—it has meant the difference between burning a blighted field and harvesting a “bumper” crop. One has to see or experience the difference between, on the one hand, financial loss and frustration and, on the other, economic gain and fulfilled expectations to realize the full significance of some gene transfers from one species to another.

These gene or character transfers may be considered as introgression applied to domestic species;² the transfers leave the recipient species essentially unchanged except for the transferred character. Depending on the relationship of the species involved, some will be simple transfers, others more complex.

Gene Transfers Between Species with the Same Chromosome Number

In a few cases interspecific crosses have been no more complex than intervarietal crosses. For example, a number of plant breeders have successfully transferred resistance to leaf mold, *Fusarium* wilt, and spotted wilt from the red currant tomato (*Lycopersicon pimpinellifolium*) to the common garden varieties (*L. esculentum*). Rick and Butler point out that it has been the common experience of these workers that no barriers of any consequence exist to the hybridization of these species, to the production of progeny by the hybrids, or to gene recombinations.¹³ The only departures from normal behavior are a reduction in pollen fertility to 85 to 90 per cent and a reduction in chiasmata number.

From *L. pimpinellifolium*, Langford was able to transfer a gene, Cf_{p_1} , for resistance to tomato leaf mold caused by *Cladosporium fulvum* to the domestic species (*L. esculentum*).⁸ This was done by backcrossing. Unfortunately, the gene Cf_{p_1} reacted with a recessive gene *ne* in domestic tomatoes to produce a severe type of necrosis. The necrosis began as small, angular, yellowish to brownish spots on the lower leaves, and in later stages most of the older leaves were entirely necrotic and distorted in shape. When the dominant allele *Ne* was transferred from *L. pimpinellifolium* to *L. esculentum* along with Cf_{p_1} , leaves were normal in appearance. This is a case where a transferred gene was of little use without an important modifier gene.

In sweet clover (*Melilotus*) Smith readily transferred the genes for low coumarin content from *M. dentata* to *M. alba*.¹⁶ The transfer to *M. officinalis*, a species more drought-tolerant than *M. alba* and better adapted to the Great Plains, has been complicated by the failure of *M. officinalis* to cross with *M. dentata*. However, by using embryo culture, Webster was able to obtain two F_1 plants from a cross of low-coumarin

M. alba and *M. officinalis*.¹⁸ These hybrids, which were highly self-fertile, provide a means for the transfer of a gene from one species through a second to a third.

Gene Transfers Between Species with Different Chromosome Numbers

Some of the best examples of transfers of genes between species with different chromosome numbers are provided by wheat and related genera. In the following discussion it will be necessary to keep in mind the genomic pattern developed for these in Chapters 22 and 25.

In considering a polyploid series of species such as exists in wheat (*Triticum*), it is not difficult to visualize the transfer of genes from the lower polyploids to the higher if crosses are possible between them and some fertility exists. The reverse is not always true, particularly if gene control is found only in the odd genome. This may be illustrated by the theoretical transfer of kernel color between members of the emmer group and common wheat (*T. aestivum*); "emmer" is used in a general sense to refer to most wheat species with 14 pairs of chromosomes. Kernel color is used because it is easy to visualize.

It may be assumed that it is desired to transfer the dominant gene for red color (*R*) from common wheat to a white-kerneled emmer with the genotype $R_aR_aR_bR_b$. The subscripts *a*, *b*, and *d* will denote the genome to which the chromosome belongs and which carries the gene in question.

Red Common Wheat		White Emmer	
$R_aR_aR_bR_bR_dR_d$		$r_ar_ar_br_br$	
F_1	$R_ar_aR_br_brR_d$		
21-Chromosome Gametes		14-Chromosome Gametes	
$R_aR_bR_d$		R_aR_b	
$R_ar_bR_d$		R_ar_b	
$r_aR_bR_d$		r_aR_b	
$r_ar_bR_d$		r_ar_b	

Only the 21- and 14-chromosome gametes are shown because these are the ones that will function most frequently in reconstituting the two species. Combinations of 14-chromosome gametes will, of course, give a type like the species of the emmer group. Since three out of the four 14-chromosome gametes carry one or two genes for red kernel color, 15 out of 16 of the emmer derivatives of the cross will have red kernels. The reverse transfer would be equally simple, if the common wheat had white kernels and emmer had red.

The transfer of white kernel color from a member of the emmer group to common wheat will not be possible if the gene for red (*R*) is present

in the D genome. This would be true regardless of the genotype of the chromosome of the other two genomes because all 21-chromosome gametes would carry the R_d gene. Actually, the difficulty encountered in transferring the complete stem-rust resistance of members of the emmer group to common wheat may have a similar basis. The fact that the transfer was accomplished (see Chapter 28 for details) indicates that either translocations of parts of chromosomes from A or B genomes to the D genome occurred, or inhibitors or modifiers of resistance in the D genome were lost.^{1,6,10,12,17}

Stem and leaf hairiness was transferred from *Gossypium raimondii* ($2n = 26$) to *G. hirsutum* ($2n = 52$) by first producing an allopolyploid of the two species.¹⁴ The allopolyploid was achieved by doubling the F_1 of a cross ($3x = 39$) to form a hexaploid ($2n = 78$). The hexaploid was backcrossed to *G. hirsutum* to produce a pentaploid ($5x = 65$). The pentaploid was backcrossed again to *G. hirsutum*, and selected backcross F_1 plants were selfed for two generations. Apparently, through crossing over between chromosomes of *G. raimondii* and *G. hirsutum*, a gene for hairiness in *G. raimondii* was transferred to a chromosome of *G. hirsutum*.

Gene Transfers Between Two Genera

With the realization that bread wheat is an allopolyploid resulting from the combination of species of the genera *Triticum* and *Aegilops*, it was natural that plant breeders should seek to transfer characters from *Aegilops* species to domesticated wheat. McFadden and Sears proposed an approach whereby different genomes of both *Aegilops* and *Agropyron* species might be combined with the genomes of wheat species, and the synthesized allopolyploids used in crosses with the species of bread wheat, *T. aestivum*.¹¹ Products of such crosses would serve as basic material for the transfer of *Aegilops* and *Agropyron* genes to common wheat.

Perhaps the most elegant method of transfer of disease resistance from one species to another is that provided by Sears.¹⁵ He wished to transfer resistance to leaf rust (*Puccinia triticina*) from *Aegilops umbellulata* to *T. aestivum* wheat. *Ae. umbellulata*, with seven pairs of chromosomes (genome C^u), bears little similarity to wheat, so it was not surprising when crosses in both directions gave inviable seed.

An allopolyploid of *T. dicoccoides* (genomes A and B) and *Ae. umbellulata* was used as a bridge. The leaf-rust resistant allopolyploid was easily crossed to *T. aestivum*. The hybrid had 42 chromosomes with no better pairing than 14_{II} and 14_I at meiosis—there were many cells with less than 14_{II} . After two backcrosses to *T. aestivum*, using the latter as the female parent, a resistant plant was found with 21_{II} and 1_I . The 21_{II} were chromo-

somes of *T. aestivum* and the 1_I was an *Aegilops* chromosome. Resistance was narrowed down to this one chromosome. In the resistant progeny of this plant, one plant had 21_{II} and one isochromosome. This isochromosome was duplicated for the long arm of the *Aegilops* chromosome, which meant that resistance was narrowed to this one arm. It was apparent that this isochromosome or the normal *Aegilops* chromosome would not pair with the chromosomes of *T. aestivum* to permit crossing over and the transfer of genes for resistance. Sears then resorted to a technique that has been developed to facilitate chromosomal exchanges; he treated the plants carrying the isochromosome with X-rays.

Sears hoped that this treatment with X-rays would effect the translocation of a segment of the isochromosome to a wheat chromosome and that the translocated segment would carry the gene or genes for resistance. He used plants carrying an isochromosome because, with resistance present in both arms, there would be twice as many chances of effecting such a translocation. The pollen from irradiated plants was used on *T. aestivum*.

Of the 6,091 plants obtained, 132 were resistant to rust, and of the latter, 40 had a translocation of part of the isochromosome to part of a wheat chromosome. It is an interesting side light to such experiments that most of the 132 resistant plants had chromosome abnormalities other than those involving the *Aegilops* chromosome; these consisted mainly of rings or chains of four or more chromosomes, heteromorphic bivalents, and monosomes. Several different types of translocation were found (Fig. 26.1), but the one of most interest to the plant breeder is termed *intercalary*. In this type of translocation a small portion of the *Aegilops* chromosome has been lifted out and inserted in a wheat chromosome. Of the 40 translocations obtained, one intercalary type was cytologically undetectable; in meiosis it formed 21_{II} in the normal manner. Pollen transmission was normal also. Its only difference from the normal *T. aestivum* was its high resistance to leaf rust and its slightly later maturity. By deft use of polyploidy, backcrossing, cytological examinations, and irradiation, a successful gene transfer had been made between two genera.

Using a somewhat similar technique, Driscoll and Jensen transferred resistance to both wheat leaf rust (*Puccinia recondita*) and wheat powdery mildew (*Erysiphe graminis tritici*) from rye (*Secale cereale*) to common wheat (*Triticum aestivum*).^{3,4} Both Elliott⁵ and Knott⁷ have transferred genes for stem-rust resistance from *Agropyron elongatum* to common wheat. Hybrid materials carrying the rust resistance of *A. elongatum* were radiated, and a segment or segments of the chromosomes of *A. elongatum* translocated to wheat chromosomes.

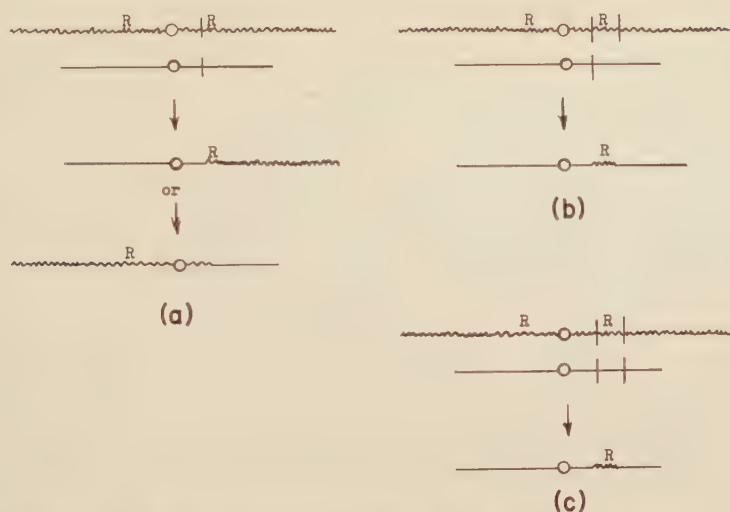


Figure 26.1 Some translocations of a small portion of an *Aegilops* isochromosome carrying a gene (R) for leaf-rust resistance to a wheat chromosome. A, reciprocal translocations; B, intercalary translocation without the loss of any of the wheat chromosome; C, intercalary translocation with the deletion of a portion of the wheat chromosome. The perpendicular lines indicate breaks in the chromosome caused by X-rays. (After Sears.¹⁵)

Résumé

Without exception, backcrossing is heavily involved in the interspecific transfer of characters, whether the transfer occurs under natural conditions or as a consequence of man's handiwork. It is a process of interspecific hybridization that leaves one species essentially unaltered and improved by the addition of one or a few genes of a second species.

In general, the difficulty in making transfers from one species to another increases with morphological and cytological differences between them. Where the differences are slight, the transfer is no more difficult than between varieties of the same species. Where the differences are great, it may take special techniques, involving bridging through polyploidy, cytological examinations, and radiation to effect the transfer.

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GENETICS OF RESISTANCE
TO DISEASES AND INSECTS



*The genetic diversity and phenotypic variability of many of the most destructive pathogens are so great as to create extremely complex problems of disease control in the present and to raise the question as to how complex they can become in the future. How much virulence can nature put into plant pathogens and how much resistance can man put into crop plants? **

STAKMAN AND HARRAR, 1957

ALL THROUGH HIS HISTORY man has feared the disease or insect that would suddenly appear and destroy his crops. Where a society depended heavily upon one crop, such attacks often led to famine and death. In

* E. C. Stakman and J. George Harrar, *Principles of Plant Physiology*. Copyright © 1957, The Ronald Press Company, New York.

recent times, with famine unlikely because of improved transportation, diseases and insects have caused serious economic losses where the attacks were severe. Even in the absence of epidemics, losses each year from diseases and insects are tremendous. It is no surprise, then, to realize that there are very few plant breeders who do not have as an objective a greater resistance to some disease or insect.

Breeding for resistance to disease and insects has been difficult because two organisms are involved, the host and the pest. The genetic systems of both must be kept in mind, and the environment must be managed carefully with a view to its effect on both the crop plant and the disease or insect.

The first report of genes controlling resistance to a pathogen appeared in 1905, when Biffen published an account of his experiments with yellow rust (*Puccinia glumarum*) of wheat.³ When the susceptible varieties, Michigan Bronze and Red King, were crossed to Rivet, an English variety long known to be resistant, he observed that the F_2 plants segregated in a ratio of approximately three susceptible to one resistant. From these data and tests of later generations he concluded that resistance to this disease was governed by a single recessive Mendelian gene. By 1934, Hansen found that there were more than 200 reports on the inheritance of resistance to disease.²² There will be at least three times as many reports now as in 1934. By comparison, reports of insect resistance being due to a specified number of genes are not numerous.

Often there is a three-way relationship among a disease, an insect, and a host. The insect may move the disease to the host, or may damage the host so that disease develops. In the case of many viruses a particular insect species may be required for transmission from one plant to another. Carter has discussed these relationships in detail.¹⁰

Disease Organisms

Diseases of plants are sometimes considered to include all abnormal conditions brought about by unfavorable environment or parasitic agencies. The definition of disease in this book narrows the definition to include only abnormal conditions caused by a pathogen, which may be a virus, bacterium, or a fungus. Since fungi cause most diseases and have been the subject of a great deal of research into host-pathogen relationships, most of the remarks here will pertain to them.

Life cycles of fungi. During their life cycle, fungi may have one or three nuclear phases: *haploid*, *diploid*, and *dicaryotic*. The diploid phase involves the union of haploid nuclei, analogous to fertilization in higher plants. Reduction to the haploid phase requires meiosis. A dicaryon is the inclusion of two haploid nuclei in the same cell without fusion. A

diploid and a dicaryon, because they have their chromosomes in duplicate, manifest similar properties. For example, if one haploid nucleus in a dicaryon has a dominant gene and the other a recessive gene, the effect of the dominant gene is manifested as if the two genes were in a diploid nucleus. The haploid, diploid, and dicaryon are symbolized as n , $2n$, and $n + n$, respectively.

About 20 per cent of all known species of fungi are asexual. In such species it is not possible, unless close relatives with a sexual phase give a clue to the situation, to know whether they are haploid or diploid. Most pathogenic species of fungi, however, divide most of their life cycle between the haploid and dicaryotic phase, and spend the balance in the diploid phase. In such types the pathogenic phase may be haploid or dicaryotic, or both. A diagram of the life cycle of stem rust of wheat is given in Fig. 27.1. This pathogen is infective of the barberry, a secondary host, in the haploid phase, and infective of wheat in the dicaryotic phase. The diploid phase, following fusion, ends when the fused nuclei complete meiosis.

Where haploid cells, fusing to form a dicaryon, are on the same mycelium, they are called homothallic; where they must be on different mycelia (+ and -), they are heterothallic. Heterothallism is analogous to separation of the sexes in higher plants.

Physiological specialization. As early as 1894, Eriksson in Sweden showed the intergeneric specialization of stem rust (*Puccinia graminis*) in relation to wheat, oats, rye, and other species. He gave these specialized strains subspecific rank, that is, *tritici*, *avenae*, *secalis*, etc.¹⁵ Barrus, in 1911, was the first to record distinct *physiologic races* (or strains, as he called them) within a species of a pathogen.² On the basis of differential reaction to bean varieties, he distinguished two strains of bean anthracnose (*Colletotrichum lindemuthianum*). On wheat, Stakman took the leadership in identifying a very large number of physiologic races of stem rust.^{47,52}

Physiologic races are identified by their reaction to a group of varieties known as *differential hosts*. At first, hosts were chosen without precise knowledge of the genetic basis of resistance. In recent years, greater precision has been achieved by using hosts having known genes for resistance.

The way in which races are identified may be illustrated by the use of three wheat varieties that are known to have different gene pairs for resistance to bunt (*Tilletia caries*). These varieties and their reaction to hypothetical bunt races are listed in Table 27.1.

Briggs pointed out that with two levels of interaction, resistant and susceptible, one variety will differentiate two races; two varieties, four

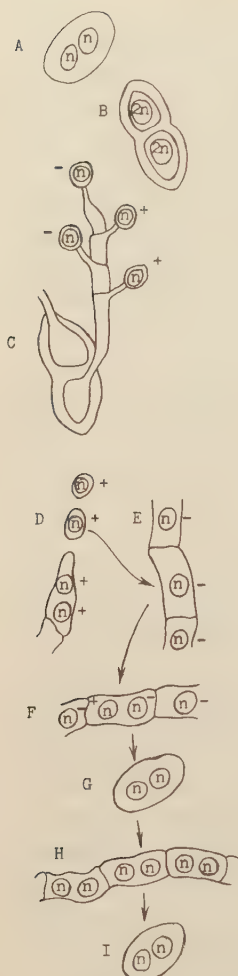


Figure 27.1 Life cycle of wheat-stem rust (*Puccinia graminis tritici*).

A. Urediospore (a dicaryon because it has two haploid nuclei). It is produced in great abundance in uredia, or pustules, on leaves and stems. Urediospores constitute the red-rust or summer stage of the disease. They are borne on dicaryotic mycelia growing through the tissues of the host.

B. Teliospore (a diploid because each nucleus is a result of fusion of the two paired haploid nuclei). They occur in pairs, are thick-walled, dark in color, and are referred to as the black-rust stage. This is the over-wintering stage.

C. Basidiospores or sporidia are borne on a short mycelium developing from the teliospore following meiosis. Nuclei are of two mating types, + and -.

Sporidia infect barberry, the secondary host, and a mycelium with haploid uninucleate cells develops. The mycelium forms a pycnium, a flask-shaped structure, which produces pycniospores (D) and flexuous hyphae (E), both with single haploid nuclei. When a pycniospore and hypha of opposite mating type meet, fusion occurs, and the nucleus of the pycniospore migrates into the hypha (F).

The mycelium from the binucleate (dicaryotic) cell develops into a cup-shaped aecia. Binucleate aeciospores are produced by aecia, and these infect wheat (G).

H. The mycelium from the germinating aeciospore is binucleate, and gives rise to binucleate urediospores (I).

racess; three varieties, eight races (as in the example above); and n varieties, 2^n races.⁷ This assumes that each differential variety has different genes for resistance.

Biotype. The term biotype is often used to designate differences in levels of virulence of a particular race. For example, races 15A and 15B of stem rust of wheat were at first considered biotypes of race 15 because they attacked the same differential hosts, with race 15B more virulent. Later, 15B was raised to full status as a race, and biotypes were found in it.

Table 27.1 Identification of bunt with differential hosts.

DIFFERENTIAL HOST	RACES OF BUNT							
	1	2	3	4	5	6	7	8
Martin	R*	R	R	S*	R	S	S	S
Selection 1403	R	R	S	R	S	R	S	S
Turkey	R	S	R	R	S	S	R	S

* R = resistant; S = susceptible.

Insects

An important distinction between insects and microorganisms is that the former are almost always propagated sexually, whereas the latter are propagated asexually, at least much of the time. As a general rule, then, microorganisms may often be handled as clones, and insects as outcrossing populations. As a population, with the possibility for recombination in each generation, there is room for a great deal more variability in insect populations. It is not surprising that resistance often has a complex inheritance.

Like pathogenic organisms, insects have shown the existence of physiologic races. This was obvious in studies of the hessian fly, where the variety Dawson of common wheat was resistant in California but susceptible in the Corn Belt. In studies of species of the root-knot nematodes attacking alfalfa in California, there were found three races of *Meloidogyne incognita acrita*, two races of *M. javanica javanica*, and two races of *M. hapla*.²⁰ There appears to have been no studies of crosses of insects to determine the inheritance of differences between races.

The role of natural selection in changing insect populations is well established. The best-known example is the development of resistance to DDT in the housefly after continued use of this insecticide. As a consequence it is not surprising to learn that four generations of the hessian fly grown on the resistant variety W38 produced a strain capable of causing higher levels of infestation than the original wild Indiana fly.¹¹

Genetics of Fungi

A knowledge of the genetics of fungi will explain the basis for physiologic races. It will also explain why resistant varieties often become susceptible after a few years of commercial production. Knowing the genetics of the pathogen, the plant breeder can plan a breeding program more intelligently.

Recombination. Newton, Johnson, and others at the Dominion Rust

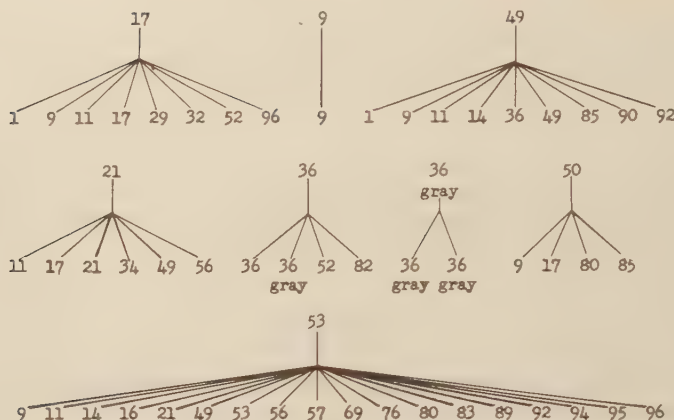


Figure 27.2 Races of stem rust of wheat which arose from selfing eight physiologic races. (Newton and Johnson.⁸¹)

Research Laboratory in Winnipeg, Manitoba, Canada, studied the effects of selfing and crossing races of stem rust of wheat.³¹ From 8 selfed races, 30 different races were identified (Fig. 27.2). Of these, 18 had been described previously, but 12 were new. Only race 9 bred true. Others segregated varying numbers of races, ranging from 2 to 18. In addition, an aberrant gray color appeared in the progeny of race 36. This evidence indicated that the infectious dicaryon of stem rust is heterozygous, as in cross-pollinated species of higher plants. Haploid spores with different genotypes are produced at meiosis and combine to form new dicaryons different from the original race.

Race 36 was crossed with race 9.²⁶ The F_1 was race 17 and the F_2 gave 11 races. This research, and similar research at other stations, indicated that sexual reproduction in stem rust was capable of giving new races. It was obvious that recombination would greatly complicate the control of this disease.

Corn smut (*Ustilago maydis*, sometimes called *U. zeae*) provides an opportunity to study the variability in the haploid phase, following a cross of two types. Monosporidial (haploid) lines have been crossed, leading to fusion of the haploid nuclei and the production of a chlamydospore with a diploid nucleus. In smut the term "chlamydospore" is used instead of teliospore, as in the rusts. Reduction division occurs on germination of the chlamydospore, and the sporidia have haploid nuclei. Single sporidia may be cultured as clones on agar plates, and are referred to as monosporidial lines or colonies. Crosses of different parental types give a wide range of monosporidial lines (Fig. 27.3).⁵⁰



Figure 27.3 Monosporidial (haploid) cultures on agar plates of corn smut (*Ustilago zeae*) obtained from crosses of two different parents. Top row: center and right, 10K₂-1 and 10I₁, parents of the cross; 10K₂-1 arose as a white mutant in a culture of 10K₂, left. From three diploid chlamydospores (termed F, M, and K) obtained from the union of the haploid parental spores, monosporidial (haploid) cultures were obtained; meiosis occurs in the chlamydospore. Four of the haploid cultures from each of the three chlamydospores are shown in vertical rows under the letters F, M, and K. The variability in the progeny are products of recombination both of chromosomes and chromosome segments. (From E. C. Stakman, M. F. Kernkamp, T. H. King, and W. J. Martin, *Amer. J. Bot.*, 30 (1943), 37. Courtesy Dr. M. F. Kernkamp, University of Minnesota.)

Pathogenicity was shown in many cases to be under rather simple genetic control. Flor's work on rust of flax (*Melampsora lini*) is outstanding.¹⁶ Using one variety of flax as the host (assume that it is called A), he showed that a cross of a virulent and nonvirulent races (races 1 and 2, respectively) would give:

F₁: Nonvirulent, indicating that virulence was recessive.

F₂: Cultures would segregate 3 resistant to 1 susceptible.

The F₂ segregation indicated that race 1 was virulent on variety A because it was homozygous for recessive alleles at one locus. For the example it may be assumed that the genotype of race 1 is a_1a_1 , and of race 2, A_1A_1 .

With variety B, the situation might be reversed, and race 2 would be virulent because it was homozygous for recessive alleles for virulence at another locus; its genotype may be symbolized as b_1b_1 . In other words, the genotypes of these two races, as determined by their reactions to varieties A and B, would be: for race 1, $a_1a_1B_1B_1$; and for race 2, $A_1A_1b_1b_1$. It is now obvious that crosses of the two races would give a dihybrid ratio of four different races in F₂. These are listed in Table 27.2.

Table 27.2 Dihybrid ratio obtained in F₂ from a cross of two races with genotypes $a_1a_1B_1B_1$ and $A_1A_1b_1b_1$.

GENOTYPE	FREQUENCY	RACE	REACTION OF VARIETIES	
			A	B
$a_1a_1B_1$ —	3	Like race 1	Susceptible	Resistant
A_1 — b_1b_1	3	Like race 2	Resistant	Susceptible
A_1 — B_1 —	9	New race	Resistant	Resistant
$a_1a_1b_1b_1$	1	New race	Susceptible	Susceptible

From these studies it is apparent that the genetic basis of pathogenicity is often quite simple. It is also clear, where a fungus has a sexual phase, that crosses of two races will give rise to new races, some of them with a wider host range than the two parents.

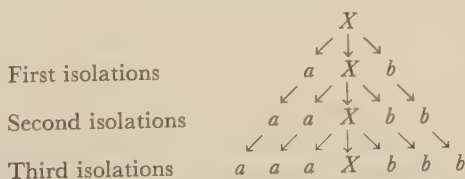
Mutations. The first evidence of mutations in fungi was obtained from colonies of pathogens growing on nutrient media. Where a mutation occurs that affects colony appearance, it is apparent as a fanshaped sector of the round colony. The point of the fan indicates where the mutation occurs. Fanshaped sectors can be caused by separation of unlike nuclei, where the fungus has more than one type of nucleus.

Stakman et al. observed large numbers of mutants from monosporidial lines of corn smut (*Ustilago zeae*), which were cultured in the haploid phase.⁴⁹ Lines differed with respect to both their rates and types of mutations, one line giving 220 in one year. Mutations varied in morphology, growth, and pathogenicity. Johnson reported many mutants, most of them recessive, that have affected pathogenicity of cereal rusts.²⁵

Flor found that ultraviolet and X-ray radiation of urediospores of flax rust would induce mutations affecting virulence.^{17,18} As would be expected from the behavior of genes in higher plants, some genes affecting pathogenicity were more mutable than others.

Where the pathogenic phase of the fungus is diploid or dicaryotic, a mutation in this phase to greater pathogenicity will usually be undetected. The reason is that mutations to greater virulence are almost always recessive, and will be expressed only when the mutant gene is present in both nuclei of a dicaryon or in both genomes of a diploid. The mutant gene may then be expressed if (1) a second mutation of the same type occurs in the other nucleus of the dicaryon; (2) the fungus passes through a sexual stage and haploid spores carrying the mutant gene later unite to form a dicaryon; and (3) the haploid nucleus carrying the mutant gene unites with a similar haploid nucleus by a process of nuclear recombination in heterocaryons (this will be discussed below). It is readily seen, considering diploid or dicaryotic species, that those with a sexual phase have an advantage in the expression of recessive mutations. Stakman has reviewed in detail the manifestations of variability in plant pathogens.⁴⁸

Recombination of nuclei in heterocaryons. Hansen and Smith showed that the recombination of genetically unlike nuclei would account for the constant and inconstant variants observed in gray mold (*Botrytis cinerea*).²¹ Starting with an inconstant culture X they were able to isolate, by culturing repeatedly from single spores, two constant lines *a* and *b*. This is illustrated in the accompanying figure.



Once isolated, *a* and *b* remained constant through a number of transfers. On the other hand, *X* continued to give an *X* type of colony or gave rise to new isolates of *a* and *b*. The Hansen and Smith interpretation was that all nuclei in the *a* and *b* colonies were genetically alike, but those in the *X* colonies were a mixture of the two genetically different

nuclei. By mixing *a* and *b*, they obtained *X*, which behaved the same as the original culture of *X*.

In gray mold the thallus is multinucleate, so the possibility exists, if different kinds of nuclei are present, for different cultures to emerge. Where different mycelia fuse, by a process known as *anastomosis*, nuclei may be interchanged, and give rise to cultures different from those that fused.

Parasexualism. Pontecorvo proposed the term parasexualism for a process in fungi whereby haploid hyphae fuse to form a heterocaryon, after which the nuclei fuse to form a diploid.³⁹⁻⁴¹ In the diploid phase, rare mitotic crossing-over may occur. Finally, by chromosome loss, the haploid phase is reestablished. The haploid derivatives will likely have genotypes different from the original haploid hyphae that fused. In the absence of mitotic crossing-over, parasexualism provides a means of recombining chromosomes; with mitotic crossing-over, it will accomplish all the essential features of the sexual cycle.

Genetics of other microorganisms. Bacteria have been shown to be capable of developing variability by mutation and by systems of recombination. Since bacteria are haploids, mutations are detected immediately. Recombination occurs through a system of conjugation, or fusion, of bacterial cells and migration of chromosome material from one cell to the other. Haploid products of the fused cells may carry characteristics of both fused cells. Bacterial cells are also capable of *transformation*²⁴ and *transduction*.⁵⁸

Viruses also may mutate or recombine.²³ This has been demonstrated by studies of bacteriophages.⁵³ The details are beyond the scope of this book.

Genetics of Resistance

During the past century, and in many cases during the early part of the present century, disease- and insect-resistant varieties were developed without any knowledge of the inheritance of resistance. Now, however, most plant breeders carry on genetic studies of resistance parallel with breeding programs. Often, such studies are conducted in cooperation with a plant pathologist or an entomologist.

A few examples of such studies are given here; they are only a few from a great many. Studies have usually required the following:

1. A single race of the pathogen or insect.
2. Pure lines of the host to serve as resistant and susceptible parents of crosses.

3. A uniform and ideal environment for the pathogen or insect, such that resistance and susceptibility are clearly differentiated.

4. A suitable method of inoculation or infestation so that the disease or insect has an equal opportunity for development on all materials of the study.

5. A consistent system of classification, preferably similar to that used by others conducting similar studies.

6. Provision to grow selfed progenies of a cross through to F_3 , since F_3 families give the best measure of the F_2 genotype.

7. A repetition of the study with other races or other pure varieties, or with both.

Race T-1 of bunt of wheat (Tilletia tritici). From the distribution of the infections of the F_3 lines of a cross between resistant Martin and susceptible Hard Federation (Fig. 27.4), Briggs concluded that resistance was governed by a single dominant gene, M .⁴ Of a total of 299 F_3 lines, 73 fell under the first mode of the curve, 147 under the second, and 79

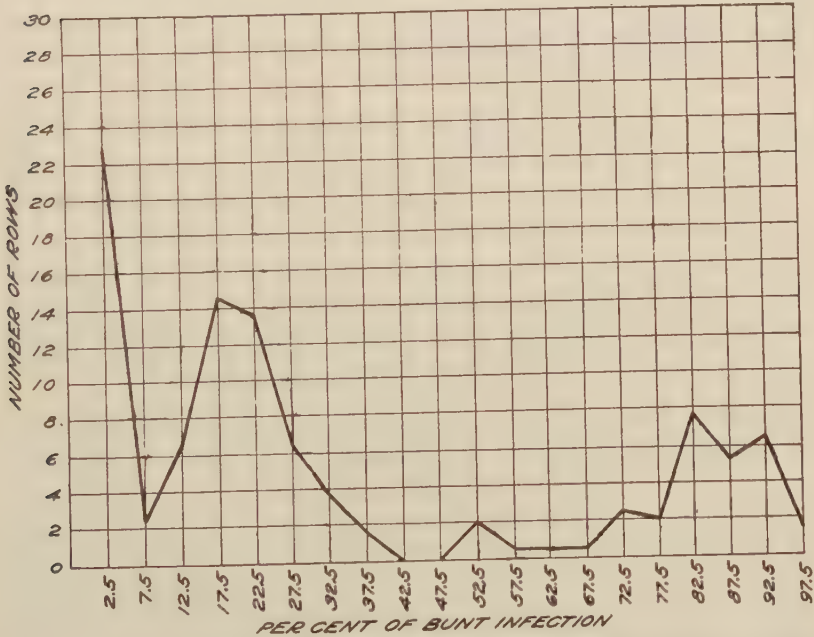


Figure 27.4 Per cent of bunt infection, Martin $\times$ Hard Federation cross. Distribution of F_2 plants on basis of F_3 rod rows. (Briggs.⁴)

under the third. If these are accepted as homozygous resistant, heterozygous, and homozygous susceptible, respectively, the ratio is very close to 1:2:1.

The resistant parent, Martin, was completely free from bunt, but the resistant F_3 lines ranged from 0 to 7.5 per cent smut and averaged 0.09 per cent. In such studies, "resistant" F_3 lines will often be more susceptible than the resistant parent. This was shown by Briggs to be due to modifying genes.⁵

When the bunt-resistant Hussar was crossed to the susceptible Baart, Briggs found that the distribution of F_3 lines (Fig. 27.5) was more dif-

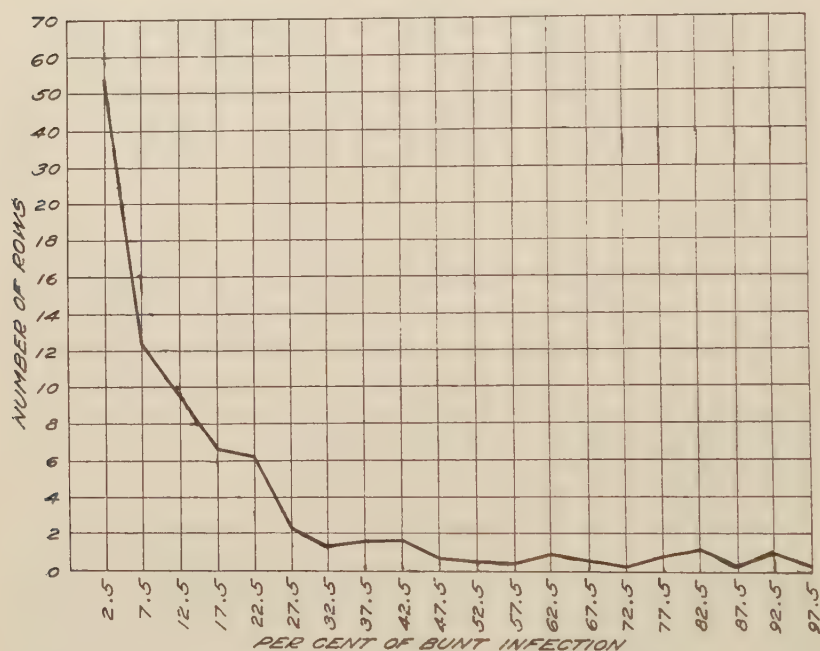


Figure 27.5 Per cent of bunt infection, Hussar $\times$ Baart cross. Distribution of F_2 plants on basis of F_3 rod rows. (Briggs.⁴)

ficult to interpret.⁴ He proposed tentatively that two genes were involved, both of them dominant; Hussar was later given the genotype $MMHH$, and Baart the genotype $mmhh$. This would give a ratio of 15 resistant ($M_H_$, M_hh , $mmH_$) to one susceptible ($mmhh$) in F_2 , and F_3 lines should react as shown in Table 27.3.

A cross between Martin and Hussar did not segregate for resistance, so

Table 27.3 Segregation of F_3 lines obtained from a cross of Hussar with genotype $MMHH$ and Baart with genotype $mmhh$.

NUMBER	SEGREGATION	GENOTYPES OF THE F_2 PLANT
7	All resistant	$MMHH$, $MmHH$, $MMHh$, $MMhh$, $mmHH$
4	15 resistant:1 susceptible	$MmHh$
4	3 resistant:1 susceptible	$Mmhh$, $mmHh$
1	All susceptible	$mmhh$

it was assumed that Hussar had the Martin gene, M . The second gene in Hussar was termed the Hussar gene, H .

It is obvious that there should be varieties with the genotype $mmHH$ and that in crosses to Martin ($MMhh$), the segregation in F_2 and F_3 should be the same as that given above for Martin $\times$ Baart. Selection 1403 was such a variety.⁶ This confirmed the interpretation of the genetic situation in the Hussar $\times$ Baart cross. Selection 1403 was used as the tester for the Hussar gene, since its genotype was $mmHH$. Subsequently, Briggs and his associates identified the T gene, from the variety Turkey, and the R gene, from the variety Rio, both giving high levels of resistance to race T-1. The genes X and Y are weak, with the former permitting an average of 22 per cent of bunt, and the latter 45 per cent. The two genes acting together in the variety Turkey 10015 provided a high level of resistance.¹⁴

Race 3 of barley mildew (Erysiphe graminis). A study of inheritance of resistance to barley mildew at Davis, California, has led to the identification of nine genes for resistance to race 3.

Stem rust of wheat (Puccinia graminis tritici). Knott and his associates have published a series of papers on the inheritance of stem-rust resistance in wheat varieties.^{28,29} All 11 genes for resistance identified by him are being transferred to the variety Marquis by backcrossing. These back-cross lines of Marquis, each with its own gene for resistance, are being used as host differentials to identify races of the pathogen. They are being used also as testers for other genes that provide resistance.

Rust of flax (Melampsora lini). The work of Flor on the genetics of rust resistance in flax is classic.¹⁶ He found that resistance is determined by 25 genes occurring as multiple alleles at five loci. Those at each locus are:

- K : 1 gene, k , recessive to K for susceptibility
- L : 11 genes, L to L^{10} , all dominant to l for susceptibility
- M : 6 genes, M to M^6 , all dominant to m for susceptibility
- N : 3 genes, N to N^3 , all dominant to n for susceptibility
- P : 4 genes, P to P^3 , all dominant to p for susceptibility

The loci *N* and *P* are linked with a crossover value of 26 per cent. Otherwise, the genes at these loci are inherited independently. Flor has 25 varieties, each with one of these resistant genes present.

An important feature of this situation is that, of the 25 genes for resistance available, a pure-line variety can have only 5, that is, 1 at each locus. The plant breeder's task is to select the five genes that will give flax the best protection against races present in an area. In the United States it is possible to get protection against all physiologic races by choosing the correct five alleles.

Resistance to yellow-dwarf virus of barley. Rasmusson and Schaller found a high level of resistance, but not immunity, to yellow dwarf in four barley varieties from Ethiopia.⁴² In crosses of these varieties to susceptible varieties, the F_1 was intermediate in resistance. F_3 families showed a good fit to a ratio of 1 breeding true for resistance:2 segregating:1 breeding true for susceptibility. It was apparent that one gene, when homozygous, gave resistance to this disease (Fig. 27.6). Crosses of resistant varieties gave only resistant progeny in F_2 and F_3 , which indicates that they carry the same gene, Yd_2 , for resistance. Suneson reported that a recessive gene gave resistance in the variety Rojo, and this gene has been termed yd_1 .⁵⁴

*Hessian fly (*Phytophaga destructor*) on wheat.* From crosses of the resistant variety Dawson to two susceptible varieties, Poso and Big Club,



Figure 27.6 Yellow-dwarf reaction of resistant and susceptible families at heading stage. Left, susceptible. Right, resistant.⁴² (Courtesy Dr. D. C. Rasmusson, University of Minnesota.)

the data of the F_1 , F_2 , and F_3 indicated that Dawson had dominant duplicate genes for resistance.¹² Subsequently, these genes have been termed H_1 and H_2 .^{32,55} With both genes present, the level of infestation was 0 to 2 per cent, with one gene 0 to 10 per cent, and with neither dominant gene, 83 per cent. These studies were conducted in California.

Since Dawson is susceptible to hessian fly in the Corn Belt, neither H_1 nor H_2 imparts resistance. A third gene, H_3 , from the variety W38 gives resistance to the Corn Belt type.⁹ The gene H_3 contributes some resistance to hessian fly in California but less than H_1 or H_2 . A fourth recessive gene, h_4 , was identified in the variety Java in California tests.⁵⁵

Gene-Race Relationships

Following the discovery of physiologic races in pathogenic organisms, and particularly as larger numbers were identified in several diseases, the problem of controlling diseases with resistant varieties appeared insuperable to some breeders. The tension was eased when Aamodt showed that a single gene in Kanred wheat gave resistance to 11 races of stem rust.¹

In this regard it is of interest to consider the protection against several races of bunt of wheat that are given by the M , H , R , and T genes. These genes were identified using only one race, T-1 (see above). Resistance given to 16 races of *Tilletia caries* and 9 races of *T. foetida*, as determined by Briggs and Holton,⁸ is given in Table 27.4.

Table 27.4 Protection against 25 physiologic races of bunt of wheat given by the M , H , T and R genes. Adapted from Briggs and Holton.⁸

VARIETIES	GENES FOR RESIST- ANCE	RACES OF THE PATHOGEN															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>T. caries</i>																	
(T-1 to T-16)																	
White Odessa	MM	R ^a	R	R							R	R	R				R
Selection 1403	HH	R	R		R		R				R	R		R		R	
Turkey 3055	TT	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Rio	RR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
<i>T. foetida</i>																	
(L-1 to L-10)																	
White Odessa	MM	R	R	R			—		R		R						
Selection 1403	HH	R	R		R		—										
Turkey 3055	TT	R	R	R	R	R	—	R		R	R						
Rio	RR	R	R	R	R	R	—	R		R	R						

^a R = resistant; — = no test; and blank cells represent a susceptible reaction.

The most striking feature of the results is the large number of races controlled by each of the genes for resistance. The Turkey and Rio genes provide resistance to the same 23 races. The Martin gene and either the Turkey or Rio genes give resistance to all 25 of the races.

Complementary gene hypothesis. Flor found that there is some sort of complementary relationship between genes for resistance in the host and genes for virulence in the pathogen.¹⁶ This has sometimes been termed the "gene-for-gene" hypothesis. Flor illustrates the situation, using the data in Table 27.5.

Table 27.5 Complementary relationships between genotypes of flax plants and flax rust.¹⁶

FLAX VARIETY	GENOTYPE		HOST REACTION ^a
	Host	Pathogen	
Polk	$N^1 N^1 pp$	$A_N^1 A_N^1 a_P a_P$	R
Polk	$N^1 N^1 pp$	$a_N^1 a_N^1 A_P A_P$	S
Koto	$n^1 n^1 PP$	$a_N^1 a_N^1 A_P A_P$	R
Koto	$n^1 n^1 PP$	$A_N^1 A_N^1 a_P a_P$	S
Redwood	$N^1 N^1 PP$	$A_N^1 A_N^1 a_P a_P$	R
Redwood	$N^1 N^1 PP$	$a_N^1 a_N^1 A_P A_P$	R
Redwood	$N^1 N^1 PP$	$A_N^1 A_N^1 A_P A_P$	R
Redwood	$N^1 N^1 PP$	$a_N^1 a_N^1 a_P a_P$	S
Winona	$nnpp$	All genotypes	S

^a R = resistant; S = susceptible.

All genes of the pathogen are symbolized by A or a , with a for virulence being recessive to A . The subscript identifies the particular gene of the pathogen. For example, in the first genotype of Table 27.5, $A_N^1 A_N^1 a_P a_P$, A_N^1 is the gene complementary to N^1 of the host, and a_P is complementary to p in the host.

Susceptibility results when either one of (or both) the complementary genes are homozygous recessive in all sets of complementary genes. If any one complementary set has a dominant gene present in both the host and the pathogen, the plant or variety is resistant. With one exception, this has been true in all Flor's studies. Table 27.5 shows that Polk is resistant to all races with the A_N^1 gene, and susceptible to races with the genotype $a_N^1 a_N^1$. Redwood is resistant to races carrying either one (or both) of the genes A_N^1 or A_P , and susceptible only to races with genotype $a_N^1 a_N^1 a_P a_P$. Winona is susceptible to all races because no genes for re-

sistance are present. Person has provided a detailed review of the gene-for-gene hypothesis.³⁷

Résumé

Studies of pathogens and insects have indicated the full capabilities of these enemies of crop plants. With a knowledge of their resources, particularly in terms of meeting the challenge of resistant varieties, has come better planning in plant breeding programs. The most important conclusion is that campaigns to control pests through resistance will be long, drawn-out affairs in which these enemies will never admit defeat.

Encouragement has come from studies of the genetic resources of the host plants and their relatives, for almost without exception, these resources have been found equal to the task of controlling the pests, *if they are properly managed*. The management of these resources is the subject of the next chapter.

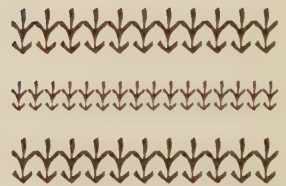
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BREEDING FOR RESISTANCE TO DISEASES AND INSECTS



Much more plant breeding will be done in the future. The productive-ness of all our crops must be increased and their quality improved. Yield and quality are naturally the first considerations, but attention may profitably be paid in every case to reducing injury from disease, and in many instances in which a disease is a limiting factor the first aim of the breeder will need to be the securing of resistant strains.

ORTON, 1909

AMONG THE ACHIEVEMENTS of plant breeders in this century there is none that has contributed more to the world's economy than disease- and insect-resistant varieties. Resistance has not only given higher and more

consistent yields, it has also provided products, particularly of fruit and vegetable crops, that are higher in quality.

Two steps are required to develop a disease-resistant variety: (1) finding a source of resistance; and (2) combining resistance with other desirable crop characteristics. As will be shown below, these steps are not always easy to achieve.

Historical Background

The discovery that cultivated varieties differ in ability to withstand diseases is not new; this was discussed by Theophrastus in the third century B.C.⁷ Actually, many resistant varieties of crops were in cultivation before plant breeders undertook their improvements.

During the early part of the nineteenth century the first clear-cut descriptions of differences between varieties in their reactions to diseases were recorded, both in Europe and in the United States. With more resistant varieties being noted toward the end of that century, and with a need for resistance in several crops, it is not surprising that the inheritance of disease resistance engaged the attention of geneticists soon after the discovery of Mendel's paper.

In France, about 1880, Millardet developed wine grapes resistant to downy mildew by crossing European varieties with the resistant varieties from America. Unfortunately, some of the introduced American types carried the root aphid of grape, called phylloxera (*Phylloxera vitifoliae*). Not long thereafter this insect threatened to destroy grapes as a crop in Europe. By 1884, the estimated loss to France from this insect was 10 billion francs (2 billion dollars), and the grapevines on 1,200,000 hectares (3 million acres) were completely destroyed. The insect was brought under control by grafting European grapes to American roots, and a few years later the resistant European types were developed from crosses of European and American types.

The idea of screening a large volume of material to find plants resistant to a pathogen was developed in the first part of the twentieth century by Orton when he was searching for resistance to *Fusarium* wilt in cotton, cowpeas, and watermelons.¹² At about the same time, Bolley at the North Dakota Agricultural Experiment Station made Plot 30 famous as a source of varieties of flax resistant to *Fusarium* wilt.⁴ Plot 30 had been artificially infested with wilt by growing a susceptible variety on it for several years. This technique is still being used successfully in the development of varieties resistant to many diseases.

In general, plant breeders have had less success in the control of insects by resistance than in the control of plant diseases. Part of this failure is due to the tendency of insects to be less specialized in their food habits,

often attacking several crops. Also, insects may avoid one variety of a crop when they have a choice, but will attack the so-called resistant variety when there is no choice. Howard has provided details of the early history of applied entomology.⁸

The use of chemicals to control diseases and insects increased greatly after World War II, and it appeared as if plant resistance might not be so necessary. When races of insects developed with resistance to insecticides, and insecticide residues became a health problem, agriculturists turned again with increased interest to the development of resistant varieties. Disease and insect resistance is a biological method of control.

Sources of Resistance

Sources of resistance may be difficult to find, particularly where a new disease or insect appears or where new races overcome the protection provided by readily available genes for resistance. In the case of many crops, lists of varieties resistant to several diseases are available. Such lists may be found in the 1953 *Yearbook of Agriculture*.¹⁹ The Food and Agriculture Organization in Rome, Italy, provides a list of plant breeders and their breeding objectives, and it is possible to get resistant varieties by writing to FAO or directly to the plant breeders developing the varieties that are resistant to a specific disease or insect.

If no resistant variety is available, it will be necessary to survey a large collection of materials from different sources that are subject to conditions of natural or artificial epidemics. For example, Schaller surveyed the entire world collection of barley for resistance to yellow dwarf, a virus disease, and found introductions from Ethiopia to provide, with one exception, the only source of resistance.¹³

A mixture of the races likely to be present in the area of production is sometimes used in a survey, and resistance, if it is found, will be effective against all the races. However, if a mixture is used and no resistant types are found, this is no assurance that resistance does not exist in the collection. It is quite possible for one variety to be resistant to one group of races, another variety resistant to another group, and so on, so that collectively a few varieties may have resistance to all races; but with a mixture of races, resistance may not be apparent in any of the varieties. In such a situation the plant breeder must use separate races of the pathogen or insect in his survey and breeding program.

A high level of resistance is desirable because there usually is some dilution of the resistance when it is transferred to a new genic background. If resistance is complete, it will eliminate the pathogen or insect, assuming that no other host is available. This will deny the pest an opportunity to develop new physiologic races through hybridization or

mutation. Holmes, in discussing the desirable level of resistance to virus diseases, pointed out that tolerance often permitted more damage to occur than was apparent.⁶ However, tolerance is better than susceptibility, and may provide considerable protection against loss in yield and quality.

The breeding procedure is considerably simplified if the resistant parent is of the same species as the crop. However, as was noted in Chapter 26, resistance may be transferred from a related species. In some cases the highest level of resistance has been found in a related wild or weedy species.

If the genetics of resistance is known in relation to the prevailing races, the plant breeder is in the best position to choose a resistant parent. If the genetics of resistance is unknown, it is possible to make a reasonable estimate of its nature in the F_2 and F_3 generations of a cross. This will provide a sound basis for managing segregating populations.

Managing the Pathogen or Insect

Breeding for disease or insect resistance very often is a cooperative project between the plant breeder and the plant pathologist or entomologist. Even without active participation, the pathologist or entomologist is able to advise the plant breeder on suitable techniques for managing the pest so that accurate classifications can be made.

Breeding populations may be grown in the field or greenhouse. In most cases some of the work must be done in the field because of the size of the populations. Usually, artificial epidemics are established, even in the field, because natural epidemics are unreliable at best. In this case the breeding nursery is surrounded and interplanted with susceptible varieties. In these the disease or insects are established and allowed to spread. When an insect vector is involved, as is the case in many virus diseases, the vector may be reared in an insectary, allowed to feed on diseased plants, and released either on the susceptible varieties or over the entire breeding nursery. In the case of soil-borne diseases such as *Fusarium* wilt, known diseased fields may be selected or the soil of a test area may be artificially inoculated. It is not possible to control races in the field completely, but it is possible to make certain that races of interest are present. Field nurseries should be grown under climatic and soil conditions conducive to good development of the pest and the host.

Controlled environment facilities are necessary in breeding for resistance to some insects and diseases, if rapid advances are to be made. By depending on the environment of the field, an entire year may be lost and a great deal of breeding material wasted, simply because the field environment happens to be unfavorable for the pest. Greenhouses often will provide the necessary control, but it may be necessary to provide

even more precise controls than are available in most greenhouses. Depending on the disease, control may apply to (1) soil conditions such as temperature, pH, level of organic matter, nutrient status, and water content; (2) atmospheric conditions, including temperature, light quality and intensity, day length, and relative humidity; and (3) inoculation, with respect to race composition, timing, and intensity. Often in the case of disease, precise controls are necessary for a short period when the pathogen is introduced into the host, in which case an inoculation chamber is used.

In addition to providing ideal conditions for development of the disease or insect, controlled environment facilities permit a breeding program to advance during the entire year or to proceed at a location distant from the area of the pest. Such facilities are absolutely necessary for genetic studies of the host and the pest. It is obvious also that breeding programs should be conducted under controlled conditions when there is danger of the disease or insect spreading from the field nursery to commercial fields.

Breeding Methods

Breeding methods appropriate to the development of disease resistance have been discussed in earlier chapters. Often, at least in the past, selection has offered a quick method of developing a suitable resistant variety. Reference has already been made previously in this chapter to Orton who, by selection, developed varieties of cotton, cowpeas, and watermelons resistant to *Fusarium* wilt, and to Bolley who developed flax varieties resistant to *Fusarium* wilt.

The pedigree method has been most widely used by breeders of self-pollinated crops. The bulk method would be next in popularity, and the backcross method last. Where a disease or insect is a constant feature of the environment, the bulk method, including composite crosses, permits the use of very large populations.

The backcross method has several desirable features, particularly in the development of resistance to disease. In the first place, it requires less time than other methods, which is often important when diseases develop rapidly. More important, however, is the possibility in the backcross method of indefinitely adding genes for resistance to a variety, without losing the genes already added, if the added genes are at a different locus than those present.

Mutation breeding has considerable appeal because it presents the possibility of adding resistance without giving up the characteristics of the variety being grown commercially. In this respect it has the advantages

of the backcross method. Where no genes for resistance to a race or races of a pathogen are available, mutation breeding is the only choice.

Stability of Resistance

The permanence of resistance has varied over a wide range. At one extreme is the resistance of the watermelon variety Conqueror to Fusarium wilt. This variety was released in 1911, and though it was not a successful variety for reasons not related to its wilt resistance, it has been used as a source of wilt resistance since that time.

At the other extreme, resistant varieties may be short-lived. Stevens and Scott report that the life of a spring oat variety in the central Corn Belt varied from 1 to 12 years, with an average of 5 years.¹⁸ In large part this has been due to the changing race picture of stem rust (*Puccinia graminis avenae*). Races changed quickly because an established race was shut out by gene(s) for resistance in a few varieties that occupied most of the area in oats, and minor races virulent on the new varieties increased explosively on millions of contiguous acres of host plants. For example, the oat varieties Boone, Vicland, and Tama were released in 1941, 1942, and 1943, respectively. All three obtained their crown-rust resistance from Victoria and their stem-rust resistance from Richland. Richland was resistant to races 2 and 5 of stem rust, which made up 97 per cent of the collections of that disease between 1921 and 1942. By 1948, when oat varieties derived from the Victoria $\times$ Richland cross were widely grown, races 8 and 10 became the prevailing ones, and Richland and all its derivatives were susceptible. Another reason for the decline of these varieties was their susceptibility to Victoria blight (*Helminthosporium victoriae*), a previously unimportant disease to which Victoria was susceptible.

Meanwhile, a new series of varieties under development had resistance to races 8 and 10 of stem rust and the resistance of Bond to crown rust. Bond conferred resistance to the same races of crown rust as Victoria and was resistant to Victoria blight. Clinton, Benton, Mindo, and Bonda were prominent among these varieties, which were released to growers in 1945, 1946, 1947, and 1948, respectively. By 1954, all these varieties had essentially disappeared from commercial production because they were susceptible to stem-rust races 7 and 7a and crown rust races 45 and 57, to which Bond was susceptible.³

Borlaug has found that the average life of an improved variety of stem-rust resistant wheat is four years in Mexico, and five years in Colombia.² Their short life in temperate regions of the tropics is due to the presence of green host plants throughout the year, which permits many races of the pathogen to persist on them.

Multiline varieties. As a means of stabilizing the race situation in stem rust and of preventing the rapid increase of new races, Jensen proposed the use of multiline varieties.⁹ Such varieties would be a composite of selections or varieties with similar height, maturity, yield capacity, and other agronomic features, with each constituent selection of the multilineal variety carrying a different gene for resistance. No matter what race was present, there would be something resistant to it in the variety. Because a race could attack only one or a few constituents of the multilineal variety, its increase would be slow and less damaging even to susceptible types.

Borlaug proposed a similar approach except that the component lines would be developed by the backcross method, and consequently would be phenotypically similar but would have different genes for resistance.¹ The composition of the variety would be subject to rapid modification as populations of rust races shifted.

More recently, Borlaug has described the development of multilineal hybrid wheat varieties (Fig. 28.1).² Basically, they are produced by three-way crosses: the first cross incorporates several genes for rust resistance into a male-sterile variety; and the second imparts heterosis and restores male fertility. Using multilineal hybrid varieties, it is possible to use not only genes at different loci, but also different alleles at the same locus. In the example of Fig. 28.1, lines $A^1(S)$, A^3 and B^2 could carry different alleles at the same locus.

Resistance to Stem Rust of Wheat

There are few plant breeding endeavors that have taken more of man's time and energy than the development of varieties of wheat resistant to stem rust. Though the undertaking has been world-wide in its scope, the greatest effort has been made in North America. With about 1 billion bushels of wheat being grown in the United States each year, and 500 million bushels in Canada and Mexico, stem rust threatened not only the income of thousands of farmers, but also the nourishment of the world. In the two worst epidemics in the United States in 1916 and 1935, 300 million and 135 million bushels of wheat, respectively, were destroyed. The hard red spring wheat and durum wheat in the northern Great Plains suffered most in rust epidemics, primarily because the wheat developed during the summer when rust inoculum was most likely to be present in quantity. This summary will focus on the control of stem rust in hard red spring wheat through breeding.

*The pathogen (*Puccinia graminis tritici*).* With two hosts necessary for the life cycle of rust (Fig. 27.1), it would seem that it might be controlled easily, either by resistant varieties of wheat or by destruction of barberry,

the alternate host. But the fungus was more formidable than it appeared. A determined effort to eliminate the barberry failed to control the disease because it survived the winter in the urediospore stage in the southern states and northern Mexico. From these survivors the disease always spread northward during the spring and early summer on the developing wheat crop or, occasionally, the spores were blown north by air currents over distances of hundreds of miles in a few days. These early flights of spores, combined with ideal weather conditions for rust development, were responsible for the devastating epidemics. The only way, then, to secure the wheat crop was to develop resistant varieties.

Resistant varieties. The first variety with resistance was Ceres, developed from a cross of Marquis $\times$ Kota, and released by the North Dakota Agricultural Experiment Station in 1926. Ceres lasted only a short time before it was destroyed by race 56 in 1935. Race 56 increased slowly in importance until 1934 (Fig. 28.2), when it rapidly increased.

The first real breakthrough came with the development of Thatcher by H. K. Hayes at the Minnesota Agricultural Experiment Station. Thatcher had the resistance of both Kanred, a winter wheat, and Iumillo, a durum wheat. Thatcher's performance was spectacular in the rust epidemic of 1935 when race 56 destroyed 100 million bushels of wheat in the northern Great Plains.

Efforts had been made for years to transfer the high level of resistance from emmer wheat (*Triticum dicoccum*) to bread wheats, but without success. Indeed, it was considered impossible because the D genome of common wheat carried only genes for susceptibility or suppressed the effects of genes for resistance in other genomes. E. S. McFadden crossed Yaroslav emmer and Marquis in 1916 and grew the F_2 , F_3 , and F_4 populations in bulk at the Highmore substation in South Dakota.¹¹ From an F_4 population of 4,000 to 5,000 plants, only 6 plants combined the characteristics of common wheat with the resistance of emmer wheat.

When the cereal project was terminated at Highmore in 1920, McFadden moved to his farm at Webster and carried the six selections forward in his garden to 1925, using the pedigree method. His persistence and faith were rewarded, and in 1925 two promising selections were named Hope and H-44-24 (usually shortened to H-44). These varieties were the most important sources of resistance throughout the world. In addition, Hope carried resistance to both loose and covered smut. Varieties of hard red spring wheat carrying the Hope or H-44 resistance were Regent, Apex, Renown, Redman, Cadet, Mida, Newthatch, Rival, Pilot, and Lee. With the resistance of Hope, H-44, and Thatcher in common wheat, it was apparent that the major task was done; wheat breeders could concentrate on other objectives. For 12 crop years, from 1938 to 1949,

1. Find two varieties, *A* and *B*, which cross to give a vigorous F_1 .
2. Incorporate cytoplasmic male sterility into *A* (S = male-sterile cytoplasm) by backcrossing. Include a gene (1) for stem-rust resistance.
3. Incorporate different genes $(2, 3 \dots 7)$ for rust resistance into *A* by backcrossing, making sure that no fertility restoring genes are present.
4. Incorporate fertility restorers into *B*, and a different gene (8) for rust resistance.
5. Cross male-sterile $A^1(S)$ with *A* lines having different genes for rust resistance. These crosses will not restore male fertility.
6. Male-sterile F_1 of single crosses above are crossed to B^8 . This cross restores fertility and contributes vigor.
7. The crossed seed from the last step will be used for commercial production, and will be male-fertile.

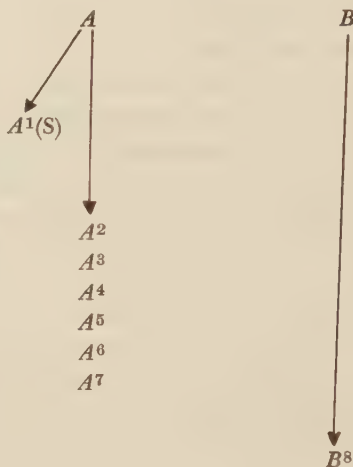
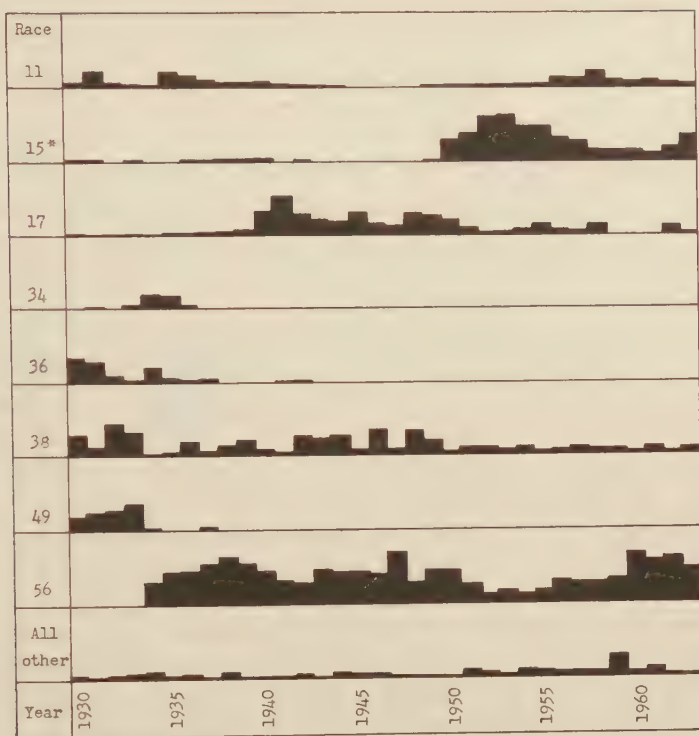


Figure 28.1 Outline of a method of producing a multilinear hybrid wheat variety resistant to stem rust. (Adapted from Borlaug.²)

stem rust was of little importance in the hard red spring wheat area of Canada and the United States.

A new race. For many years the races of importance had been 17, 38, and 56 (Fig. 28.2). Many other races had been recorded, but they caused no worry. A race called 15B was first recorded in 1939, and collections of race 15, which could not be differentiated from 15B until that time, were found as far back as 1918. Until 1949, race 15B was just another race of little consequence. Then, in 1950, it constituted 27 per cent of



*Almost exclusively 15B subsequent to 1939

Figure 28.2 Percentage of different physiological races of stem rust of wheat (*Puccinia graminis tritici*) in the United States from 1930 to 1963. From data obtained and reported by the U.S. Dept. of Agriculture and the Minnesota Agricultural Experiment Station.

rust isolates, and rose to 40 per cent in 1951. From 1951 to 1955, race 15B was more prevalent than any other race. In 1953 and 1954, race 15B destroyed 75 per cent of the durum wheat and 25 per cent of the bread wheat in the Dakotas and Minnesota.¹⁷

Wheat breeders in the spring wheat area of the northern Great Plains had to take up the task again of breeding varieties resistant to stem rust. Actually, some progress had been made prior to 1950, but the amount of race 15B was not sufficient for satisfactory field evaluations of breeding materials, and wheat breeders were reluctant to develop artificial epidemics of such a virulent race.¹⁷ Resistance was found in Kenya types of common wheat and Khapli emmer; from crosses of these types to commercial varieties, resistant varieties have been developed. Some resistant

varieties of common wheat are Canthatch, Conley, and Selkirk (to some biotypes). Resistant durums are Lakota and Wells. The use of these resistant varieties is probably the main reason for the decline in importance of race 15B.

Resistance to the Spotted Alfalfa Aphid (*Therioaphis maculata*)

In the United States the spotted alfalfa aphid was first reported in New Mexico in early 1954. By the end of the year it was in alfalfa-producing areas of southern California, Arizona, and New Mexico. The damage in southern California alone in 1954 totaled \$337,900.¹⁴ Two years later it was present in most of the alfalfa-growing areas of the United States (Fig. 28.3). Combined losses and costs of control in California for this pest were: in 1955, \$12,855,000; in 1956, \$10,600,000; and in 1957, \$9,705,000.

The first variety showing resistance was Lahontan, which had been developed cooperatively by the Nevada Agricultural Experiment Station and U.S. Dept. of Agriculture for its resistance to the stem nematode. This variety was not well adapted to the warmer areas of the Southwest.

In only three years from the appearance of the pest, cooperative work of the U.S. Dept. of Agriculture and state experiment stations, under the leadership of Dr. O. F. Smith, U.S.D.A. plant breeder, produced the variety Moapa (Fig. 28.4). Moapa is a synthetic of selections of the variety African, which is well adapted to many areas of the Southwest.⁵ By 1960 the estimated annual value of Moapa was \$1.5 million, and the projected value for 1963 was \$3 million, all for a direct investment of some \$30,000 in the development of the variety.

The third variety with resistance was Sonora, also a synthetic, developed from 13 plants selected from the variety African in California, Arizona, and Nevada.¹⁰ It was released in 1963. Sonora has the same level of resistance as Moapa, but has higher forage yields in southern areas of the Southwest.

Jointly, the Kansas Agricultural Experiment Station and the U.S. Dept. of Agriculture developed the variety Cody and released it in 1959, four years after the appearance of the insect in Kansas.¹⁵ Cody is a synthetic variety developed from 22 plants selected from the variety Buffalo. In the absence of the aphid, Cody is equivalent in performance to Buffalo, a variety well adapted to the southern Great Plains.

The frightening spread and costly consequences of the spotted alfalfa aphid is a dramatic warning to agriculture that an insect, or a disease, can appear at any time. With modern methods of transportation it is not improbable that other insects will threaten agriculture in the future.

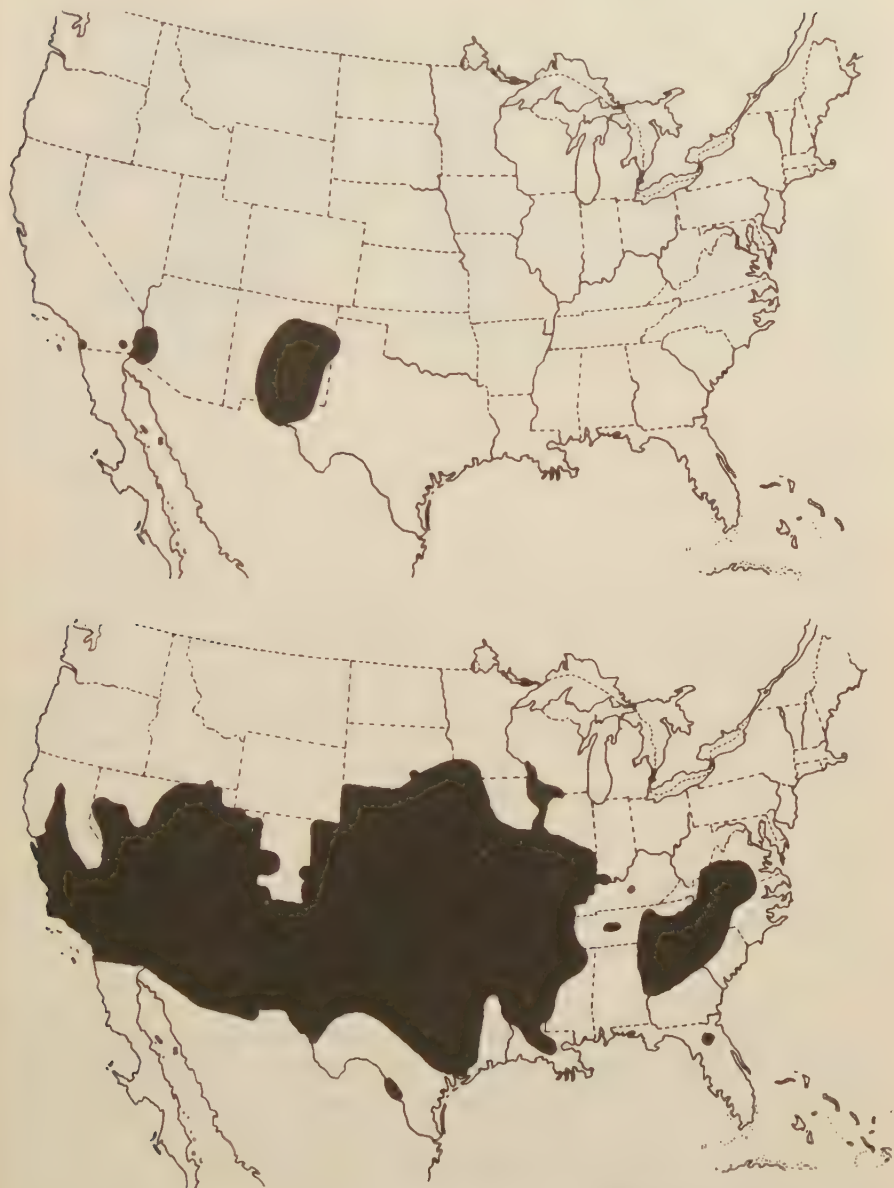


Figure 28.3 Spread of the spotted alfalfa aphid (*Therioaphis maculata*) in the United States. Top: Distribution on June 30, 1954. Bottom: Distribution on December 31, 1956. (Courtesy Dr. R. F. Smith, University of California, Berkeley.¹⁴)



Figure 28.4 Alfalfa variety Caliverde in every other row killed by the alfalfa aphid. Surviving, healthy rows (left to right) are Lahontan, Nevada strain, Moapa, and another Nevada strain. (Courtesy Dr. C. H. Hanson, Research Leader of Alfalfa Investigations, USDA, Beltsville, Maryland.⁵)

Experience with both this aphid and other immigrant pests in the past would encourage the development of resistance in varieties before the arrival of a pest. This could be managed through the extension of breeding programs to countries where the pest is endemic.

Fortunately, in the case of the spotted alfalfa aphid, resistance was immediately available in adapted types; otherwise, the cost to agriculture through delays in the development of resistant varieties would have been very great. Nevertheless, the achievement of varieties resistant to this pest in such a short period of time is something of a record in plant breeding. It is a demonstration of the accomplishments from vigorous cooperation between plant breeders and entomologists of both the state experiment stations and the U.S. Dept. of Agriculture.

Résumé

It is estimated that the annual benefit to American agriculture from the use of disease-resistant varieties is equivalent to 600 to 750 million dollars, about 10 per cent of the total production.¹⁹ Considering other benefits in terms of increased returns from transportation, packaging, and marketing, the estimate would be raised to 1.2 to 1.5 billion dollars per year. Equally impressive values could be produced for other countries.

But current losses from insects and disease, and the probability of damage from unknown species or races in the future, dictate that breeding for resistance continues. Greater resistance to insects and pathogens will long continue as primary objectives of most breeding programs.

Breeding methods to develop disease or insect resistance are not new or distinctive; they will be modifications of methods used to achieve many other objectives. However, it is apparent that varieties will have to carry genes sufficient to protect them against a wide range of races.

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29

MAINTENANCE AND DISTRIBUTION OF VARIETIES



*Unseen buds, infinite, hidden well,
Under the snow and ice, under the darkness, in every square or cubic inch,
Germinal, exquisite, in delicate lace, microscopic, unborn,
Like babes in wombs, latent, folded, compact, sleeping;
Billions of billions, and trillions of trillions of them waiting,
(On earth and in the sea—the universe—the stars there in the heavens,)
Urging slowly, surely forward, forming endless,
And waiting ever more, forever more behind.*

WALT WHITMAN, 1892

WHEN the plant breeder's task is done, and he has assembled and evaluated a superior genotype or pool of genes, his labors may be rep-

resented by a handful of seed, a bundle of cuttings, or a few tubers. Though small in amount, any one of these may represent years of work and all the skill and knowledge that the plant breeder can muster. It is natural, then, that there should be concern about the future of the seeds or plants, how they will be preserved, and how they will be increased and distributed. At one time it was in large part the responsibility of the plant breeder. Now, with agriculture much more complex, the preservation and distribution of a new variety is often the responsibility of someone else, even several organizations.

The purpose of this chapter is to look back briefly on the development of agencies of variety preservation and distribution and to review the procedures of today. Considering the many systems in use over the world, or even the diverse systems in one country for different types of crops, it will be necessary to generalize. Details will be given only for systems in use in the United States, and particularly in California. Two volumes have been the source of much of the discussion, one by the Food and Agriculture Organization of the United Nations, titled *Agricultural and Horticultural Seeds*,⁶ and the other by the U.S. Dept. of Agriculture, titled *Seeds*.¹³

Historical Background

During the early development of agriculture in the United States, farmers saved their own seed or acquired seed from a neighbor if he seemed to have a more productive variety. The first seedhouse was opened in Philadelphia in the 1770's. By 1850 there were 45 seed firms, most of them in the eastern states. Clover and timothy seeds for export were major items. New York and Pennsylvania, and later the Ohio Valley, supplied the largest amount of grass seed of any area of the world.

In 1862, when the newly formed Dept. of Agriculture took over the function of seed distribution, seeds of cereals, cotton, tobacco, and vegetables were procured from domestic and foreign sources and distributed in about 1,200,000 packages. Also, 25,750 bulbs, cuttings and vines were sent out that year. Congressmen and agricultural societies aided in this undertaking. This distribution of free seed continued until 1923.

Prior to 1900, the importance of variety purity was not fully appreciated. Seed was distributed in small lots, rarely large enough to plant more than an acre. Such releases soon became so contaminated with other varieties that their identity and value were usually lost. Much of the seed moving in the established seed-trade channels was not evaluated for its adaptation, quality, and productive capacity.

During the twentieth century there have been many factors encouraging the development and improvement of the seed industry. Among

these are: (1) the rapid development of genetics as a science; (2) expanding plant-breeding programs; (3) the introduction and increase in the use of hybrid varieties; (4) the localization of seed production of many crops in certain areas; (5) the development of seed laws and state and federal agencies regulating the production of seed; and (6) the improvement of equipment for handling seed.

With the development of genetics came increased insight into what differentiated one variety from another and an understanding of how the characteristics of a variety should be maintained. The basis for deterioration of varieties, primarily from outcrossing to other varieties, became clear, and this led to better standards for isolation of seed fields. The development of hybrid varieties followed the development of the science of genetics.

A greater knowledge of the control of plants and diseases by genes led to the initiation of breeding programs by the U.S. Dept. of Agriculture, state experiment stations, and seed companies, with a view toward developing better varieties of field and horticultural crops. The large number of new varieties encouraged the development of agencies that would facilitate and regulate the production and distribution of seed stocks.

The introduction of hybrid varieties of corn and their rapid acceptance encouraged the development or expansion of seed companies that produced hybrid seed for commercial use. Most of these companies hired their own plant breeders. These companies have participated in the development of hybrid varieties of other crops. With control of the inbred lines or clones involved in the production of a hybrid variety, these companies have been able to maintain control of the distribution and use of the hybrid.

Over the past 50 years there has been a trend toward the localization of seed production of many crops in certain areas of the United States and mostly in the western states. This has encouraged companies and farmers to specialize in this business. It has also led to the movement of vast amounts of seed from one part of the country to another. Farmers have depended less and less on their own farms or localities for seed and more and more on companies specializing in the business.

The rapid expansion in the seed business, and the movement of seed from one region to another, has led to the development of federal and state laws regulating the distribution of seed. These laws were designed to ensure that the purchaser of the seed obtained the variety specified on the label.

It was natural that as the volume increased, there should be improvements in equipment and techniques for handling seed and maintaining its purity. Equipment included such items as improved flat and cylin-

drical screens, gravity separators, indented disks, velvet rollers, fractionating spiral slides, magnetic separators, and electronic machines that sort seed by color.

Parallel with the development of the seed industry has come an increase and improvement in the distribution and quality of varieties propagated asexually. With such varieties, one of the most difficult problems has been to maintain and to distribute them free of diseases and insects.

Production of Seed of Varieties of Field Crops

Except for the small grains, much of the field-crop seed sown each year is purchased from a seed company. Some idea of the size of this business in the United States is given by the data of Table 29.1, which lists only the

Table 29.1 Acreage and production of seed of forage crops used for hay and pasture in the United States (Average 1959 to 1963).^a

	ACRES HARVESTED	PRODUCTION, THOUSANDS OF POUNDS
Alfalfa	723,700	133,781
Red Clover	952,400	77,035
Sweet Clover	119,620	23,936
White Clover	29,170	5,230
Ladino Clover	19,140	5,172
Lespedeza	374,700	79,415
Timothy	222,440	32,877
Orchardgrass	56,200	11,962
Merion Ky. Bluegrass	14,084	3,441
Other Ky. Bluegrass	^b	12,366
Chewings Fescue	22,000	8,732
Red Fescue	12,370	4,587
Tall Fescue	152,020	33,407
Bentgrass	23,990	6,379
Crimson Clover	58,160	13,097
Hairy Vetch	109,700	25,504
All Ryegrass	154,000	158,900
Total	3,043,694	635,821

^a From "Field Seeds—Supply and Disappearance," Washington, D.C., U.S. Dept. Agr. Statistical Reporting Service, Crop Reporting Board, December, 1965.

^b Data not available.

seed of small-seeded forage crops. Seed laws and seed-regulating agencies were developed primarily for the field crops.

The distribution of high-quality seed of field crops has been greatly

facilitated by the development of a system of certified seed. The International Crop Improvement Association has played an important role in the effective operation of certified seed programs.

International Crop Improvement Association. The International Crop Improvement Association was formed in 1919.⁸ It is now a federation of seed associations of Canada and more than 40 states of the United States. The present objectives¹ are:

1. To assist its members in promoting the production, identification, distribution and use of certified seed and other propagating materials of superior crop varieties.
2. To establish minimum standards of seed production, storage, and handling.
3. To assist in standardization of certification requirements and procedures to the end that all certified seed will be as good or better than an accepted, minimum standard of quality.
4. To inform the public as to the value of certified seed and encourage its wide-scale use through approved educational media.
5. To develop cooperation with all individuals, groups and organizations directly or indirectly interested in the improvement of crops.

Seed certification. The purpose of seed certification is to make available for general use seed of the best quality, having in mind its genetic constitution and its condition. It is a system that provides operating procedures both to achieve seed of high quality and to guarantee it to the user.^{1,4}

As the name implies, certified seed is labeled and guaranteed as to variety, level of germination, and other characteristics. It is seed that has been subjected to field inspections, seed inspections, and germination tests. Records on certified seed may be traced back directly to the seed developed and released by the plant breeder. Three classes of seed are usually involved in the ancestry of certified seed: (1) breeder seed; (2) foundation seed; and (3) registered seed.

Breeder seed. This is the seed stock of a variety developed by the plant breeder. It is under the control of the plant breeder or of the institution or company with which he is associated. It is the plant breeder's responsibility to maintain breeder seed indefinitely into the future, as long as the variety is grown. This may not be an easy task, for it may involve the maintenance of inbred lines or clones and the production of hybrid combinations of these as breeder seed is required. Breeder seed may consist of a few pounds or several sacks.

Foundation seed. Foundation seed (including elite seed in Canada) is the initial increase of breeder seed. Often, foundation seed is grown by

the plant breeder or his company or institution, and may consist of several acres. It is often grown directly or indirectly under the supervision of the plant breeder originating the variety. Where a variety is used in several states, experiment stations of a number of states may each develop its own foundation seed from breeder seed. Foundation seed is grown from either breeder seed or foundation seed, and may be used to produce certified seed directly or through registered seed.

Registered seed. Registered seed is usually grown from foundation seed by farmers, sometimes under a contract with a seed company. Both field and seed inspections are required, with standards higher than for certified seed. Usually only one generation is registered, but under certain conditions registered seed may be grown from registered seed.

Certified seed. Certified seed may be produced from foundation, registered, or certified seed. The number of generations eligible for certification is specified. As is registered seed, it is grown under prescribed conditions of culture and isolation, and is subject to field and seed inspections.

The certifying agency. Certifying agencies are usually operated in collaboration with the state agricultural experiment stations. The agricultural extension services and the state departments of agriculture are often involved. Most of the seed certification agencies are organized as state crop improvement associations. These associations are governed by a board of directors, which usually includes representatives from the experiment station, the extension service, the state department of agriculture, the seed trade, and several others elected by growers.

The experiment station usually provides office facilities and pays part of the expenses of maintaining the secretary manager, who is a staff member of the station. Other expenses are met by fees that may include acreage, inspection, sealing, and tagging fees.

Approval of a variety for certification. Certification of a new variety is a responsibility of the crop improvement association of the state where the certified seed will be grown. The plant breeder, or the agency or company requesting certification, must supply information on the history or origin of the variety, results of evaluations in comparison with other varieties, a detailed description, the area of intended use, and a description of procedures to be used in maintaining breeder, foundation, registered, and certified seed. In addition, the plant breeder specifies the number of generations that can be grown from breeder seed, and in the case of perennial crops, he states the number of harvests from one generation.

When a company requests certification, it retains control of breeder and foundation seed, or may contract with another company to grow

these stocks. Fields producing breeder and foundation seed must be made available for inspections.

The certification process. Certification begins for the grower when he files an application with the certification agency to grow a variety of a crop. He must identify the source of seed he intends to use. Also included is the cropping history of the field.

The land, to be eligible, must not have grown the same crop for a specified period unless the seed crop was of the same variety and of the proper certified class. The field must be free of all primary noxious weeds and secondary noxious weeds that may be difficult to separate from the crop seed. It must provide the isolation requirements established for the crop in question.

Field inspection by an authorized inspector becomes the next important feature of certification. Fields are examined before harvest for mixtures with other varieties and crops, for the presence of diseases, especially those that are seed-borne, for noxious weeds, and for degree of isolation.

Harvesting and recleaning equipment is inspected to make certain that it has been thoroughly recleaned in order to avoid mechanical mixtures and to see that it is properly adjusted to do a thorough job without damage to the seed. Once the seed is recleaned and put into approved storage, an official sample is drawn and submitted to the certifying agency.

A portion of the seed sample is sent to an approved seed laboratory to determine germination, inert matter, weed seed, and other features required for good seed.

If the seed meets the minimum standards established by the International Crop Improvement Association and the standards established by the state (which may be higher), it becomes officially certified. Before moving into commerce, it is tagged and sealed to protect its identity.

Distribution of certified seed. Certifying agencies actively promote the use of certified seed, but do not buy or sell certified seed or market it directly. It is distributed through the established seed-marketing channels. This includes seed firms, brokers, retailers, and others engaged in merchandising seed. In some crops such as small grains and soybeans, certified seed is often sold by the growers to other farmers.

Noncertified seed of field crops. Some companies contracting acreages of field crops for processing have their own breeding programs, develop their own varieties, and have worked out their own arrangements for the increase of seed of new varieties. In this way these companies have full control of seed stocks. Most, if not all, sugar beet processing companies operate on this basis, and companies contracting acreage of the new oil crops, safflower and castor beans, appear to be following a similar pattern.

Exclusive of seed used by processing companies, a vast amount of seed of field crops is not certified. In this case the guarantee of the variety's identity is the reputation of the seed company selling the seed.

Registration of new varieties. New varieties of field crops in the United States can be registered with the Crop Science Society of America. Registration includes a description of the variety, which is published in *Crop Science*.

Production of Seed of Varieties of Flowers and Vegetables

The flower and vegetable seed industry of the western states is rather highly developed. In part, seed production is on land belonging to seed companies, and in part in the hands of farmers growing the seed under contract. In both cases, production is managed by persons specializing in one or a few species. Often the seed company, or even the grower, contracts with a canner desiring a particular variety.

Much of the vegetable and flower seed is not handled or regulated through a program of seed certification. Instead, arrangements are made directly between persons or companies involved in the seed business.

Where seed production is regulated in large part by canning companies that channel the seed to their growers, federal and state experiment stations often pass the seed of newly developed varieties directly to the companies. Sometimes this is done before the station has developed the new production to the status of a variety. In this way the company can make its own final selection, which will conform to the needs of the company.

Production of Crops Propagated Asexually

The maintenance and distribution of new varieties of several horticultural crops propagated asexually present special problems because the stocks may become contaminated with certain diseases and pests that are difficult or impossible to eliminate. The most difficult of the diseases are those caused by viruses.

Many states have developed programs to promote and facilitate the distribution of disease-free stocks, which is often termed "certification." The procedures used by the State Department of Agriculture in California, in cooperation with the University of California and the U.S. Dept. of Agriculture, are described briefly below.⁹ It will be noted that the terms are similar to those used for certified seed, with the word "stock" substituted for "seed."

The University and the U.S. Dept. of Agriculture develop stocks free of the specified diseases and pests. These are propagated at the University by the Foundation Plant Materials Service, and are called *foundation*

stock. Either registered or certified stock may be grown from foundation stock.

Registered stock, which is grown from propagations of foundation stock, is maintained under isolation by a nurseryman participating in the program. It is subject to frequent inspections to ensure that it remains free of the specified diseases or pests. It is a source of certified stock.

Certified stock is propagated directly from foundation or registered stock. It is sold for the production of commercial crops.

Some examples of certification programs for nursery stock in California are avocado stock free of avocado root rot, caused by *Phytophthora cinnamoni*; citrus trees free of the virus disease called scaly bark; cherry trees free of serious virus diseases; and grapes free of virus diseases causing fanleaf, leafroll, yellow vein and yellow mosaic, and other harmful pests.

Patents. Patents may be granted to the person, agency, or company developing a new and distinct variety of plant reproducing asexually by layering, budding, and grafting. It does not include plants reproducing by tubers or producing seed apomictically. Best known are the patented varieties of roses.

Localizing Seed Production

In the United States many companies producing vegetable and flower seeds have centered their operations in the western states. Seed of many field crops, particularly the forage legumes and grasses, are being grown in the western states also. There are several reasons for this. Drier climates reduce the incidence of leaf diseases and lead to a higher quality of seed. With a long growing season and good control of water supply, maximum yields are obtained.

Some Canadian and European seed companies have arranged for the production of seed in the western states also. Other companies in Europe have taken their seed production operations to countries bordering on the Mediterranean. It is likely that this trend will continue to develop for small-seeded crops, where the price of seed is high.

With cross-pollinated crops there is danger that there will be shifts in their genetic constitution under another environment (see Chapter 16). For this reason, certified seed of some crops is grown under specified restrictions of latitude and number of generations out of the area of intended use.

Forage-crop breeders are planning to develop varieties that will not flower under the day lengths in the area of intended use.¹¹ This would mean that all resources of the plant would be used in the production of leaves that have a greater nutritive value than the stems. Breeder seed would be produced under conditions of extended daylight provided by

lights in the field. Foundation, registered, and certified seed would then be produced at a latitude providing long days. It is obvious that the environment chosen for seed production should permit all plants, not a portion of them, to produce seed.

Isolation

Isolation of seed fields is required to prevent mechanical admixtures and to prevent the transfer of pollen from other varieties of the same or a closely related species. The isolation necessary to prevent mechanical admixtures is approximately 10 to 20 ft separation, which is about the isolation requirement in the production of a self-pollinated crop.

Isolation requirements for cross-pollinated crops are usually based on field experience or experimental evidence obtained from the crop in question or from a similar crop. Isolation requirements will vary, depending on whether the crop is wind- or insect-pollinated. Isolation requirements for cross-pollinated crops vary from 300 to 1600 m.⁷

For wind-pollinated species, windbreaks have been used to reduce isolation distances.⁶ In this respect cereal plots may serve to isolate small plots of low-growing vegetables and grass-seed crops. Trees may be necessary for taller crops. Location of seed fields relative to prevailing winds is important.

The feeding and flight patterns of bees complicate isolation requirements for some insect-pollinated species. Large seed fields with abundant flowers usually encourage bees to stay in the field, particularly if the hive is in the field. Often, bees coming into a field may visit the margin of the field first. For this reason it is often advisable to use seed fields that are square in shape and to discard plants on the margin.² The bee colony and two fields of different varieties of the same crop should form a triangle, since bees will fly directly from a field to the hive.¹⁰ If the two fields line up with the colony on one end of the line, bees are more likely to visit both fields on the same flight. Fortunately, bees are clean of pollen when they leave the hive.

One-variety communities. Partly for reasons of isolation, and partly to maintain a uniform product, some communities have elected to grow only one variety of a field crop. Such communities have developed for cotton varieties in several states of the United States. When a single community grows several varieties of cotton, it has been practically impossible to keep the seed of varieties separate during the ginning process.

The largest one-variety community growing cotton is in the southern San Joaquin Valley of California, where by law only one variety of cotton can be grown in the entire area, which amounts to over 500,000 acres. A carefully controlled program of seed production has been developed by

the cotton industry of the area, involving the cooperation of farmers, gin managers, and county, state, and federal personnel. This program permits any improved versions of the variety to be channeled quickly in an orderly manner to cotton growers.

Seed Laws

Seed laws were developed in the United States to protect not only the farmer or nurseryman who used the seeds, but also the seedsman.¹² They provide for correct labeling of seed containers.

In 1912, Congress passed the Seed Importation Act. This Act specified the requirements of imported seed with respect to purity and content of weed seeds. In 1916, it was amended by specifications regarding live seed. Again, in 1926, it was amended to require coloring of imported seed to indicate the degree of adaptability. Also in 1926, another amendment prohibited interstate shipment of seeds that were falsely or fraudulently labeled. A new Federal Seed Act was enacted in 1939, which incorporated and extended the provisions of the Seed Importation Act. Most state seed laws conform rather closely to federal laws.

Generally, state and federal laws require the label to show percentage content of: (1) pure seed; (2) weed seeds; (3) other crops seeds; (4) inert matter; (5) germination; and (6) hard seeds, if any. In addition, the label includes the date of the germination test, the names and rates of occurrence of noxious weed seeds, and the name and address of the shipper, or seller, or person who labeled the seeds. Mixtures must be indicated with the percentage of each kind of seed in amounts over 5 per cent, and the label must identify treated seed.

Plant Breeders' Rights

Several decades ago, European plant breeders, many of whom are employed by seed companies or organizations closely connected with the seed industry, prevailed upon their governments to set up a system of breeders' rights. Such rights require that anyone reproducing a variety must obtain the permission of the originator, often on payment of a fee. Such rights exist for a specified period, usually 15 years. The restrictions apply only to seed offered for sale; a farmer is free to use the seed for as many generations as he wishes. Prior to being granted rights to a variety, a breeder must have the variety registered. To be registered, the variety must be distinct from other varieties, uniform, and stable through repeated reproduction.

The system of breeders' rights has operated to the satisfaction of most breeders in Europe. It has been of particular value in the case of asexually propagated crops or self-pollinated crops, both of which reproduce

themselves faithfully. In many cases it has permitted companies or plant breeders to recover the costs of breeding new varieties. It has not been mandatory in Europe that a variety be registered and that a breeder exercise his rights to its use.

Opinion about breeders' rights in the United States is mixed.⁵ On the one hand are those who strongly feel that breeders' rights should and will develop if the seed industry is to be kept in good order and the cost of breeding new varieties is to be recovered. On the other hand, many persons in the flower and vegetable seed business prefer the unregulated "free and easy" system, in which the identity and quality of the seed is judged by the reputation of the seed company and its representatives. Another group in the seed business opposes breeders' rights because it has achieved essentially all its advantages biologically through the control of inbred lines, clones, or other stocks going into the production of a new hybrid or synthetic variety.

Résumé

The trends of the past in the seed industry will continue in the future. As hybrid varieties of the self-pollinated crops are developed, like many of the hybrid and synthetic varieties of the cross-pollinated crops, they will consist mostly of developments by seed companies. This means that most, if not practically all, of the seed of field and horticultural crops will some day be grown by persons or organizations specializing in seed production, often in areas quite different from that where the seed will be used. The germ plasm of the crops of the country will be under a different system of management. The sheer volume of seed, the dependence of agriculture on the seed industry, and the large number and diversity of seed companies and organizations will dictate changes in laws and regulations. These laws and regulations will be designed to facilitate the movement and production of seed and to guarantee its identity.

More and more seed will be moving from one country to another over distances of thousands of miles. This will require extensions of present regulations and integration of regulations of several countries.

With seed companies expanding their role in plant breeding, it may seem that the U.S. Dept. of Agriculture and the state experiment stations will reduce their plant-breeding responsibilities accordingly, but this would be unfortunate. Rather, the public research agencies should not decrease their efforts but should change the pattern or emphasis on plant breeding. Public research agencies will make their most useful contributions to agriculture by developing better germ plasm and improved breeding procedures. Better germ plasm can involve the discovery of (1)

genes that give improvements with respect to such things as resistance to diseases and insects, quality, and adaptability to mechanical handling; (2) combinations of cytoplasm and genes that permit control of the mating system; and (3) changes in chromosome number and structure that will lead to the production of better varieties. Improved breeding procedures will permit better management of genes, chromosomes, and genomes to the end that improved varieties are produced in a shorter period of time. In short, plant breeders of the federal and state research organizations will be advancing the science of plant breeding.

It is obvious that the future developments of the seed industry will require a great many plant breeders with different levels and types of training. This means that educational institutions will have greater responsibilities in the training of personnel for this industry. It means also that there will be many opportunities for students well trained in the practice and the science of plant breeding.

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FIELD PLOT TECHNIQUE
AND EXPERIMENTAL
DESIGN



We live in a statistical world and should use statistical methods wherever they prove useful. We live also in a commonsense world in which good judgement is essential. If statistical methods and judgement disagree we should accept neither one nor the other but strive to determine which is most likely correct, or in many cases conduct additional experiments until conclusions can be supported by both.⁷

SALMON AND HANSON, 1964

IN DEVELOPING NEW VARIETIES a plant breeder must continually compare the materials he develops with those they are intended to replace.

Such comparisons will begin early in the development of varieties, often when only small amounts of seed of a large number of selections are available. Comparisons at later stages will involve fewer selections, probably very similar in performance, and comparative tests then will require all the refinements possible in experimental design.

Great advances have been made in the past 50 years in experimental designs and analytical procedures to facilitate comparisons of several varieties. Only the simpler designs are considered here; sophisticated and complex types may be learned from courses or books on the subject. The best experimental design is no better than the field-plot technique, so that is considered first.

Field-Plot Technique

The details of good field-plot technique are best learned in an apprenticeship to one experienced in this field. Though the details are important, they can be covered here only in general terms. It must be emphasized, however, that the purpose of good technique is to make the sample truly representative of the population. If six plots of Atlas barley are harvested each year from a rod-row test to represent the performance of that variety in one location (the total area will be about 100 sq ft, or less than 1/400 of an acre), it is extremely important that conditions be such that the sample is reliable.

Uniformity of test areas. In field experimentation one can rarely escape some degree of soil heterogeneity. Many factors contribute to it, too many to discuss here in detail. These are often undetectable at planting time, becoming visible only after the crop begins to grow. Such "invisible variability" may be the product of such things as soil compaction from previous crops, a creek bed covered up in land levelings, alleyways in the previous crop, herbicide and fertilizer tests in the immediate past, and soil-infesting diseases or insects. As described in Chapter 8, pre-cropping the test area uniformly to a single variety of a crop will often eliminate some variability and will provide an opportunity to record the pattern of remaining variability.

Growing the crop. The best cultural practices should be used with respect to seedbed preparation, time and rate of planting, fertilization, irrigation, cultivation, weed control, and harvesting. Because of the small size of plots, specialized equipment is often necessary, and in many cases hand planting and harvesting may be required.

Protection against rodent or bird damage may be essential, even to the extent of caging plots. Rarely, if ever, do birds or rodents feed uniformly on all varieties or in all locations in an experimental field.

It hardly seems necessary to say that the crop should be carefully har-

vested and the produce accurately weighed. Likewise, care must be taken to harvest exactly the same areas for each plot.

Size and shape of plots. A good deal of research has been done on size and shape of plots, often in order to make the best use of a limited amount of land. The tendency has been toward smaller plots and more replications. The smaller the total experimental area, the smaller the total problem of soil heterogeneity is likely to be. In general, two sizes of plots are in common use.

1. *Very small plots* are normally referred to as nursery plots. The row plot is typical and is used extensively with cereal varieties. It usually consists of one to three rows 18 to 20 ft long, though more rows are sometimes used. With three or more rows, the outside rows are discarded at harvest to eliminate border effects and competition between varieties. Row ends are also discarded.

2. *Large plots* are often referred to as field plots. With cereal crops, these may be 1/100 to 1/10 acre, and are often the width of a commercial-type drill or combine.

Blocks (replications). Where the pattern of soil heterogeneity is unknown, it is preferable to have the replicates square in shape. If the plant breeder knows the gradients in, or islands of, soil variability, replicates should be shaped or located so that each is as uniform as possible in all aspects of the environment. The individual plots of the replicate should be long and narrow, with the long dimension in the direction of the environmental gradient, if the gradient is known.

Row spacings. Row spacings should be similar to those used in commercial production. In small plots, however, very close spacings are usually difficult to achieve, sometimes difficult to evaluate, and difficult to harvest. For small grains the spacings are often extended to 12 in., about twice that used commercially. Experimentation has indicated the departures from commercial practice that may be made with most crops without sacrificing accuracy.

Border effect. It is a common observation, not only in nursery tests but in commercial fields, that plants at the margins are better than those in the center. This happens usually because the border plants have more space, and consequently more moisture and nutrients. The situation may be the opposite, too. The margins of soybean nurseries are sometimes severely infested by spider mites because more dust accumulates there; and yellow dwarf, a virus disease of barley, may be more severe on margins because the aphid vector prefers such locations.

Usually, the narrow plots of a replicate are planted contiguous to one another, with extra rows on each side of the replicate. This leaves only the ends of the plots manifesting border effect. Sufficient material is

trimmed off the ends of the plots to eliminate the border effect. It might be argued that the border effect will be the same for all plots and that its removal is unnecessary. There is evidence, however, that varieties differ in their abilities to take advantage of the extra moisture and nutrient supplies at the border.

Interplot competition. There is abundant evidence that varieties have different competitive abilities. A variety may gain its advantage by vigorous early growth, by greater height, or ability to tiller or branch profusely. Weak-stemmed varieties, if they lodge, will often take down adjacent rows. Because of these interplot interactions, plots often consist of three or more rows, and the outside rows of each plot are discarded. Where seed is scarce, plant breeders may use single-row plots, but alternate every plot with a row of a check variety. In this way each single-row plot competes with the same variety on each side.

Uniform stands. Every row in a yield test is sown with the same number of seeds that will germinate to produce seedlings. This means that seed-weight determinations and germination tests are necessary on all entries, with the amount of seed sown in each row being adjusted accordingly. In spite of such procedures and careful planting techniques, stands may not be uniform.

Of most concern are thin stands in one or a few varieties or selections in a test. A plant breeder's experience or previous experimentation will usually indicate the limits in stand that will affect yields, and certainly the records of the test should indicate the type of stands obtained for each selection so that the results may be properly interpreted. Thin stands may indicate an inherent weakness in a potential new variety, a weakness such as susceptibility to threshing injury or soil-borne pests.

Gaps in the stand present a problem. If they are small (with cereal plots, less than 12 in.) they are ignored; the border effect enjoyed by the plants on the margin of the gap is assumed to compensate in large part for the missing plants. Large gaps should be measured and yields adjusted in accordance with the actual length of row harvested. With both thin stands and long gaps in the stand, adjacent rows will often benefit from the reduction in competition.

Harvesting all selections in a test. Often in field tests of similar selections some may appear obviously inferior—they may show stem weakness after strong winds, severe damage from a slight frost, or susceptibility to disease. The plant breeder will wonder whether such selections should be harvested. With a simple design such as the randomized complete block, some selections may be deleted without affecting the validity of the test. But with some complex designs involving special groupings of varieties and a small number of replicates, all materials must be harvested

if the analysis is to be made. It is important to be aware of the requirements that a design places on the need for harvesting.

Check variety. It is scarcely necessary to state that a check variety is necessary in a test. Usually, check varieties are successful commercially grown varieties. Where feasible, the same check variety may be continued for several years to provide a measure of the progress a plant breeder is achieving. If a check variety proves susceptible to a disease or pest, it ceases to be a good measure of the yielding ability of new selections, aside from yield differences resulting from their resistance to the disease or pest.

Relating nursery yields to commercial yields. Often, though not always, experimental plots will give yields higher than those achieved by farmers. This is particularly true of small nursery plots given the best care. Such yields are usually reported as they occur, but a well-known check variety provides a basis for comparisons.

Comparisons with small amounts of seed. Where small amounts of seed are available (for example, the amount obtained from a single head of wheat), replicated comparisons are obviously impossible; the plant breeder must rely on the results from a single plot. Often, a very large number of such head rows will be grown at one time. The procedure is to plant the same number of seed in each plot and to choose a plot size that will use the available seed. Every fifth to tenth plot is sown to a check variety, the check plot being more frequent where the soil is variable. Comparisons from such plots are usually limited to height, date of flowering and maturity, strength of stem, and other easily measured characteristics, though yields may sometimes be taken if stands are uniform.

Experimental Design

With larger amounts of seed, a plant breeder will have the choice of increasing plot size or increasing the number of plots of each variety or selection, or both. Increasing the number of plots of each variety and using an appropriate design for the test make it possible to submit the data to statistical analyses, usually termed *variance analyses*.

In a general sense, variance may be considered synonymous with variation, and a variance analysis is simply a procedure for measuring the magnitude of factors contributing to variability in a test or tests. In tests carried on by a plant breeder, the factor of major interest is the differences between the materials he has developed and existing varieties. Another important factor increasing variability is soil heterogeneity; yields will be high on good soil and low on poor soil. The yield test may be conducted over a period of years, or in different areas, and vari-

ance analyses will measure variation due to the effects of years or different locations. Not all varieties of the test may show the same relative differences with different years or locations; they *interact* variously with year or location, and variance analysis will measure the interaction.

More precisely variance, V , is the square of the standard deviation, or $V = s^2$; and conversely, $s = \sqrt{V}$.

In terms of deviations from the mean, $(x - \bar{x})$, and degrees of freedom, $(n - 1)$, from formula 5.7,

$$V = \frac{\sum (x - \bar{x})^2}{n - 1}$$

Replication. A reliable measurement of the performance of a new variety obviously requires that the comparisons be replicated, or grown more than once. A number of plots of each variety makes it possible to measure the standard errors of their mean yields ($s_{\bar{x}_v}$) and thus measure the significance of any differences. It will be recalled that the standard error of the mean decreases by $1/\sqrt{n}$ of the standard deviation of the population (formula 5.6). Thus, 4 replications will reduce it to $1/2$, 9 replications to $1/3$, and 16 replications to $1/4$. It is evident that increases in replications beyond 5 or 6 reduce the standard error very little.

Further, the theoretical gain is never fully realized: Each increase in replications increases the area of land required, and the increase in soil heterogeneity and other factors contributing to variability will offset some of the gain in reduction of the standard error of the mean. There is also the very practical consideration of how many replications can be afforded. The decision is very likely to be a compromise, with economy exerting a strong influence.

Replications are rarely less than four, or more than six. Fortunately, the necessity of running comparisons for a minimum of three years somewhat lessens the necessity for great precision during any one year. If grouped in certain ways, besides adding precision to measures of mean yields and providing a measure of experimental error, replications permit a measure of the variability due to soil heterogeneity. This will be apparent when the balanced block design is studied.

Randomization. Statistical treatment of data implies that random samples are being considered. Randomization is essential to avoid any personal bias, no matter how unintentional. It has already been mentioned that single replications of each variety or selection in an experiment are usually grouped together in one block. Each variety or selection is located at random within the block. Random assignment may be achieved by using numbered marbles, bean seeds, or cards. The numbered

items are thoroughly mixed and drawn without reference to the numbers on them. More convenient for many purposes are tables of random numbers, prepared by Tippett¹⁰ and Fisher and Yates.⁵

It is important to examine the distribution of each entry in a test area, even though the assignments are made at random. Each entry should be fairly well distributed over the test area. If one falls consistently on one side of a test with a strong soil gradient, the performance of this item may be strongly biased in one way or another. In such case it would be advisable to repeat the randomization procedure.

It should be apparent that the use of blocks puts a control on randomization, since a variety or selection can appear only once in each block. Control exists in two directions in the Latin-square design, as will be seen later.

Randomized complete block. Since the randomized block design is in common use, it will be used as the first example of a variance analysis. Small values have been chosen so that computations will be easier. The randomized block is of interest to the plant breeder because it provides a method of comparing a relatively large number of varieties or selections at one time. Furthermore, it can be carried out with any number of replications that can be managed.

Hypothetical yields in sacks per acre of five varieties (*A*, *B*, *C*, *D*, and *E*) are given in Table 30.1. The mean was chosen as 10 so that all devia-

Table 30.1 Randomization, yields and deviations from mean yield, of five varieties in a 4-replicate yield test (all data in sacks per acre).

BLOCKS	YIELDS AND DEVIATIONS OF VARIETIES					TOTAL YIELD
1	<i>D</i> 12 (2)	<i>A</i> 16 (6)	<i>B</i> 14 (4)	<i>C</i> 9 (−1)	<i>E</i> 13 (3)	64
2	<i>C</i> 7 (−3)	<i>B</i> 12 (2)	<i>A</i> 12 (2)	<i>E</i> 12 (2)	<i>D</i> 9 (−1)	52
3	<i>A</i> 10 (0)	<i>D</i> 9 (−1)	<i>E</i> 8 (−2)	<i>C</i> 8 (−2)	<i>B</i> 9 (−1)	44
4	<i>B</i> 7 (−3)	<i>E</i> 8 (−2)	<i>C</i> 8 (−2)	<i>D</i> 7 (−3)	<i>A</i> 10 (0)	40

tions would be whole numbers. Deviations from the mean, $x - \bar{x}$, or in this case, $x - 10$, are shown in parentheses.

Since each block (replicate) has the same entries present, it is possible to calculate the variance due to blocks. Also, the variance due to varieties may be measured. Subtracting these from the total gives an error variance that may be used to determine the significance of variance due to both blocks and varieties. Using the data in Table 30.1, the necessary computations and analyses are as follows:

The *total sum of squares* is the sum of the deviations from the mean squared and may be obtained by the formulas:

$$\sum(x - \bar{x})^2 \quad (\text{for deviations from the mean}) \quad (30.1)$$

$$\sum x^2 - \frac{(\sum x)^2}{n} \quad (\text{for machine calculations}) \quad (30.2)$$

in which n is the total number of plots (20 in the example)

Using formula 30.1, the total sum of squares becomes:

$$(2)^2 + (6)^2 + (4)^2 \cdots + (-2)^2 + (-3)^2 + (0)^2 = 124$$

Using formula 30.2, the result is

$$(12^2 + 16^2 + 14^2 \cdots + 8^2 + 7^2 + 10^2) - \frac{200^2}{20} = 124$$

Sum of squares for varieties. The total yields of each of the five varieties are then assembled to determine the amount of variance they exhibit. For each variety, the total yield, mean yield, and number of replicates are symbolized as x_v , $\bar{x}_v$, and n_v , respectively, as in Table 30.2.

Table 30.2 Summary of yields of varieties in Table 30.1.

VARIETY	TOTAL YIELD, x_v	MEAN YIELD, $\bar{x}_v$	DEVIATION, $\bar{x}_v - \bar{x}$
<i>A</i>	48	12.0	2.0
<i>B</i>	42	10.5	0.5
<i>C</i>	32	8.0	-2.0
<i>D</i>	37	9.25	-0.75
<i>E</i>	41	10.25	0.25

Sum of squares for varieties is obtained by the formulas:

$$n_v[\sum(\bar{x}_v - \bar{x})^2] \quad (\text{for deviations from the mean}) \quad (30.3)$$

$$\frac{\sum(x_v)^2}{n_v} - \frac{(\sum x_v)^2}{n} \quad (\text{for machine calculations}) \quad (30.4)$$

Using formula 30.3, the sum of squares for varieties becomes:

$$4[(-2.0)^2 + (-0.5)^2 + (-2.0)^2 + (-0.75)^2 + (0.25)^2] = 35.5$$

and using formula 30.4:

$$\frac{(48^2 + 42^2 + 32^2 + 37^2 + 41^2)}{4} - \frac{200^2}{20} = 35.5$$

Sum of squares for blocks (replications). For each block, x_b , $\bar{x}_b$, and n_b represent the total yield, mean yield, and number of entries (varieties). These are given in Table 30.3:

Table 30.3 Summary of yields of blocks in Table 30.1.

BLOCK	TOTAL YIELD, x_b	MEAN YIELD, $\bar{x}_b$	DEVIATION, $\bar{x}_b - \bar{x}$
1	64	12.8	2.8
2	52	10.4	0.4
3	44	8.8	-1.2
4	40	8.0	-2.0

Proceeding as for varieties, the sum of the squares for blocks is

$$n_b[\sum(\bar{x}_b - \bar{x})^2] \qquad \text{(for deviations from the mean)} \qquad (30.5)$$

$$\frac{\sum(x_b)^2}{n_b} - \frac{(\sum x_b)^2}{n} \qquad \text{(for machine calculation)} \qquad (30.6)$$

Using formula 30.5 sum of squares for blocks becomes:

$$5[(2.8)^2 + (0.4)^2 + (-1.2)^2 + (-2.0)^2] = 67.20$$

and using formula 30.6:

$$\frac{(64^2 + 52^2 + 44^2 + 40^2)}{5} - \frac{200^2}{20} = 67.20$$

Of the total sum of squares (124.0) in the example, it is now known that the amount contributed by varieties was 35.5, and by blocks, 67.2. The balance 21.3, or $124.0 - (35.5 + 67.2)$, is the sum of squares contributed by unknown factors, and is termed variously as the error sum of squares, sum of squares due to experimental error, the within-varieties sum of squares, or the sum of squares due to random variation. In any case, this is used to arrive at the standard error that is used in comparing the yield of the varieties.

These data are brought together in Table 30.4. *Degrees of freedom* are one less than the total number of items in each case; there are a total of 20 plots and 19 degrees of freedom, 5 varieties and 4 degrees of freedom, and 4 blocks and 3 degrees of freedom. The degrees of freedom for error are those remaining after the degrees of freedom for varieties and blocks are subtracted from the total degrees of freedom.

Variance. To obtain the variances (or mean squares), the sum of the

squares are divided by their corresponding degrees of freedom. The values for the example are: for varieties, $35.5/4 = 8.875$; for blocks, $67.2/3 = 22.4$; and for error, $21.3/12 = 1.775$, as shown in Table 30.4.

Table 30.4 Variance analysis of the yield of five varieties in a randomized block with five blocks (all data in sacks per acre).

SOURCE OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	F VALUE	F AT THE:	
					5% Point	1% Point
Varieties	4	35.5	8.875	5.00*	3.26	5.41
Blocks	3	67.2	22.4	12.62**	3.49	5.95
Error	12	21.3	1.775			
Total	19	124.0				

* Exceeds the 5 per cent probability point.

** Exceeds the 1 per cent probability point.

The total variance usually is not calculated. It may be of interest to note that the formula for total variance,

$$V_t = \frac{\sum x^2 - (\sum x)^2/n}{n - 1}$$

is similar to that for s (formula 5.5), but without the radical sign.

F values. The F values are obtained by dividing the variance for varieties and blocks by the variance for error; these are called "observed F values."

The F value for blocks exceeds the 1 per cent probability point, indicating that the variance from this source made a very substantial contribution to the total variance. If the variability due to block differences had not been removed, error variance would have been large, and this would have reduced the observed F value for varieties to a point where differences in variety means would have been judged not significant.

In other words, these F values are a comparison of the variance due to varieties and blocks with variance due to random sources and which is represented by error variance. Obviously, if the F value for varieties increases, this indicates an increase in the differences between the mean yields of varieties relative to differences in yield due to random causes. The question then is: At what level of F for varieties is there a significant difference between the mean yields of the varieties? Appendix Table A.2 gives values of F at 5 per cent and 1 per cent probability points for different degrees of freedom. In the example, the F value at the 5 per

cent point of probability for varieties in Appendix Table A.2 ($n_1 = 4$ and $n_2 = 12$) is 3.26. This shows that if the differences in variety means were due only to random causes, an F value of 3.26 or higher would be expected in only 5 per cent of the comparisons.

Statisticians agree that where the observed F value exceeds the F value at the 5 per cent probability point, it may be assumed that the differences between variety means are due to more than random differences; they consider that the varieties differ significantly in yield. Similarly, if the observed F value for varieties exceeds the F value at the 1 per cent point (5.41 in the example) the difference in yields of the varieties is considered to be highly significant. Since the observed F value for varieties is greater than the F value at the 5 per cent probability point, the varieties may be considered to differ significantly in yield.

Standard error of a difference. The analysis of the example up to this point has revealed a significant difference in yield among at least some of the five varieties but not between any two particular varieties. In Chapter 5 this was determined for two varieties, using the standard errors for each variety mean (formulas 5.9 and 5.10). In the present example the standard deviation, s , for error is the square root of the error variance or $\sqrt{1.775} = 1.33$. The standard error of the mean $s_{\bar{x}_v}$ of a variety is equal to $s/\sqrt{n}$ (formula 5.6), where n is the number of replications of a variety. For all varieties of the present test, $s_{\bar{x}_v}$ is $1.33/\sqrt{4} = 0.67$ bu per acre. The standard error of a difference, $s_{\bar{d}}$ (formula 5.9), is $\sqrt{s_{\bar{x}}^2 + s_{\bar{y}}^2}$, where $s_{\bar{x}}$ and $s_{\bar{y}}$ are the standard errors of the varieties being compared. Since all varieties have the same standard error of a mean,

$$\sqrt{s_{\bar{x}}^2 + s_{\bar{y}}^2}$$

becomes

$$\sqrt{2s_{\bar{x}}^2}, \text{ or } s_{\bar{d}} = s_{\bar{x}}\sqrt{2} = 0.67 \times 1.43 = 0.96 \quad (30.7)$$

Using a t test (formula 5.10), any two varieties may be compared. For varieties A and B , the mean difference, $\bar{d}$, is 1.5, so $t = 1.5/0.96 = 1.56$. The value of t for a probability of 0.05 (Appendix Table A.1) for 12 degrees of freedom is 2.18. Since the computed value of t is less than 2.18, A and B are judged not significantly different in yield.

Actually, the formula for the t test ($t = \bar{d}/s_{\bar{d}}$) is more conveniently used if the tabular value of t is multiplied by $s_{\bar{d}}$ and compared with the mean difference $\bar{d}$. If $\bar{d} > t \times s_{\bar{d}}$, the difference is judged significant. The value $t \times s_{\bar{d}}$ is called the least significant difference (LSD), or

$$\text{LSD} = t \times s_{\bar{d}} \quad (30.8)$$

For the five variety means under comparison in the present example the LSD is:

1. At the 5 per cent probability point, $2.18 \times 0.96 = 2.09$.
2. At the 1 per cent probability point, $3.06 \times 0.96 = 2.94$.

All varieties are compared in Table 30.5.

There are three comparisons in which there is a significant difference, and one where there is a highly significant difference.

Table 30.5 Differences in mean yields of varieties in Table 30.1.

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
<i>B</i>	1.5			
<i>C</i>	4.0**	2.5*		
<i>D</i>	2.75*	1.25	1.25	
<i>E</i>	1.75	0.25	2.25*	1.0

* Exceeds the 5 per cent probability point.

** Exceeds the 1 per cent probability point.

Latin square. The Latin square design requires that the replications run linearly in two directions, which are referred to as rows and columns. This design also requires that the number of entries be the same as the number of rows and columns. This means that it is an excellent design (1) for small numbers of entries and (2) for situations where the soil variability is unknown or known to run in two directions. Usually, the number of entries does not exceed eight. The Latin square design is useful to the plant breeder when the number of selections under test have been narrowed down to a small number. Hypothetical data in sacks per acre from a 5×5 Latin square test of five lettered varieties are given in Table 30.6. It will be seen that there is an increasing yield gradient from column 1 to column 5 and from row 1 to row 5. The mean yields in sacks per acre of the varieties are: $A = 12.0$; $B = 10.6$; $C = 8.0$; $D = 9.2$; and $E = 10.2$.

The variance analysis proceeds in the same manner as for the randomized block. A summary of the variance analysis is given below:

Total sum of squares (formula 30.1):

$$(0)^2 + (3)^2 + (2)^2 \cdots (-3)^2 + (2)^2 + (0)^2 = 114.$$

Variety sum of squares (formula 30.3):

$$5[(2.0)^2 + (0.6)^2 + (-2.0)^2 + (-0.8)^2 + (0.2)^2] = 45.2$$

Table 30.6 Randomization and yields of five varieties in a 5×5 Latin square test.

ROW	YIELDS IN SACKS PER ACRE										Column Total	Column Mean
	Col.		Col.		Col.		Col.		Col.			
	1	2	3	4	5	6	7	8	9	10		
5	D	10	A	13	B	12	C	11	E	12	58	11.6
4	C	8	E	9	A	14	B	13	D	12	56	11.2
3	A	11	B	10	E	11	D	10	C	8	50	10.0
2	B	8	D	7	C	7	E	11	A	10	43	8.6
1	E	8	C	6	D	7	A	12	B	10	43	8.6
Row Total	45		45		51		57		52			
Row Mean	9.0		9.0		10.2		11.4		10.4			

Rows sum of squares (formula 30.5, using means for rows):

$$5[(1.6)^2 + (1.2)^2 + (0.0)^2 + (-1.4)^2 + (-1.4)^2] = 39.6$$

Columns sum of squares (formula 30.5, using means for columns):

$$5[(-1.0)^2 + (-1.0)^2 + (0.2)^2 + (1.4)^2 + (0.4)^2] = 20.8$$

$$\text{Error sum of squares} = 114 - (45.2 + 39.6 + 20.8) = 8.4.$$

The variance due to each of varieties, rows and columns, was highly significant (Table 30.7). It is readily seen that this design is capable of giving a measure of control of environmental variability running in two

Table 30.7 Variance analysis of the yield of five varieties in a Latin square (all data in sacks per acre).

SOURCE OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE	F VALUE	F AT THE:	
					5% Point	1% Point
Varieties	4	45.2	11.3	16.14**	3.26	5.41
Rows	4	39.6	9.9	14.14**	3.26	5.41
Columns	4	20.8	5.2	7.46**	3.26	5.41
Error	12	8.4	0.7			
Total	24	114.0				

** Exceeds the 1 per cent probability point.

$$s_x = \sqrt{0.7} = 0.837$$

$$s_{\bar{x}_i} = 0.837/\sqrt{5} = 0.374$$

$$s_d = 0.374 \times \sqrt{2} = 0.529$$

$$\text{LSD} = t \times 0.529 = 2.18 \times 0.529 = 1.15$$

dimensions. This has reduced error variance, the standard error of a difference, $s_{\bar{d}}$, and the LSD. If the data are analyzed as a randomized block, with rows serving as blocks, the degrees of freedom, sum of squares and variance for error will be 16, 29.2, and 1.825, respectively. With error variance increased to 1.825, the following values will be obtained.

Standard error of a variety mean:

$$s_{\bar{x}_i} = \sqrt{1.825}/\sqrt{5} = 0.604$$

Standard error of a difference:

$$s_{\bar{d}} = 0.604\sqrt{2} = 0.854$$

Least significant difference:

$$\text{LSD} = 2.12 \times 0.854 = 1.81$$

Analysis of the data as a Latin square design gave an LSD of 1.15, 64 per cent of the value obtained, if the same data are analyzed as a randomized block design. In other words, where there was a gradient in the environment in two directions, the Latin square was more sensitive than the randomized block in identifying differences in the yields of varieties.

Résumé

Plant breeders are in the business of developing populations that they call varieties, some of which they hope will be better than varieties presently in use. All populations, when compared, will be different to some extent; they may differ in time to mature, in height, in yield, and in other characteristics. The problem in evaluating populations is to choose or develop an experimental design and plot dimensions that will permit two things: (1) the development of reliable comparable data and (2) a measure of the significance of differences.

This chapter has been a short review of this subject leading to an understanding of two simple designs in common use. The training of plant breeders will include practice in the use of much more complicated designs.

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APPENDIX

Table A.1 Table of t^*

DEGREES OF FREEDOM	PROBABILITY (P)		DEGREES OF FREEDOM	PROBABILITY (P)	
	0.05	0.01		0.05	0.01
1	12.71	63.66	26	2.06	2.78
2	4.30	9.92	27	2.05	2.77
3	3.18	5.84	28	2.05	2.76
4	2.78	4.60	29	2.04	2.76
5	2.57	4.03	30	2.04	2.75
6	2.45	3.71	35	2.03	2.72
7	2.36	3.50	40	2.02	2.70
8	2.31	3.36	45	2.01	2.69
9	2.26	3.25	50	2.01	2.68
10	2.23	3.17	60	2.00	2.66
11	2.20	3.11	70	1.99	2.65
12	2.18	3.06	80	1.99	2.64
13	2.16	3.01	90	1.99	2.63
14	2.14	2.98	100	1.98	2.63
15	2.13	2.95	125	1.98	2.62
16	2.12	2.92	150	1.98	2.61
17	2.11	2.90	200	1.97	2.60
18	2.10	2.88	300	1.97	2.59
19	2.09	2.86	400	1.97	2.59
20	2.09	2.84	500	1.96	2.59
21	2.08	2.83	1000	1.96	2.58
22	2.07	2.82	∞	1.96	2.58
23	2.07	2.81			
24	2.06	2.80			
25	2.06	2.79			

* Abridged from Table IV of Fisher's *Statistical Methods for Research Workers*, Oliver & Boyd, Ltd., Edinburgh, and from *Statistical Methods*, 5th ed., by George W. Snedecor, © 1956 by the Iowa State University Press, by kind permission of the authors and publishers.

Table A.2 Points for the distribution of F^*
(Values for 5 per cent in lightface type. Values for 1 per cent in boldface type.)

n ₂	FI DEGREES OF FREEDOM (FOR GREATER MEAN SQUARE)																								∞
	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500		
1	161	200	216	225	230	234	237	239	241	242	243	244	245	246	248	249	250	251	252	253	253	254	254	254	
	4,052	4,999	5,403	5,625	5,764	5,859	5,928	5,981	6,022	6,056	6,082	6,106	6,142	6,169	6,208	6,234	6,258	6,286	6,302	6,323	6,334	6,352	6,361	6,366	
2	18.51	19.00	19.16	19.25	19.30	19.33	19.36	19.37	19.38	19.39	19.40	19.41	19.42	19.43	19.44	19.45	19.46	19.47	19.47	19.48	19.49	19.49	19.50	19.50	
	98.49	99.01	99.17	99.25	99.30	99.33	99.34	99.36	99.38	99.40	99.41	99.42	99.43	99.44	99.45	99.46	99.47	99.48	99.48	99.49	99.49	99.50	99.50	99.50	
3	10.13	9.55	9.28	9.12	9.01	8.94	8.88	8.84	8.81	8.78	8.76	8.74	8.71	8.69	8.66	8.64	8.62	8.60	8.58	8.57	8.56	8.54	8.54	8.54	
	34.12	30.81	29.46	28.71	28.24	27.91	27.67	27.49	27.34	27.23	27.13	27.05	26.92	26.83	26.69	26.60	26.50	26.41	26.35	26.27	26.23	26.18	26.14	26.12	
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.93	5.91	5.87	5.84	5.80	5.77	5.74	5.71	5.70	5.68	5.66	5.65	5.64	5.63	
	21.20	18.00	16.69	15.98	15.52	15.21	14.98	14.80	14.66	14.54	14.45	14.37	14.24	14.15	14.02	13.93	13.83	13.74	13.69	13.61	13.57	13.52	13.48	13.46	
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.78	4.74	4.70	4.68	4.64	4.60	4.56	4.53	4.50	4.46	4.44	4.42	4.40	4.38	4.37	4.36	
	16.26	13.27	12.06	11.39	10.97	10.67	10.45	10.27	10.15	10.05	9.96	9.89	9.77	9.68	9.55	9.47	9.38	9.29	9.24	9.17	9.13	9.07	9.04	9.02	
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.03	4.00	3.96	3.92	3.87	3.84	3.81	3.77	3.75	3.72	3.71	3.69	3.68	3.67	
	13.74	10.92	9.78	9.15	8.75	8.47	8.26	8.10	7.98	7.87	7.79	7.72	7.60	7.52	7.39	7.31	7.23	7.14	7.09	7.02	6.99	6.94	6.90	6.88	
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.63	3.60	3.57	3.52	3.49	3.44	3.41	3.38	3.34	3.32	3.29	3.28	3.25	3.24	3.23	
	12.25	9.55	8.45	7.85	7.46	7.19	7.00	6.84	6.71	6.62	6.54	6.47	6.35	6.27	6.15	6.07	5.98	5.90	5.85	5.78	5.75	5.70	5.67	5.65	
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.34	3.31	3.28	3.23	3.20	3.15	3.12	3.08	3.05	3.03	3.00	2.98	2.96	2.94	2.93	
	11.26	8.65	7.59	7.01	6.63	6.37	6.19	6.03	5.91	5.82	5.74	5.67	5.56	5.48	5.36	5.28	5.20	5.11	5.06	5.00	4.96	4.91	4.88	4.86	
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.13	3.10	3.07	3.02	2.98	2.93	2.90	2.86	2.82	2.80	2.77	2.76	2.73	2.72	2.71	
	10.56	8.02	6.99	6.42	6.06	5.80	5.62	5.47	5.35	5.26	5.18	5.11	5.00	4.92	4.80	4.73	4.64	4.56	4.51	4.45	4.41	4.36	4.33	4.31	
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.97	2.94	2.91	2.86	2.82	2.77	2.74	2.70	2.67	2.64	2.61	2.59	2.56	2.55	2.54	
	10.04	7.56	6.55	5.99	5.64	5.39	5.21	5.06	4.95	4.85	4.78	4.71	4.60	4.52	4.41	4.33	4.25	4.17	4.12	4.05	4.01	3.96	3.93	3.91	
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.86	2.82	2.79	2.74	2.70	2.65	2.61	2.57	2.53	2.50	2.47	2.45	2.42	2.41	2.40	
	9.65	7.20	6.22	5.67	5.32	5.07	4.88	4.74	4.63	4.54	4.46	4.40	4.29	4.21	4.10	4.02	3.94	3.86	3.80	3.74	3.70	3.66	3.62	3.60	
12	4.75	3.88	3.49	3.26	3.11	3.00	2.92	2.85	2.80	2.76	2.72	2.69	2.64	2.60	2.54	2.50	2.46	2.42	2.40	2.36	2.35	2.32	2.31	2.30	
	9.33	6.93	5.95	5.41	5.06	4.82	4.65	4.50	4.39	4.30	4.22	4.16	4.05	3.98	3.86	3.78	3.70	3.61	3.56	3.49	3.46	3.41	3.38	3.36	
13	4.67	3.80	3.41	3.18	3.02	2.92	2.84	2.77	2.72	2.67	2.63	2.60	2.55	2.51	2.46	2.42	2.38	2.34	2.32	2.28	2.26	2.24	2.22	2.21	
	9.07	6.70	5.74	5.20	4.86	4.62	4.44	4.30	4.19	4.10	4.02	3.96	3.85	3.78	3.67	3.59	3.51	3.42	3.37	3.30	3.27	3.21	3.18	3.16	

14	4.60	3.74	3.34	3.11	2.96	2.85	2.77	2.70	2.65	2.60	2.56	2.53	2.48	2.44	2.39	2.35	2.31	2.27	2.24	2.21	2.19	2.16	2.14	2.13
	8.86	6.51	5.56	5.03	4.69	4.46	4.28	4.14	4.03	3.94	3.86	3.80	3.70	3.62	3.51	3.43	3.34	3.26	3.21	3.14	3.11	3.06	3.02	3.00
15	4.54	3.68	3.29	3.06	2.90	2.79	2.70	2.64	2.59	2.55	2.51	2.48	2.43	2.39	2.33	2.29	2.25	2.21	2.18	2.15	2.12	2.10	2.08	2.07
	8.68	6.36	5.42	4.89	4.56	4.32	4.14	4.00	3.89	3.80	3.73	3.67	3.56	3.48	3.36	3.29	3.20	3.12	3.07	3.00	2.97	2.92	2.89	2.87
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.45	2.42	2.37	2.33	2.28	2.24	2.20	2.16	2.13	2.09	2.07	2.04	2.02	2.01
	8.53	6.23	5.29	4.77	4.44	4.20	4.03	3.89	3.78	3.69	3.61	3.55	3.45	3.37	3.25	3.18	3.10	3.01	2.96	2.89	2.86	2.80	2.77	2.75
17	4.45	3.59	3.20	2.96	2.81	2.70	2.62	2.55	2.50	2.45	2.41	2.38	2.33	2.29	2.23	2.19	2.15	2.11	2.08	2.04	2.02	1.99	1.97	1.96
	8.40	6.11	5.18	4.67	4.34	4.10	3.93	3.79	3.68	3.59	3.52	3.45	3.35	3.27	3.16	3.08	3.00	2.92	2.86	2.79	2.76	2.70	2.67	2.65
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.37	2.34	2.29	2.25	2.19	2.15	2.11	2.07	2.04	2.00	1.98	1.95	1.93	1.92
	8.28	6.01	5.09	4.58	4.25	4.01	3.85	3.71	3.60	3.51	3.44	3.37	3.27	3.19	3.07	3.00	2.91	2.83	2.78	2.71	2.68	2.62	2.59	2.57
19	4.38	3.52	3.13	2.90	2.74	2.63	2.55	2.48	2.43	2.38	2.34	2.31	2.26	2.21	2.15	2.11	2.07	2.02	2.00	1.96	1.94	1.91	1.90	1.88
	8.18	5.93	5.01	4.50	4.17	3.94	3.77	3.63	3.52	3.43	3.36	3.30	3.19	3.12	3.00	2.92	2.84	2.76	2.70	2.63	2.60	2.54	2.51	2.49
20	4.35	3.49	3.10	2.87	2.71	2.60	2.52	2.45	2.40	2.35	2.31	2.28	2.23	2.18	2.12	2.08	2.04	1.99	1.96	1.92	1.90	1.87	1.85	1.84
	8.10	5.85	4.94	4.43	4.10	3.87	3.71	3.56	3.45	3.37	3.30	3.23	3.13	3.05	2.94	2.86	2.77	2.69	2.63	2.56	2.53	2.47	2.44	2.42
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.28	2.25	2.20	2.15	2.09	2.05	2.00	1.96	1.93	1.89	1.87	1.84	1.82	1.81
	8.02	5.78	4.87	4.37	4.04	3.81	3.65	3.51	3.40	3.31	3.24	3.17	3.07	2.99	2.88	2.80	2.72	2.63	2.58	2.51	2.47	2.42	2.38	2.36
22	4.30	3.44	3.05	2.82	2.66	2.55	2.47	2.40	2.35	2.30	2.26	2.23	2.18	2.13	2.07	2.03	1.98	1.93	1.91	1.87	1.84	1.81	1.80	1.78
	7.94	5.72	4.82	4.31	3.99	3.76	3.59	3.45	3.35	3.26	3.18	3.12	3.02	2.94	2.83	2.75	2.67	2.58	2.53	2.46	2.42	2.37	2.33	2.31
23	4.28	3.42	3.03	2.80	2.64	2.53	2.45	2.38	2.32	2.28	2.24	2.20	2.14	2.10	2.04	2.00	1.96	1.91	1.88	1.84	1.82	1.79	1.77	1.76
	7.88	5.66	4.76	4.26	3.94	3.71	3.54	3.41	3.30	3.21	3.14	3.07	2.97	2.89	2.78	2.70	2.62	2.53	2.48	2.41	2.37	2.32	2.28	2.26
24	4.26	3.40	3.01	2.78	2.62	2.51	2.43	2.36	2.30	2.26	2.22	2.18	2.13	2.09	2.02	1.98	1.94	1.89	1.86	1.82	1.80	1.76	1.74	1.73
	7.82	5.61	4.72	4.22	3.90	3.67	3.50	3.36	3.25	3.17	3.09	3.03	2.93	2.85	2.74	2.66	2.58	2.49	2.44	2.36	2.33	2.27	2.23	2.21
25	4.24	3.38	2.99	2.76	2.60	2.49	2.41	2.34	2.28	2.24	2.20	2.16	2.11	2.06	2.00	1.96	1.92	1.87	1.84	1.80	1.77	1.74	1.72	1.71
	7.77	5.57	4.68	4.18	3.86	3.63	3.46	3.32	3.21	3.13	3.05	2.99	2.89	2.81	2.70	2.62	2.54	2.45	2.40	2.32	2.29	2.23	2.19	2.17
26	4.22	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.18	2.15	2.10	2.05	1.99	1.95	1.90	1.85	1.82	1.78	1.76	1.72	1.70	1.69
	7.72	5.53	4.64	4.14	3.82	3.59	3.42	3.29	3.17	3.09	3.02	2.96	2.86	2.77	2.66	2.58	2.50	2.41	2.36	2.28	2.25	2.19	2.15	2.13
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.30	2.25	2.20	2.16	2.13	2.08	2.03	1.97	1.93	1.88	1.84	1.80	1.76	1.74	1.71	1.68	1.67
	7.68	5.49	4.60	4.11	3.79	3.56	3.39	3.26	3.14	3.06	2.98	2.93	2.83	2.74	2.63	2.55	2.47	2.38	2.33	2.25	2.21	2.16	2.12	2.10
28	4.20	3.34	2.95	2.71	2.56	2.44	2.36	2.29	2.24	2.19	2.15	2.12	2.06	2.02	1.96	1.91	1.87	1.81	1.78	1.75	1.72	1.69	1.67	1.65
	7.64	5.45	4.57	4.07	3.76	3.53	3.36	3.23	3.11	3.03	2.95	2.90	2.80	2.71	2.60	2.52	2.44	2.35	2.30	2.22	2.18	2.13	2.09	2.06

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Table A.2 (Continued)

F ₂	FI DEGREES OF FREEDOM (FOR GREATER MEAN SQUARE)																							∞
	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	
29	4.18	3.33	2.93	2.70	2.54	2.43	2.35	2.28	2.22	2.18	2.14	2.10	2.05	2.00	1.94	1.90	1.85	1.80	1.77	1.73	1.71	1.68	1.65	1.64
	7.60	5.42	4.54	4.04	3.73	3.50	3.33	3.20	3.08	3.00	2.92	2.87	2.77	2.68	2.57	2.49	2.41	2.32	2.27	2.19	2.15	2.10	2.06	2.03
30	4.17	3.32	2.92	2.69	2.53	2.42	2.34	2.27	2.21	2.16	2.12	2.09	2.04	1.99	1.93	1.89	1.84	1.79	1.76	1.72	1.69	1.66	1.64	1.62
	7.56	5.39	4.51	4.02	3.70	3.47	3.30	3.17	3.06	2.98	2.90	2.84	2.74	2.66	2.55	2.47	2.38	2.29	2.24	2.16	2.13	2.07	2.03	2.01
32	4.15	3.30	2.90	2.67	2.51	2.40	2.32	2.25	2.19	2.14	2.10	2.07	2.02	1.97	1.91	1.86	1.82	1.76	1.74	1.69	1.67	1.64	1.61	1.59
	7.50	5.34	4.46	3.97	3.66	3.42	3.25	3.12	3.01	2.94	2.86	2.80	2.70	2.62	2.51	2.42	2.34	2.25	2.20	2.12	2.08	2.02	1.98	1.96
34	4.13	3.28	2.88	2.65	2.49	2.38	2.30	2.23	2.17	2.12	2.08	2.05	2.00	1.95	1.89	1.84	1.80	1.74	1.71	1.67	1.64	1.61	1.59	1.57
	7.44	5.29	4.42	3.93	3.61	3.38	3.21	3.08	2.97	2.89	2.82	2.76	2.66	2.58	2.47	2.38	2.30	2.21	2.15	2.08	2.04	1.98	1.94	1.91
36	4.11	3.26	2.86	2.63	2.48	2.36	2.28	2.21	2.15	2.10	2.06	2.03	1.98	1.93	1.87	1.82	1.78	1.72	1.69	1.65	1.62	1.59	1.56	1.55
	7.39	5.25	4.38	3.89	3.58	3.35	3.18	3.04	2.94	2.86	2.78	2.72	2.62	2.54	2.43	2.35	2.26	2.17	2.12	2.04	2.00	1.94	1.90	1.87
38	4.10	3.25	2.85	2.62	2.46	2.35	2.26	2.19	2.14	2.09	2.05	2.02	1.96	1.92	1.85	1.80	1.76	1.71	1.67	1.63	1.60	1.57	1.54	1.53
	7.35	5.21	4.34	3.86	3.54	3.32	3.15	3.02	2.91	2.82	2.75	2.69	2.59	2.51	2.40	2.32	2.22	2.14	2.08	2.00	1.97	1.90	1.86	1.84
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.07	2.04	2.00	1.95	1.90	1.84	1.79	1.74	1.69	1.66	1.61	1.59	1.55	1.53	1.51
	7.31	5.18	4.31	3.83	3.51	3.29	3.12	2.99	2.88	2.80	2.73	2.66	2.56	2.49	2.37	2.29	2.20	2.11	2.05	1.97	1.94	1.88	1.84	1.81
42	4.07	3.22	2.83	2.59	2.44	2.32	2.24	2.17	2.11	2.06	2.02	1.99	1.94	1.89	1.82	1.78	1.73	1.68	1.64	1.60	1.57	1.54	1.51	1.49
	7.27	5.15	4.29	3.80	3.49	3.26	3.10	2.96	2.86	2.77	2.70	2.64	2.54	2.46	2.35	2.26	2.17	2.08	2.02	1.94	1.91	1.85	1.80	1.78
44	4.06	3.21	2.82	2.58	2.43	2.31	2.23	2.16	2.10	2.05	2.01	1.98	1.92	1.88	1.81	1.76	1.72	1.66	1.63	1.58	1.56	1.52	1.50	1.48
	7.24	5.12	4.26	3.78	3.46	3.24	3.07	2.94	2.84	2.75	2.68	2.62	2.52	2.44	2.32	2.24	2.15	2.06	2.00	1.92	1.88	1.82	1.78	1.75
46	4.05	3.20	2.81	2.57	2.42	2.30	2.22	2.14	2.09	2.04	2.00	1.97	1.91	1.87	1.80	1.75	1.71	1.65	1.62	1.57	1.54	1.51	1.48	1.46
	7.21	5.10	4.24	3.76	3.44	3.22	3.05	2.92	2.82	2.73	2.66	2.60	2.50	2.42	2.30	2.22	2.13	2.04	1.98	1.90	1.86	1.80	1.76	1.72
48	4.04	3.19	2.80	2.56	2.41	2.30	2.21	2.14	2.08	2.03	1.99	1.96	1.90	1.86	1.79	1.74	1.70	1.64	1.61	1.56	1.53	1.50	1.47	1.45
	7.19	5.08	4.22	3.74	3.42	3.20	3.04	2.90	2.80	2.71	2.64	2.58	2.48	2.40	2.28	2.20	2.11	2.02	1.96	1.88	1.84	1.78	1.73	1.70
50	4.03	3.18	2.79	2.56	2.40	2.29	2.20	2.13	2.07	2.02	1.98	1.95	1.90	1.85	1.78	1.74	1.69	1.63	1.60	1.55	1.52	1.48	1.46	1.44
	7.17	5.06	4.20	3.72	3.41	3.18	3.02	2.88	2.78	2.70	2.62	2.56	2.46	2.39	2.26	2.18	2.10	2.00	1.94	1.86	1.82	1.76	1.71	1.68
55	4.02	3.17	2.78	2.54	2.38	2.27	2.18	2.11	2.05	2.00	1.97	1.93	1.88	1.83	1.76	1.72	1.67	1.61	1.58	1.52	1.50	1.46	1.43	1.41
	7.12	5.01	4.16	3.68	3.37	3.15	2.98	2.85	2.75	2.66	2.59	2.53	2.43	2.35	2.23	2.15	2.06	1.96	1.90	1.82	1.78	1.71	1.66	1.64

60	4.00	3.15	2.76	2.52	2.37	2.25	2.17	2.10	2.04	1.99	1.95	1.92	1.86	1.81	1.75	1.70	1.65	1.59	1.56	1.50	1.48	1.44	1.41	1.39
	7.08	4.98	4.13	3.65	3.34	3.12	2.95	2.82	2.72	2.63	2.56	2.50	2.40	2.32	2.20	2.12	2.03	1.93	1.87	1.79	1.74	1.68	1.63	1.60
65	3.99	3.14	2.75	2.51	2.36	2.24	2.15	2.08	2.02	1.98	1.94	1.90	1.85	1.80	1.73	1.68	1.63	1.57	1.54	1.49	1.46	1.42	1.39	1.37
	7.04	4.95	4.10	3.62	3.31	3.09	2.93	2.79	2.70	2.61	2.54	2.47	2.37	2.30	2.18	2.09	2.00	1.90	1.84	1.76	1.71	1.64	1.60	1.56
70	3.98	3.13	2.74	2.50	2.35	2.23	2.14	2.07	2.01	1.97	1.93	1.89	1.84	1.79	1.72	1.67	1.62	1.56	1.53	1.47	1.45	1.40	1.37	1.35
	7.01	4.92	4.08	3.60	3.29	3.07	2.91	2.77	2.67	2.59	2.51	2.45	2.35	2.28	2.15	2.07	1.98	1.88	1.82	1.74	1.69	1.62	1.56	1.53
80	3.96	3.11	2.72	2.48	2.33	2.21	2.12	2.05	1.99	1.95	1.91	1.88	1.82	1.77	1.70	1.65	1.60	1.54	1.51	1.45	1.42	1.38	1.35	1.32
	6.96	4.88	4.04	3.56	3.25	3.04	2.87	2.74	2.64	2.55	2.48	2.41	2.32	2.24	2.11	2.03	1.94	1.84	1.78	1.70	1.65	1.57	1.52	1.49
100	3.94	3.09	2.70	2.46	2.30	2.19	2.10	2.03	1.97	1.92	1.88	1.85	1.79	1.75	1.68	1.63	1.57	1.51	1.48	1.42	1.39	1.34	1.30	1.28
	6.90	4.82	3.98	3.51	3.20	2.99	2.82	2.69	2.59	2.51	2.43	2.36	2.26	2.19	2.06	1.98	1.89	1.79	1.73	1.64	1.59	1.51	1.46	1.43
125	3.92	3.07	2.68	2.44	2.29	2.17	2.08	2.01	1.95	1.90	1.86	1.83	1.77	1.72	1.65	1.60	1.55	1.49	1.45	1.39	1.36	1.31	1.27	1.25
	6.84	4.73	3.94	3.47	3.17	2.95	2.79	2.65	2.56	2.47	2.40	2.33	2.23	2.15	2.03	1.94	1.85	1.75	1.68	1.59	1.54	1.46	1.40	1.37
150	3.91	3.06	2.67	2.43	2.27	2.16	2.07	2.00	1.94	1.89	1.85	1.82	1.76	1.71	1.64	1.59	1.54	1.47	1.44	1.37	1.34	1.29	1.25	1.22
	6.81	4.75	3.91	3.44	3.14	2.92	2.76	2.62	2.53	2.44	2.37	2.30	2.20	2.12	2.00	1.91	1.83	1.72	1.66	1.56	1.51	1.43	1.37	1.33
200	3.89	3.04	2.65	2.41	2.26	2.14	2.05	1.98	1.92	1.87	1.83	1.80	1.74	1.69	1.62	1.57	1.52	1.45	1.42	1.35	1.32	1.26	1.22	1.19
	6.76	4.71	3.88	3.41	3.11	2.90	2.73	2.60	2.50	2.41	2.34	2.28	2.17	2.09	1.97	1.88	1.79	1.69	1.62	1.53	1.48	1.39	1.33	1.28
400	3.86	3.02	2.62	2.39	2.23	2.12	2.03	1.96	1.90	1.85	1.81	1.78	1.72	1.67	1.60	1.54	1.49	1.42	1.38	1.32	1.28	1.22	1.16	1.13
	6.70	4.66	3.83	3.36	3.06	2.85	2.69	2.55	2.46	2.37	2.29	2.23	2.12	2.04	1.92	1.84	1.74	1.64	1.57	1.47	1.42	1.32	1.24	1.19
1000	3.85	3.00	2.61	2.38	2.22	2.10	2.02	1.95	1.89	1.84	1.80	1.76	1.70	1.65	1.58	1.53	1.47	1.41	1.36	1.30	1.26	1.19	1.13	1.08
	6.66	4.62	3.80	3.34	3.04	2.82	2.66	2.53	2.43	2.34	2.26	2.20	2.09	2.01	1.89	1.81	1.71	1.61	1.54	1.44	1.38	1.28	1.19	1.11
∞	3.84	2.99	2.60	2.37	2.21	2.09	2.01	1.94	1.88	1.83	1.79	1.75	1.69	1.64	1.57	1.52	1.46	1.40	1.35	1.28	1.24	1.17	1.11	1.00
	6.64	4.60	3.78	3.32	3.02	2.80	2.64	2.51	2.41	2.32	2.24	2.18	2.07	1.99	1.87	1.79	1.69	1.59	1.52	1.41	1.36	1.25	1.15	1.00

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Table A.3 Table of χ^2 *

DEGREES OF FREEDOM	PROBABILITY (P)							
	0.99	0.95	0.50	0.20	0.10	0.05	0.02	0.01
1	0.0002	0.004	0.46	1.64	2.71	3.84	5.41	6.64
2	0.020	0.103	1.39	3.22	4.60	5.99	7.82	9.21
3	0.115	0.35	2.37	4.64	6.25	7.82	9.84	11.34
4	0.30	0.71	3.36	5.99	7.78	9.49	11.67	13.28
5	0.55	1.14	4.35	7.29	9.24	11.07	13.39	15.09
6	0.87	1.64	5.35	8.56	10.64	12.59	15.03	16.81
7	1.24	2.17	6.35	9.80	12.02	14.07	16.62	18.48
8	1.65	2.73	7.34	11.03	13.36	15.51	18.17	20.09
9	2.09	3.32	8.34	12.24	14.68	16.92	19.68	21.67
10	2.56	3.94	9.34	13.44	15.99	18.31	21.16	23.21
11	3.05	4.58	10.34	14.63	17.28	19.68	22.62	24.72
12	3.57	5.23	11.34	15.81	18.55	21.03	24.05	26.22
13	4.11	5.89	12.34	16.98	19.81	22.36	25.47	27.69
14	4.66	6.57	13.34	18.15	21.06	23.68	26.87	29.14
15	5.23	7.26	14.34	19.31	22.31	25.00	28.26	30.58
16	5.81	7.96	15.34	20.46	23.54	26.30	29.63	32.00
17	6.41	8.67	16.34	21.62	24.77	27.59	31.00	33.41
18	7.02	9.39	17.34	22.76	25.99	28.87	32.35	34.80
19	7.63	10.12	18.34	23.90	27.20	30.14	33.69	36.19
20	8.26	10.85	19.34	25.04	28.41	31.41	35.02	37.57
21	8.90	11.59	20.34	26.17	29.62	32.67	36.34	38.93
22	9.54	12.34	21.34	27.30	30.81	33.92	37.66	40.29
23	10.20	13.09	22.34	28.43	32.01	35.17	38.97	41.64
24	10.86	13.85	23.34	29.55	33.20	36.42	40.27	42.98
25	11.52	14.61	24.34	30.68	34.38	37.65	41.57	44.31
26	12.20	15.38	25.34	31.80	35.56	38.88	42.86	45.64
27	12.88	16.15	26.34	32.91	36.74	40.11	44.14	46.96
28	13.56	16.93	27.34	34.03	37.92	41.34	45.42	48.28
29	14.26	17.71	28.34	35.14	39.09	42.56	46.69	49.59
30	14.95	18.49	29.34	36.25	40.26	43.77	47.96	50.89

For larger values of n , the expression $\sqrt{2\chi^2} - \sqrt{2n-1}$ may be used as a normal deviate with unit variance.

* Abridged from Table III of Fisher's *Statistical Methods for Research Workers*, Oliver & Boyd, Edinburgh, by kind permission of the author and publishers.

INDEX

INDEX

- Aalders, L. E.,* 249-250, 279-280
 Aamodt, O. S., 355, 357
 accessory chromosomes, 297
 Adams, M. W., 107, 113
 adaptation
 general, 106
 genes, 76
 specific, 106
 adventitious embryony, 43
Aegilops, 284-285, 287, 314, 337-339
 aging of seed, 310
Agropyron, 285, 287, 337-338
 Åkerberg, E., 271, 276, 278-280, 286-287, 290, 302, 316
 Åkerman, Å., 140, 146
 Alexander, D. E., 268, 280
 alfalfa, 17, 23, 47, 170-171, 199, 203, 215, 236, 262, 271, 345
 spotted aphid of, 370-372
 alien addition lines, 295-297
 alien substitution lines, 299
 Allard, R. W., 7, 107, 108, 113, 128, 132, 167-168, 171, 173, 190, 194, 322, 332, 337, 339
 Allen, M. W., 345, 347
 allohaploid, 278
 allopolyploid (allopolyploid), 14, 18, 266, 282-290, 327-330, 337-338
 examples of, 284-287
 genetics of, 287-289
 production of, 283-284, 322
 segmental, 266, 283
 almond, 47
 amaranths, 11
 amphiploid (amphidiploid), *see* allopolyploid
 Andersen, A. L., 313, 315
 Anderson, D. C., 229-230, 238

* Page numbers are given for authors both where their papers are cited by name or reference number and where their names appear in lists of references.

- Anderson, E., 15-16, 20, 29, 34, 186,
194, 204, 210, 317, 325-327, 332,
335, 339
- Anderson, G., 313, 315
- androgenesis, 192
- Andrus, C. F., 144, 146
- aneuploidy, 291-300
- apomixis, 43-44
- apospory, 43
- apple, 47, 110, 189, 272, 313, 330
- apricot, 46
- asexual reproduction
- apomixis, 43-44
 - vegetative propagation, 32, 43, 109-
112, 236, 307-308, 330-331, 381-382
- asparagus, 47, 182
- association, measures of, 64-68
- asynaptic gene, 268
- Atkins, I. M., 81-83
- Atkins, R. E., 148, 154, 158, 161
- Atwood, S. S., 220, 221
- Auerbach, E., 304, 315
- Auscherman, L. E., 321, 332
- autogamy, *see* self-pollinated crops
- autohaploid, 278
- autoploid (autopolyploid), 14, 17-18,
111, 263-281, 289, 314
- breeding procedures with, 277-278
 - cytology of, 272-273
 - genetics of, 273-276
 - induction of, 266-268
 - reversion to diploid, 276
 - success in plant breeding, 270-272
- Avena*, *see* oats
- Avery, A. G., 111, 113
- avocado, 382
- awns and yield, 79-82
- B chromosomes, 297
- Babcock, E. B., 187, 194
- backcross breeding, 162-173, 322, 324,
364
- character under transfer, 166-167
 - cross-pollinated crops, 170-171, 193
 - development of isogenic lines, 79-80
 - evaluation of, 171-173
 - examples of, 167-171
 - improving inbred lines, 231
 - recurrent parent recovery, 164-166
 - stepwise improvement, 168-172
- symbols used in, 163
- bacteria, 350
- Baenziger, H., 78, 83
- banana, 44, 272, 330
- barley, 46, 297, 320-321
- Atlas, 118-119, 153-154, 156, 173
 - chromosome changes, 293-295, 297
 - competition of varieties, 150-155
 - composite crosses, 154-158
 - DDT resistance in, 78
 - hybrid varieties, 234-235, 236, 293-
295
 - irradiation breeding of, 302, 305,
308-310, 312, 314
 - male sterility, genetic, 190-191, 294-
295
 - mildew, 54-55, 173, 297, 353
 - natural selection in, 150-159
 - net blotch, 33, 135-136
 - origin of, 11, 14-15
 - polyploids of, 271, 277-285
 - scald, 173
 - smooth awns, 82
 - yellow dwarf, 29, 354, 362, 389
- Barrus, M. F., 343, 357
- Bashaw, E. C., 44, 51
- basic color genes, 77
- Bat Cave, 18-19
- Bateman, A., 187, 194
- Bayles, B. B., 79, 80, 82, 83, 137, 146
- Beadle, G. W., 76, 83, 268, 280
- Beal, W. J., 224
- bean, 11, 14
- common, 11, 46, 115, 116, 313
 - lima, 11, 46, 172, 190
- Beasley, J. O., 270, 280, 320, 321, 332
- bees, 48, 190, 271, 383
- Beeson, K. E., 378, 386
- beet, 47
- Bhatti, A. G., 287, 290
- Biffen, R. H., 342, 357
- biotype, 345
- Bishop, C. J., 249, 250, 313, 315
- blackberry, 47
- Blakeslee, A. F., 111, 113, 291, 295,
296, 298, 300
- blueberry, 286
- bluegrass, 44, 45
- Bolley, H. L., 361, 373
- Bonn, G. W., 76, 83

- Booyesen, P. de V., 209, 211
 Borg, G., 312, 315
 Borlaug, N. E., 3, 7, 103, 113, 164, 173, 365, 366, 368, 373
 Boyce, S. N., 145, 146
 Boyes, J. W., 268, 281
 Bradshaw, A. D., 107-108, 113
 Braidwood, R. J., 10, 20
Brassica, see mustard, rape
 breeder seed, 378, 382
 Brewbaker, J. L., 7, 189, 194
 Briggie, L. W., 134, 137, 146, 234, 238,
 Briggs, F. N., 54, 61, 162-163, 166-173, 343-344, 351-353, 355, 357
 Brimhall, B., 242, 250
 Bringham, R. S., 16
 Brink, R. A., 321, 332
 Brittingham, W. H., 44, 51
 broccoli, 47, 187
 bromegrass (*Bromus*), 45-47, 263, 284, 330
 Brown, W. L., 15, 20, 204, 210
 Bruce, A. B., 218, 221
 Brunson, A. M., 228, 238
 Brussels sprouts, 47
 Bryan, A. A., 230, 238
 buffering, 106 (see also homeostasis)
 individual, 107
 populational, 108
 bulk population breeding, 147-164, 364
 Burkill, I. H., 29, 34
 Burnham, C. R., 127, 131-132, 231, 238
 Butler, L., 96-97, 100, 110, 113, 335, 340
 cabbage, 47, 236, 286, 320
 Caldecott, R. S., 306, 314, 315
 Caldwell, R. M., 355, 357
 Camerarius, R. J., 122
 Cannon, W. B., 107, 113
 Carleton, M., 25
 carnations, 313
 Carrier, L., 23, 34
 carrot, 47, 191
 Carson, G. P., 110, 113
 Carter, W., 342, 357
Carthamus, see safflower
 Cartwright, W. B., 345, 355, 357
 Castle, W. E., 90, 100
 castorbean, 26, 46, 71-72, 235
 cauliflower, 47
 celery, 47
 centers of diversity, 27-30
 centers of origin, 27-29
 centers of recombination, 29
 certification, 210
 asexually propagated plants, 381-382
 seed, 377-381
 Chapman, V., 284, 290
 chard, 47
 Chase, S. S., 192, 194, 278, 280
 chasmogamy, 45
 Chaubey, C., 320, 332
 check variety, 103, 391
 chemical composition, 77, 268
 Chen, C. H., 276, 280
 cherry, 47, 110, 189, 313, 330, 382
 chestnut, 47
 chiasma, 41-42
 chick pea, 46
 chili pepper, 11, 277
 chimera, 110-112, 267, 308
 chi-square (χ^2)
 table, 408
 test, 54-56
 Chittenden, R. J., 191, 194
 Christensen, J. J., 349, 358
 chlorophyll, 71, 214, 288, 305, 321
 chromatid, 40-42
 inheritance in tetraploids, 275-276
 chromosome
 accessory, 297
 additions, 292-298
 deletions, 298
 inheritance in tetraploids, 274-276
 in meiosis, 40-42
 pairing, control of, 289
 structural differences, 126-127, 309-310, 314
 substitutions, 298-299
 citrus, 44, 47, 110, 330
 Claassen, C. E., 67-69, 118-119, 121, 235, 238
 Clark, J. A., 137, 146
 Clark, W. E., 138
 Clausen, J., 290
 Clausen, R. E., 14, 283, 288, 290, 295

- cleistogamy, 44-45
 clone, 236, 251-252, 255, 257-263
 closed pedigree, 232
 clover, 46, 47, 375
 alsike, 47, 185, 189, 271
 ladino, 209-210
 red, 47, 185, 189, 263, 271, 276
 white, 47, 185, 189, 263
 coadaptation
 genes and genotypes, 176
 insects and flowers, 48
 coefficient of variability, 61, 107
 Coffman, F. A., 365, 373
 colchicine, 267, 276, 314
 collard, 47
 Collier, J. W., 247, 250
 Collins, G. N., 219, 221
 combining ability, 252, 257
 average, 228, 257
 diallel cross test of, 258
 general, 228-229, 244-246
 open-pollination test of, 257
 polycross test of, 258-261
 single-cross test of, 257-258
 specific, 229-230, 246
 top-cross test of, 228, 256
 complementary gene effects, 77, 323-324
 composite cross, 154-158
 Compton, L. E., 355, 357
 Comstock, R. E., 105-106, 113, 246, 250
 convergent improvement, 220, 231
 Coons, G. H., 200, 211
 Cooper, D. C., 321, 332
 Cooper, J. P., 13, 20, 382, 386
 Copp, L. G. L., 145, 146, 159-160
 Corkill, L., 220-221
 corn, 2, 77, 122, 298
 diseases, 214, 243, 346-347, 349
 double-cross hybrids, 108, 226, 229-231, 233, 253
 ear-to-row breeding, 201, 245
 ear-to-row-to-ear breeding, 202
 endosperm genes, 77
 heritability, 105-106
 hybrid varieties, 224-234
 hybrid vigor, 4, 213-217, 220
 inbred lines, 226-232
 inbreeding, 213-217, 220
 male-sterility, cytoplasmic, 193
 male-sterility, cytoplasmic-genetic, 191-193, 232-233
 mass selection of, 18-19, 199-200, 203-206
 mutations of, 119-120, 178, 302, 305
 oil content, 201, 204-206, 242-243
 origin of, 10, 11, 14, 16-17, 203-204
 pollination, 47-48, 320
 polyploidy, 268, 270-271
 popcorn, 248
 protein content, 201, 204-206
 recurrent selection of, 242-248
 single-cross hybrids, 225-226, 253
 synthetic varieties, 252-256
 three-way cross, 226, 253
 top cross test, 228
 variety crosses, 224-225
 correction factor, 60
 correlation, 65-66, 326
 coefficient of, 65
 cotton, 5, 30, 170, 270, 284, 320-321, 324, 331, 336, 361, 375, 383
 cross-pollination in, 46-47, 49
 origin of, 11, 18, 266
 coumarin, 78
 cowpea, 46, 361
 Crabb, A. R., 232, 238
 Craig, D. L., 249, 250
 cranberry, 286
 Crandall, B. H., 260, 264
 Crane, M. B., 50-51, 320, 332
Crepis, 187, 319
 crested wheatgrass, 263
 crop improvement associations, 378
 Crop Science Society of America, 385-386
 crossing, 136-139, 319-321
 crossing over, 40-42, 55, 314
 cross-pollinated crops, 47-48, 50, 131, 175-180, 383
 selection in, 196-211
 cross-pollination
 control of, 181-195
 determination of, 48-50
 Crow, J. F., 220-221
 cryptic structural hybridity, 322-323
 cucumber, 46, 279
 cyanogenic glucoside, 209, 220
 DDT resistance, 78, 235
 Darlington, C. D., 29, 34

- Darrow, G. M., 285, 290
 Darwin, C., 36, 51, 108, 183, 194, 212-213, 221, 224
 date palm, 27, 47, 182
Datura, 111, 291, 295-296, 298
 Davis, G. N., 191, 192, 194
 Davis, L. L., 166
 Davis, R. L., 228, 238, 320, 333
 Dawson, R. F., 110, 113
 deficiency (deletion), 304-305, 309-310, 322, 324
 De Hann, H., 7
 degrees of freedom, 55-56, 62-63, 395
 Dermen, H., 111, 113, 267, 280
 De Vries, H., 301, 315
 De Winton, D., 186, 194
 diallel crosses, 159, 258
 dicaryon, 342-343, 349
 differential hosts, 343-344
 Dillman, A. C., 76, 83, 361, 373
 dioecy, 47, 182-183
Dioscorea, 13
 diploidization, 272, 289, 314
 diplospory, 43
 Dirks, V. A., 158, 160
 disease resistance, 4-5, 13, 29
 breeding for, 335, 360-373
 genes for, 337-339
 genetics of, 350-357
 mutations for, 313
 stability of, 365-370
 tests for, 33, 363-364
 Dobzhansky, T., 175-176, 178, 180, 197, 211, 231, 238, 319, 332
 Doggett, H., 17, 20
 domestication, *see* evolution of cultivated crops
 dominant gene action, 87-89, 273
 in heterosis, 218-219
 donor parent, 163, 166, 167
 double cross, *see* corn
 Douglas, A. G., 247, 250
 Do Valle Ribeiro, M. A. M., 159, 161
 Down, E. E., 313, 315
 Downey, R. K., 78, 83
 Driscoll, C. J., 338, 340
 Drolsom, P. N., 330, 333
Drosophila, 207, 208, 302
 drug plants, 11
 duplex, 273, 276
 duplicate factors, 287, 355
 duplication, 297, 305, 310, 322, 324
 Duvick, D. N., 191-194
 dwarfing genes, 71-73
 early generation evaluations, 144-145, 158-159, 241
 East, E. M., 92-95, 100, 104-105, 113, 119, 121, 183-184, 186, 194, 214, 216-217, 221, 224, 238, 241, 250
 Ebert, W. W., 73-75, 83
 Eckhart, R. C., 230, 238
 Edwardson, J. R., 191, 195
 egg nucleus, 40
 eggplant, 46
 Eide, C. J., 349, 358
 Eigsti, O. J., 265-266, 270, 280
 Einset, J., 270, 280
 Ekdahl, W. G., 67-69
 El Ebrashy, M. F., 247, 250
 El Kishen, A. A., 353, 357
 Ellerstrom, S., 270, 280
 Elliot, F. C., 8, 298, 300, 338, 340
 Ellsworth, H. L., 24
 emasculation, 136-139
 embryo, 40
 embryo culture, 321, 335
 Emerson, R. A., 76, 83
 Emsweller, S. L., 272, 280, 320, 332
 endosperm, 40
 Endrizzi, J. E., 287, 290
 Enloe, J. A., 209, 211
 environment, 86, 99, 101-113, 208-210, 270, 310
 control of, 102, 363
 day length, 76, 270, 382
 evaluation of, 102
 temperature effects, 45, 270
 epistasis, 76, 89-90, 219-220
 equational division, 42
 equilibrium, genetic, 177
 Eriksson, J., 343, 357
 Erlanson, C. O., 30, 34
 error variance, 396
 Everett, H. L., 193-194, 231, 238
 Everson, E. H., 82-83
 evolution of cultivated plants, 2, 9-20
 centers of origin, 27-29
 changes with, 12
 comparison with natural species, 2, 14

- genetic basis of, 14-18, 301
- role of selection, 18-19
- experimental design, 391-400
 - Latin square, 398-400
 - randomized block, 393-398
- expressivity, 74

- F* table, 400-407
- F* value, 396
- Fairchild, D., 34
- Falconer, D. S., 85, 100
- family, 143
- fasciation, 74
- Fasoulas, A., 276, 280
- Federer, W. T., 108, 113
- fennugreek, 29
- fertilization, 39-40
- fescue, 47, 263
- fiber crops, 11, 26
- Ficsor, G., 305, 316
- field plot technique, 388-391
- Fife, D., 3
- Fifield, C. C., 79-80, 82-83
- fig, 47
- filbert, 47
- Findley, W. R., 242, 250
- Finkner, M. D., 49, 51
- Fisher, R. A., 393, 401, 403, 408
- flax, 3, 14, 46, 76, 191, 271, 277, 305, 321, 361
 - rust of, 348-349, 353-354, 356
- Flor, H. H., 348-349, 353, 356-357
- Florell, V. H., 159-160
- flower structure, 37
- Food and Agriculture Organization, 30, 135, 375, 383, 386
- forage crops, 210, 331, 377, 382
 - combining ability in, 257-261
 - origin of, 11, 12, 13, 18, 27
 - polyploidy in, 271
 - strain building, 257
 - synthetic varieties, 256-263
- Forbes, E., 350, 358
- forest plants, 331
- foundation seed, 378-379
- foundation stock, 381-382
- Fowler, W. L., 158, 160
- foxtail millet, 46
- Fragaria*, see strawberry
- Frandsen, H. N., 258, 263

- Frankel, O. H., 1, 8, 34, 91, 100, 145, 146
- Fraser, A. C., 76, 83
- frequency distribution, 57
- frequency histogram, 57
- frequency polygon, 57
- Fröier, K., 312, 315
- fruit crops, 11-12, 109-112, 189, 272, 285-286, 307-308, 330-331, 381-382
- Fryer, J. R., 199, 211
- Fuller, H. P., 37
- fungi
 - dicaryon, 342-343
 - heterocaryon, 349-350
 - genetics of, 345-350
 - life cycles in, 342-343
 - parasexual cycle in, 350
 - physiological races, 343-345
 - sexuality in, 343

- Gabelman, W. H., 190, 194
- Gaertner, K. F., 123
- gamete selection, 231-232
- gametophyte, 176, 322
 - female, 39-40
 - male, 38, 40
- gamma rays, 303-304
- Garber, R. J., 241, 250, 251, 255, 263
- Gardner, C. O., 199-200, 211, 261-262, 264
- Garrett, J. C., 97, 100
- Gasteria*, 187
- Gaul, H., 301-302, 307, 309-312, 314-315
- genes
 - additive effects of, 85-87
 - combinations of, 122-132
 - complementary effects of, 77
 - deleterious effects of, 214
 - dominant effects of, 87-89
 - effects on qualitative characters, 70-83
 - effects on quantitative characters, 84-100
 - epistatic effects of, 76, 89-90
 - equilibria of, 177-178
 - frequency of, 127, 176, 197
 - gene-for-gene hypothesis, 356
 - geometric gene action, 96-97
 - inhibiting effects of, 77
 - measurement of value of, 79-82

- modifying effects, 90-91, 335, 352
- multiple alleles, 124, 177-178
- multiple factors, 91-95
- mutations, 119-120, 178, 288
- overdominant effects of, 88-90, 216-218
- pleiotropic effects of, *see* pleiotropy
- pool, 176-177
- reservoirs, 26-27
- successful gene combination, 131-132
- transfer, 163, 166-167
- genetic drift, 20, 178
- genetic shift, 209, 382
- genome, 17, 266, 367
- genotypes, 124, 128-129, 165
- geometric gene action, 96-97
- germ plasm preservation, 32-33
- Gerstel, D. U., 187, 194, 295-296, 300-301
- gibberellic acid, 320
- gigas characteristics, 268
- Giles, N. H., 309, 315, 331-332
- Goodsell, D. U., 192, 194
- Goplen, B. P., 78, 83, 345, 347
- Gossypium*, *see* cotton
- gourd, 11
- Grafius, J. E., 158, 160
- grafting, 109-112, 308, 320-321, 361, 382
- grapes, 17, 29, 44, 47, 110, 272, 330, 361, 382
- Graumann, H. O., 261-262, 264
- Green, C. H., 272, 280
- Green, G. J., 353, 358
- Greenleaf, W. H., 283, 290
- Greenshields, J. E. R., 78, 83
- Gregory, W. C., 313, 315-316
- Griffiths, D. J., 383, 386
- Grimball, E. L., 191, 195
- Grissom, D. B., 248, 250
- Grundbacher, F. J., 82, 83
- Gustafsson, A., 43, 51, 302, 305, 310, 312, 315, 316
- Hackerott, H. L., 370, 373
- Hackleman, J. C., 378, 386
- Hagberg, A., 271, 276, 278-279, 280, 286-287, 290, 297, 300, 302, 309, 310, 316
- Hagedorn, D. J., 74, 83
- Hakansson, A., 270, 280
- Haldane, J. B. S., 275, 280
- Hall, O. L., 320, 332
- Hansen, H. N., 349, 357
- Hansen, H. P., 342, 358
- Hansen, N. E., 25
- Hanson, A. A., 387, 401
- Hanson, C. H., 370, 372-373
- haploid, 43, 119, 131, 192, 278, 288-289, 296
 - in fungi, 342-343, 346-347, 349-350
- Hardy, G. L., 177, 180
- Hardy-Weinberg law, 177-179, 218, 253
- Harlan, H. V., 118, 150-157, 160, 163, 173
- Harlan, J. R., 11-12, 14, 20, 29, 34, 45, 51
- Harland, S. C., 331-332
- Harrar, J. G., 341, 358, 373
- Harrington, J. B., 140, 146, 148, 158, 160
- Harrison, B. J., 207-208, 211
- Hartley, W., 27, 29, 34
- Hartmann, H. T., 110, 113
- Harvey, B. L., 78, 83
- Harvey, P. H., 105-106, 113, 246, 250
- Harvey, T. L., 370, 373
- Hayes, H. K., 8, 52, 69, 139, 146, 201, 211, 224, 226-228, 230-232, 238, 241, 250-251, 255, 263, 337, 340, 367
- Hayes, J. D., 78, 83
- Helback, H., 14, 21
- hemizygous, 278
- hemp, 47, 122, 182
- heritability, 104-106
- hermaphroditic, 48
- Hermesen, J. G., 319, 332
- hessian fly resistance, 167, 172, 345, 354-355
- heterocaryon, 349
- heterogeneity, 179
- heterogenetic pairing, 286
- heterosis, 140, 212-222
 - dominance hypothesis of, 218-219
 - epistasis hypothesis of, 219-220
 - overdominance hypothesis of, 216-218, 314
- heterostyly, 183-184
- heterothallism, 343

- heterozygosity, 179
 - advantage of, 117, 130, 140
 - decrease in, 127-131, 140
 - level of, 107, 117
- Heyne, E. G., 158, 160
- Hiesey, W. M., 290
- Hill, A. B., 78, 83
- historical period, 12
- Hockett, E. A., 197, 211
- Hodge, W. H., 30, 34
- Hoffman, A., 119, 121, 235, 238
- Holland, R. F., 234, 239
- Hollingshead, L., 319, 332
- Holmes, F. O., 350, 358, 363, 373
- Holt, E. C., 44, 51
- Holton, C. S., 343-344, 355, 357
- homeostasis, 107-108
- homoeologous chromosomes, 288-289, 298-299
- homologous series, law of, 29
- homozygosis, 127-128
- homozygosity, 123, 127-128, 144, 165, 214-215
 - shortcuts to, 131, 278
- homozygous genotypes, 128-131
- Hopkins, C. G., 201, 205
- hops, 47, 122, 182
- Hort, A. F., 361, 373
- Horner, E. S., 246, 250
- host-pathogen relationship, 355-357, 365
- Hotchkiss, R. D., 350, 358
- Houston, B. R., 170
- Hovin, A. W., 45, 51
- Howard, H. W., 110, 113
- Howard, L. O., 362, 373
- Hughes, M. B., 187, 194
- Hull, F. H., 220-221, 240-241, 246, 250
- Hutchinson, J. B., 14, 20, 70, 83
- Huxley, J., 109, 113
- hybrid varieties, 4, 223-239, 376
 - advanced generations, 232-233
 - double crosses, 108, 226, 229-231, 233, 236, 253
 - general combining ability, 228-229
 - historical, 224-225
 - production of, 232-236
 - single crosses, 225-226, 253, 256
 - specific combining ability, 229-230
 - three-way crosses, 226, 253
 - variety crosses, 225
- hybrid vigor, *see* heterosis
- hybridization, 120, 122-132 (*see also* crossing)
 - choice of parents, 135
 - objectives, 134-135
- Immer, F. R., 139, 146, 158, 160, 201, 211
- inbred lines, 226-232
 - as testers, 246
 - development of, 226-227, 245-246, 248
 - evaluation of, 227-231
 - improvement of, 231-232
 - in synthetic varieties, 252-255
 - second cycle of, 231
- inbreeding, 48, 179, 189, 207, 212-222
- incompatibility, 180, 183-190, 319-320
 - and plant breeding, 189, 190
 - effects of polyploidy on, 188-189, 270, 278
- gametophytic system of, 184-186
- heteromorphic system of, 183-185
- homomorphic system of, 183
- physiology of, 187-188
- sporophytic system of, 183-187
- use in hybrid varieties, 236
- inhibiting genes, 77
- insects (*see also* bees)
 - pollinators, 45
 - resistance to, 5, 341-342, 345, 361, 370-372
- Inspection House, 32
- integration, 179
- interallelic interaction, 76
- International Crop Improvement Association, 378
- interspecific gene transfers, 334-340
- interspecific hybridization, 317-340
 - cytology and genetics of, 324-330
 - degeneration in F_2 , 322-323
 - F_1 sterility, 321-322
 - overcoming barriers to, 319-321, 335
 - relation to evolution, 14-15
- introduction, plant, 22-35
- introduction stations, 32
- introgression, 16, 163
- inversions, 126-127, 231, 309-310, 322
- irradiation, *see* mutagen
- isoallele, 99
- isochromosome, 293, 296, 310, 338
- isogenic line, 79-82, 104

- isolation, 14, 49, 278, 383-384
 Isom, W. H., 370, 373
- Jalal, S. M., 330, 333
 Jenkin, T. J., 256-257, 264
 Jenkins, M. T., 204, 211, 214-215, 222, 227-229, 238, 241, 250, 255, 257, 264
 Jensen, N. F., 120-121, 158, 160, 321, 332, 338, 340, 366, 373
 Johannsen, W., 114-116, 118, 121
 Johnson, F. A., 384, 386
 Johnson grass, 287
 Johnson, I. J., 228, 230-231, 238, 248, 250
 Johnson, T., 345-346, 349, 358
 Johnstone, F. E., Jr., 277, 280
 Jones, D. F., 108, 113, 191, 193-194, 214, 219, 222, 224, 226, 231-232, 238, 241, 250
 Jones, H. A., 191-192, 194
 Jones, J. W., 166
 Jones, Q., 26, 35
 Jørgensen, C. A., 321, 332
 Juenheimer, R. W., 205, 211
 Julen, G., 271, 281
- kale, 47, 187, 286
 Kalton, R. R., 145-146, 154, 158, 161
 Kaplan, L., 10-11
 Kappert, H., 8
 Karpechenko, G. D., 286, 290
 Karper, R. E., 73-74, 76, 83, 217, 222
 Keck, D. D., 290
 Keeble, F., 218, 221
 Kehr, W. R., 261-262, 264
 Keim, W. F., 320, 333
 Kernkamp, M. F., 346-347, 358
 Kester, D. E., 110, 113
 Kiesselbach, T. A., 48, 51, 203, 211, 215, 222, 226-227, 232-234, 238, 258, 264
 Kihara, H., 271-272, 281
 Kimber, G., 278, 281
 King, T. H., 346-347, 358
 Kinman, M. L., 203, 254, 264
 Kirk, L. E., 256-257, 264
 Knight, R. L., 170
 Knott, D. R., 338, 340, 353, 358
 Knowles, P. F., 78, 83, 269, 281
 Knowles, R. P., 261, 264
 Koelreuter, J. G., 122-123, 213, 318
 kohlrabi, 286
 Konzak, C. F., 308, 316, 321, 332
 Kramer, H. H., 158, 160
 Kurtzweil, C., 337, 340
 Kuspira, J., 299, 300
- Laibach, F., 321, 332
 Lamarkism, 108-109
 land variety, 116-118
 Langford, A. N., 335, 340
 Langridge, J., 101, 108, 113
 Lapins, K., 313, 316
 Larter, E., 320, 332
 Latin square, 398-400
 Laude, H. H., 152-153, 160
 Laude, H. M., 209, 211
 Lawrence, W. J. C., 50-51
 least significant difference, 397-398
 legumes, 11
 Lehman, W. F., 370, 373
 Leng, E. R., 205, 211
 lentils, 29
 Lerner, I. M., 98, 100, 108, 113
 lespedeza, 46
 lettuce, 46, 270
 Leukel, R. W., 135, 146
 Lewis, D., 181, 183, 188-189, 194, 319-320, 332
 lily, 320
 line, 143
 line breeding, 163
 linkage, 54-55, 124-126, 128-129, 167, 176, 218, 221, 241, 326-327
 Little, T. M., 276, 281
 Locke, L. F., 97, 100
 Loegering, W. Q., 76, 83
 Lonnquist, J. H., 202, 211, 242, 245, 250, 255, 264
 Love, H. H., 154, 160
 Love, R. M., 331, 333, 337, 340
 Lowe, C. C., 261-262, 264
 Lundy, H. W., 246, 250
 Lutrick, M. C., 246, 250
Lycopersicon, see tomato
 Lynch, D. L., 78, 83
 Lysenko, T. D., 102, 109-113
- M generation, 306, 308
 Macadamia nut, 27
 MacArthur, J. W., 97, 100

- McClure, G. W., 224
 McCubbin, W. A., 32, 34
 McFadden, E. S., 285, 290, 337, 340, 367, 373
 McGill, D. P., 255, 264
 MacGregor, W. G., 4
 MacKey, J., 140, 146, 313-314, 316
 MacNeish, R. S., 10, 11, 21
 Maheshwari, P., 321, 333
 Mahmud, I., 158, 160
 maize, *see* corn
 male sterility, 182, 190-193
 cytoplasmic, 193
 cytoplasmic-genetic, 190-193, 232-233, 366, 368
 genetic, 157, 190-191, 234-236, 294-295
 mangel, 47
 Mangelsdorf, P., 17-19, 21, 81-83, 184, 193-194, 223, 232, 238, 284, 290, 320, 333
Manihot, 11
 Marble, V. L., 370, 373
 Marks, E., 320, 332
 Martin, W. J., 346-347, 358
 Martini, M. L., 150-157, 160
 Marx, G. A., 74, 83
 mass method of breeding, *see* bulk population breeding
 mass-pedigree method, 148-149, 278
 mass selection
 cross-pollinated crops, 198-209
 self-pollinated crops, 117-118
 maternal line selection, 199
 Mather, K., 84-85, 98, 100, 196, 207-208, 211, 275, 281
 Mather, S. M., 381, 386
 Mathews, O. R., 135, 146
 mating, nonrandom, 178
 mating system, 37-42, 45-50, 181-190
 Matsumura, S., 271, 281
 maturity genes, 74
 Maunder, A. B., 191, 194
 mean, 59
 mechanical harvest, adaptation to, 5
 median, 60
Medicago, *see* alfalfa
 megaspore, 38
 Mehlquist, G. A. L., 313, 316
 Mehta, R. K., 266, 281
 meiosis, 40-42
 melon, 44
 Mendel, G., 74, 122-123, 127, 132
 Mendelian population, 175
 Mendelian variation, 14-15
 metrical character, *see* quantitative character
 Michurinism, 109-112
 microcenters of diversity, 29
 microspore, 38
 Millardet, P. M. A., 361
 Miller, P. A., 242, 250
 millet, 11
 milo, *see* sorghum
 Mochizuki, A., 271, 281
 mode, 60
 modified mass method, 148
 modifying genes, 73, 90-91, 335, 352
 monoecy, 48, 182-183
 monoploid, *see* haploid
 monosomic, 298-299
 monosporidial lines, 346-347
 Morrison, G., 383, 386
 Muller, H. J., 274, 281, 302, 316, 323, 333
 multiline variety, 366
 multiple alleles, 124, 177-178
 multiple factors, 91-95
 multivalence, 272-276, 288, 295, 314
 Mumaw, C. R., 154, 160
 mung bean, 46
 Müntzing, A., 8, 266, 271, 281, 283, 290, 297, 300
 Murphy, H. C., 158, 160
 Murphy, R. P., 220, 222
 muskmelon, 46
 mustard, 270, 278, 286, 305, 313
 mutagen, 188, 302-305
 achievements with, 311-314
 chemicals, 188, 304-305
 effects of, 309-311
 ionizing, 302-304
 physiological effects of, 308, 310
 primary effects of, 308
 procedures with, 305, 309
 recurrent use of, 314
 ultraviolet, 304, 308, 349
 mutation, 45, 73, 347-349, 364
 cycle, 307
 frequency, 119-120, 178, 310, 331
 induced, 188, 301-314
 new functions, 288

- mutation breeding, 301-316
 Myers, W. M., 268, 281
- Natarajan, A. T., 304, 316
 National Seed Storage Laboratory, 33
 natural crossing, 109
 natural selection, 2, 108, 126, 345
 in bulk populations, 150-154
 in composite crosses, 155-156
 in cross-pollinated crops, 208-210, 382
- Neal, N. P., 193, 194, 254, 264
 nectarine, 46
 nectarless gene, 76
 Nelson, W. L., 158, 160
 nematode resistance, 345
 Neolithic period, 10
 Neufer, M. G., 305, 316
 new crops, 26
 Newman, L. H., 145-148, 160
 Newton, M., 345-346, 358
Nicotiana (see also tobacco), 184, 187,
 283-284, 318, 325-327
 nicotine, 110
 Nielsen, E. L., 330, 333
 Nilan, R. A., 308, 316
 Nilsson-Ehle, H., 91, 100, 145, 147, 218,
 222
 Noble, W. B., 345, 355, 357-358
 Noll, C. J., 236, 238
 normal curve, 58
 Norris, M. J., 82-83
 North, D. T., 306, 314-315
 nulliplex, 273
 nullisomic, 288-289, 298-299
 nursery plot, 102, 140-144, 388-391
 Nybom, N., 307, 316
- oats, 2, 12-13, 46, 158, 285, 312, 343, 365
 Odland, M. L., 187, 195, 236, 238
 oil crops, 11, 26, 304
 oil content of, 67-68, 254
 quality of oil, 77-78
 okra, 46
 Okuno, S., 190, 195
 olive, 47
 Olmo, H. P., 17, 272, 281
 Olsson, G., 313, 315
 O'Mara, J. G., 270, 281
 one-variety community, 383
 onion hybrids, 191
 open-pollination progeny test, 257
- Opuntia*, 11
 orchard grass, 47, 263, 271
 ornamentals, 12, 266, 272, 330-331, 381
 Ortman, E. E., 370, 373
 Orton, W. A., 360-361, 373
 overdominance, 88-91, 216-218
 Owen, F. V., 191, 195
- Painter, R. H., 370, 373
 pairing test, 62-63
 Paleolithic period, 9
 palms, 27
 Pandey, K. K., 189, 195
Panicum, 11
 parameter, 56
 parasexualism, 350
 Parker, J. H., 337, 340
 parsnip, 47
 Parthasarathy, N., 270-271, 278, 280
Parthenium, 187
 parthenocarp, 44
 parthenogenesis, 43, 119, 276
 partitioning gene action, 97-98
 patents, 382
 pathogenicity
 genetics of, 345-350
 of different races, 343-344, 365-370
 relation to genes for resistance, 355-
 356
 peach, 46, 330
 peanut, 46, 313
 pear, 47, 110, 189, 320
 Pearson, L. C., 227, 239
 Pearson, O. H., 320, 333
 peas, 46, 74, 116-117, 305
 pecan, 47
 pedigree breeding, 364
 compared to bulk method, 159
 improvement of inbred lines, 231
 modifications of, 144
 self-pollinated crops, 133-146, 155
- Pellew, C., 218, 222
 penetrance, 74
 Penny, L. H., 119-121, 246, 250
 peppers, 46
 persimmon, 110
 Person, C., 357-358
 pesticide resistance, 78
 Peterson, B., 349, 358
 Peterson, M. L., 382, 386
 Peterson, P. A., 297, 300

- Peterson, R. F., 164, 166, 173, 337, 340
 Peto, F. H., 268, 281
 phenotype, 124, 199, 242-244
 Philips, J. C., 90, 100
Phylloxera, 29, 361
 physiologic race
 diseases, 343-346, 355-356, 362, 367-370
 insects, 345, 354-355
 Pickett, R. C., 191, 194
 Piemeisel, F. J., 343, 358
 pine, 331
 pineapple, 44, 189
 Pirovano, A., 302, 316
 Pissarev, W. E., 320, 333
 plant breeder
 rights, 384-385
 training of, 6-7
 plant breeding
 careers in, 6
 changes in, 385-386
 long-term undertaking, 5, 143-144
 related to production, 2-6
 plant exploration, 25, 30-32
 plant introduction, 22-35
 plant introduction number (P.I. No.), 31
 plateau in selection, 208
 pleiotropy, 74-75, 98-99, 207-208, 310, 326-327
 plum, 47, 110, 330
Poa, *see* bluegrass
 Poehlman, J. M., 8, 139, 146, 158, 160, 232, 239
 pollen, 38, 74-75, 268, 270, 308, 319-320
 pollen-killer gene, 76
 pollination, 40, 139
 control of, 181-195
 in polycross tests, 258-260
 insect, 48, 383
 wind, 48, 295, 383
 Polunin, N., 12, 21
 polycross test, 258-261
 polygenes, 84, 98-99, 207-208, 310, 313
 polyhaploid, 278, 283, 289
 polyploids, 14, 266 (*see also* allopolyploids, autopolyploids)
 incompatibility, effect on, 188, 270, 277
 induction of, 266-268
 mutations in, 309
 Pontecorvo, G., 350, 358
 Pope, M. N., 163, 173
 Pope, W. K., 158, 160
 potato, 31, 46, 110, 112, 170, 190, 266, 270, 277, 320-321, 331
 Powers, L., 97, 100
 preferential pairing, 273
Primula, 183-184, 189, 274, 322
 protandry, 48
 protein, 26, 205-206
 protogyny, 48
Prunus, 188, 321
 pseudogamy, 43
 Pugsley, A. T., 164, 166-168, 171, 173
 pumpkin, 11, 12, 46
 pure line, 115-116, 118-119
 Purseglove, J. W., 12, 21
 Putt, E. D., 254, 256, 264
 quadrivalent, 272, 289
 quadruplex, 273, 276
 qualitative character, 53-54, 70-83
 quality, breeding for, 5
 Qualset, C. O., 362, 373
 quantitative character, 53-54, 84-99, 145, 167, 299, 313
 quarantine, 31
 Quinby, J. R., 73-74, 76, 83, 217, 222
 r unit, 304
 rad, 304
 radiation, 302-314 (*see also* X-rays)
 radioisotopes, 188, 303-304, 313
 radish, 187, 286
 Raeber, J. G., 145-146, 159-160
 Rajan, S. S., 278, 281
 Ramage, R. T., 236, 239, 293-294, 300
 Ramanujam, S., 271, 281
 Randolph, L. F., 266, 270, 272, 276, 281, 321, 332
 randomization, 392-393
 randomized block design, 393-398
 Rangaswamy, N. S., 321, 333
 Ranger alfalfa, 262
 Rao, H. K. S., 305, 316
 rape, 26, 286-287, 313
Raphanus, *see* radish
 Rasmusson, D. C., 354, 358, 362, 373
 raspberry, 47
Rauwolfia, 13

- recombination, 42, 120, 122-132, 345-347
 - restrictions to, 45, 124-126, 317
- recurrent parent, 163-166, 299
- recurrent selection, 232, 240-250, 277
 - for general combining ability, 244-246
 - for phenotype, 242-244, 248
 - for specific combining ability, 246
 - in self-pollinated crops, 144, 156
 - reciprocal recurrent selection, 246-248
- reductional division, 42
- Reeves, R. G., 287, 290, 320, 333
- registered seed, 379
- registered stock, 382
- regression, 66-68
- Reitz, L. P., 134, 137, 146
- replication, 143, 258, 392
- reproduction, 36-51
 - apomictic, 43-44
 - asexual, 42-44, 110-112
 - sexual, 37-42
 - vegetative, 43
- restitution nucleus, 267
- Rhoades, M. M., 41, 51, 193, 195, 231, 238, 268, 281
- rice, 11, 46, 166-167
- Richey, F. D., 220, 222, 225, 227, 229, 231, 239
- Richter, A., 313, 316
- Rick, C. M., 110, 113, 190, 195
- Righter, F. J., 331, 333
- Riley, R., 278, 281, 284, 289-290, 296, 300
- Rinke, E. H., 255, 264
- Robert, A. L., 243, 250
- Roberts, H. F., 123, 132
- Roberts, L. M., 256, 264
- Robinson, H. F., 105-106, 113, 246, 250
- Rockefeller Foundation, 3, 256
- Rodenhiser, H. A., 369, 373
- roentgen (r), 304
- Rogers, J. S., 191, 195, 247, 250
- Rollin, S. F., 384, 386
- Roman, H., 298, 300
- root crops, 11
- Roper, J. A., 350, 358
- Ross, J. G., 276, 280
- rubber, 12-13
- Rubus*, 12, 44, 111-112, 285
- Russell, W. A., 119-121, 246, 250
- rutabaga, 47, 286-287
- rye, 12, 30, 47, 270-271, 276-278, 287, 296-297, 299, 320-321, 338, 343
- ryegrass, 47, 263, 268
- Ryerson, K. A., 24, 34
- safflower, 25-26, 67-68, 73-75, 77-78, 118-119, 266, 269
- Sagawa, Y., 313, 316
- Salmon, S. C., 135, 146, 387, 401
- samples, 57
- Sampson, D. R., 187, 195
- Sanborn, J. W., 224
- Satina, S. A., 111, 113
- Saunders, C. E., 3, 4, 144
- Saunders, J. H., 337, 340
- Sax, H. J., 310, 316
- Sax, K., 310, 316
- Schaller, C. W., 29, 33, 35, 82-83, 135, 146, 173, 354, 358, 362, 373
- Schank, S. C., 269, 281
- Schlosser, L. A., 277, 281
- Schwanitz, F., 268, 281
- Scott, W. O., 365, 373
- Sears, E. R., 76, 83, 187, 195, 285-286, 288, 290, 298-300, 305, 314, 334, 337-340, 362, 373
- Secale*, *see* rye
- seed
 - certification, 378-381
 - color, 76
 - equipment, 376
 - laws, 376, 384
 - production, 376-377, 382-383
 - repository, 33
- selection, 18, 364
 - artificial versus natural, 19-20, 208-209
 - coefficient, 198
 - correlated response to, 206-208
 - cross-pollinated crops, 196-210
 - differential, 198
 - effect of heterozygosity, 117, 130, 140
 - for dominant genes, 197
 - for one locus, 197-198
 - for recessive genes, 197
 - intensity, 198-199
 - response to, 206-210
 - self-pollinated crops, 114-121
 - self-incompatibility, *see* incompatibility
 - self-pollinated crops, 44-47, 50, 117, 383

- Sermonti, G., 350, 358
 Severson, G. M., 67-69
 sexual reproduction, *see* reproduction
 Shank, D. B., 107, 113
 Sheppard, P. M., 126, 132
 Shirreff, P., 133, 146
 Shull, G. H., 214, 216-217, 222, 224-225, 239
 sib-mating, 213-214
 significant difference, tests of, 55, 62-63, 397-400
 Silow, R. A., 187, 195
 simplex, 273
 single cross, 225-226, 234-236, 253, 293-295
 Singleton, W. R., 302, 308, 313, 316
 Skirm, G. W., 321, 333
 Smith, D. C., 139, 146
 Smith, H. H., 85, 100
 Smith, R. E., 349, 357
 Smith, R. F., 370-371, 373
 Smith, W. K., 321, 333, 335, 340
 snapdragon, 189
 Snedecor, G. A., 403-407
Solanum, *see* potato
 somatic chromosome reduction, 276
 Somme, A. S., 274, 281
 Sorensen, E. L., 370, 373
 sorghum, 3, 45-47, 103, 276, 287
 dwarf types, 5, 73
 hybrid varieties, 234
 male sterility, 191
 maturity, 74, 76, 217
 origin of, 11, 17
 soybeans, 2, 14, 25, 26, 46, 145, 154, 158, 389
 Sparrow, A. H., 303, 316
 Spencer, J. L., 302, 316
 spinach, 47, 122, 182
 sporophyte, 38, 176
 Sprague, G. F., 108, 113, 119-121, 192, 195, 220, 222, 228-229, 231-232, 239, 242, 246, 250, 254-255, 257, 264
 squash, 11, 46
 Stadler, L. J., 119-121, 178, 180, 217, 222, 232, 239, 302, 309, 316
 Stakman, E. C., 341, 343, 346-347, 349, 358, 369, 373
 standard deviation, 60, 104, 396
 standard error of a difference, 62-64, 397-398
 standard error of a mean, 61, 397
 Stanford, E. H., 54, 69, 170, 173, 209, 211, 345, 357, 370, 373
 Stapleton, R. G., 256, 264
 statistics, 57-69
 Staudt, G., 14, 15, 21
 Stebbins, G. L., Jr., 43, 46, 51, 266, 271, 281-284, 290, 319, 322-324, 331, 333
 stem strength, 73-74, 75
 Stent, G. S., 350, 358
 Stephens, J. C., 234, 239
 Stephens, S. G., 324, 333
 Stevens, H., 156, 160
 Stevens, N. E., 365, 373
 Stevenson, E. C., 272, 280
 Stevenson, T. M., 257, 264
 strain building, 257
 strawberry, 15, 16, 47, 249, 285-286
 Stringfield, G. H., 193, 195
 strip test, 143
 Strommen, A. M., 193-194
 Stuart, N. W., 320, 332
 Stubbe, H., 302, 310, 316
 sugarbeet, 14, 191, 200-201, 268, 271
 Sullivan, J. T., 220-221, 268, 281
 Suneson, C. A., 13, 21, 34-35, 79-80, 82-83, 153-158, 160, 171-173, 190, 195, 234-235, 239, 354-355, 358
 sunflower, 11, 13, 27, 203, 254, 256
 Swaminathan, M. S., 266, 281, 304, 316, 320, 333
 Swanson, A. F., 152-153, 160
 sweet clover, 46, 78, 185, 248-249, 321, 335
 sweet pea, 46
 sweet potato, 331
 symbiotic relationships, 78
 synthetics, 242-249, 251
 synthetic varieties, 251-264
 components of, 261
 of corn, 252, 255-256
 of forage crops, 252, 256-263, 370
 of sunflower, 254, 256
 predicting performance of, 253-255
 production of, 252-253
t table, 403
 Tatum, L. A., 229, 239
 Taylor, L. H., 154, 158, 161
 teosinte, 17
 tertiary trisomic, 293-295

- tetrasomic, 292, 295
 tetrasomic inheritance, 273-276, 288
Theobroma, 188
 Thomas, M., 170, 174
 Thomas, W. I., 248, 250
 Thompson, J. B., 159, 161
 Thompson, K. F., 187, 195
 Thompson, W. P., 319, 333
 thin hulls, 73-75
 timothy, 12-13, 47, 263, 271, 375
 Tippo, O., 37
 tobacco, 2, 11, 18, 46, 92-95, 104-105,
 110, 119, 266, 268, 284, 288, 296,
 298, 375
 tomato, 44-46, 96-98, 110, 170-171, 190,
 277, 335
 top cross test, 228, 241, 257
 Torrie, J. H., 159-160
 toxic effect, genes for, 78
 transgressive segregation, 91-92, 109
 translocation, 131, 231, 297-298, 310,
 338-339
 trefoil, 47, 320
 triangle of U., 286
Trifolium, *see* clover
 triplex, 273, 275-276
 triploidy, 18, 272, 273, 278
Tripsacum, 17
 trisomic, 292-295, 298
Triticale, 287
Triticum, *see* wheat
 trivalent, 272, 289
 Tsiang, Y. S., 255, 264
 tulips, 272
 turnip, 47, 272
 Tysdal, H. M., 203, 211, 215, 222, 258,
 260, 262, 264
 U, Nagaharu, 286, 290
 Ullstrup, A. J., 298, 300
 ultraviolet, 304, 308, 349
 univalent, 272, 288-289
 Unrau, J., 284, 290, 298-300
 Upcott, M., 322, 333

Vaccinium, *see* blueberry
 variability, 52-69
 free versus potential, 207
 qualitative, 53-56
 quantitative, 53-54, 56-59
 variability, methods of increasing
 hybridization, 122-132, 317-331
 inbreeding, 189
 mutations, 301-314
 recurrent selection, 240-249
 variance, 61, 105, 392, 395
 analysis of, 391-400
 varieties
 distribution of, 374-386
 maintenance of, 118, 374-386
 one-variety community, 383
 purity of, 375
 registration of, 381
 release of, 232
 role of seed companies in developing,
 232, 376, 385
 superior variety, 131-132, 164-165
 testing, 172, 387-400
 Vavilov, N. I., 8, 9, 13, 20, 27-31, 35
 vegetable crops, 11, 271-272, 286-287,
 375, 381
 vegetative reproduction, 43, 381-382
 velvet bean, 46
 vetch, 46
 Vilmorin, L. de, 200, 201, 211
 virus, 308, 342, 350, 381
 Vose, P. B., 382, 386

 Walkof, C., 171, 174
 Wallace, R. W., 246, 250
 walnut, 47
 Ward, D. J., 33, 35
 Warner, J. N., 104, 113
 Waterhouse, W. L., 337, 340
 watermelon, 46, 270, 272, 361, 365
 Weber, C. R., 145-146, 154, 158-159,
 161
 Webster, G. T., 335-336, 340
 weeds, origin of, 11-12, 287
 Weinberg, W., 177, 180
 Weiss, E., 350, 358
 Weiss, M. G., 154, 158, 161
 Welch, J. E., 191, 195
 Wellensiek, S. J., 258, 264
 Wellhausen, E. J., 256, 264
 Wernsman, E. A., 297, 300, 320, 333
 Westover, H. L., 215, 222, 258, 264
 Wettstein, D. V., 305, 310, 316
 wheat, 2, 3, 30, 109, 158, 342
 awned versus awnless, 79-82
 Baart, 79-80, 168-172, 353

- backcross improvement of, 165, 166-172
- bulk methods used for, 147-148
- bunt (covered smut), 125-126, 166, 168-171, 343-344, 351-353, 355-356
- chromosome substitutions, 299
- competition of varieties, 152-154
- composite cross, 158
- genomes, 285, 328-330
- heading date, inheritance of, 90
- hessian fly, resistance to, 166, 171
- Hussar, 352-353
- hybrid varieties, 234, 366, 368
- interspecific gene transfers, 336-338, 367
- interspecific hybridization, 319, 322, 328-329
- introductions, 23, 26, 29
- kernel color, 91-92
- Marquis, 3, 136-137, 144, 165, 353, 367
- Martin, 167, 345, 351
- monosomics, 298
- mutation breeding of, 305, 309, 314
- nullisomics, 298
- origin of species, 11, 284-285
- origin of spring wheat varieties, 137
- pedigree methods used for, 145
- polyploidy in, 18, 266, 271, 277, 284-285, 287, 288
- quality of, 5
- Rio, 125, 355-356
- stem rust, 30, 125, 168-171, 299, 342-344, 346, 353, 365-370
- Turkey, 23-24, 125, 165, 345, 353, 355-356
- varieties of, 3, 90, 134-137, 147-148, 153, 165-172, 345, 367
- wheat-rye crosses, 287, 296, 299
- winter hardiness, 147-148, 296
- wheatgrass, slender, 46
- Whitehouse, R. N. H., 159, 161
- Whitman, W., 374, 386
- Whittinghill, M., 314, 316
- Whyte, R. O., 22, 30, 31, 35
- Wiebe, G. A., 33, 35, 119, 121, 135, 146, 153-154, 158, 160, 166, 174, 235, 239, 355, 357
- Williams, L. F., 78, 83
- Williams, R. J., 27, 29, 34
- Williams, W., 8
- Winkler, H., 270, 281
- Wolff, I. A., 26, 35
- Woodworth, C. M., 204, 205, 211
- Workman, P. L., 108, 113
- world collection of barley, 33, 135, 362
- Wright, S., 178, 180, 213, 222, 253, 264
- Wu, S. K., 230-231, 239
- xenia, 77
- X-rays, 188, 302-314, 338, 349
- Yarnell, S. H., 186, 194
- Yates, F., 393, 401
- yellow dwarf, *see* barley, yellow dwarf
- Yoshida, Y., 306-307, 316
- Zimmerman, L. H., 71-72, 83
- Zinder, N. D., 350, 359
- Zirkle, C., 109, 113, 213, 222, 224, 239

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