

Chapter 14

Plasmagenes and Chromogenes in Heterosis

The word *heterosis* is essentially a contraction of the phrase *stimulus of heterozygosis*. It was first used by G. H. Shull (1914). The concept of a stimulation resulting from the genetic union of unlike elements was developed by East (1909). Previous to the Mendelian conception of units of heredity, it was generally considered by plant and animal breeders that the invigorating effect of crossing unlike varieties of plants and breeds of livestock was due to the correction of imperfections that existed in both parental types. This idea is clearly stated by Samuel Johnson in the second edition of his book *How Crops Grow* (1891).

The early recordings of instances of hybrid vigor and the various means of accounting for this phenomenon have been stated and restated so many times that there is no need or useful purpose in repeating them here. Excellent reviews of the literature are readily available (see especially East and Hayes, 1912; Jones, 1918; East and Jones, 1919; East, 1936; and Whaley, 1944).

THE EXPRESSION OF HETEROSIS

At the present time, the term *heterosis* designates the increased growth or other augmented action resulting from crossing, however it is produced. As generally used, it is essentially synonymous with *hybrid vigor*. Heterosis has two general modes of expression. In one, there is an increase in size or number of parts. This is usually the result of a greater number of cells and a faster rate of cell division and cell activities. This results in an improvement in general well-being of the organism similar to the result of being placed in a more favorable environment. Such luxuriance may be accompanied by partial or complete sterility in diverse crosses.

A somewhat different manifestation of heterosis is an increase in bio-

logical efficiency, such as reproductive rate and survival ability. This may even be shown with a reduction in productiveness as measured by economic characters. Some confusion has arisen by not distinguishing clearly between these two different manifestations of heterosis.

In addition to these two general types of heterotic effects, there may also be a reduction in both growth and survival ability; in other words, hybrid weakness or a reversed or negative heterosis. This effect is much less common and is seldom found in cultivated plants and domesticated animals.

TYPES OF GENE ACTION

An understanding of the mode of action of heterosis has now resolved into a study of the nature of gene action. The genes usually used to illustrate Mendelism are the loss variations that have a major effect such as the inability to produce some essential substance. This results in a block in the normal chemical processes, finally resulting in an individual of greatly altered appearance, size, or ability to survive. The effect ranges in intensity from a completely lethal condition at some stage of development, up to individuals that differ only slightly in appearance from normal with no appreciable reduction in growth or survival ability. Such genes are illustrated by the long lists of Mendelizing characters now tabulated for maize, *Drosophila*, mice, and many other animals, plants, and lower organisms.

DOMINANT AND RECESSIVE GENES

In these cases, the normal allele is usually designated by a capital letter, with the mutant, deficient allele denoted by the corresponding lower case letter. In comparison with the normal allele, the recessive mutants are deficient in some respect. In their inability to produce certain specific substances, as shown in the haploid *Neurospora* by Beadle and his co-workers, they are referred to as *A*-less, *B*-less, *C*-less, etc. In diploid organisms *A* is usually completely dominant over *a*; that is, one *A* allele functions as well or nearly as well as two.

There is no question that the accumulation in a hybrid of the normal alleles of this type results in heterosis. In the simplest example of a cross of *A*-less by *B*-less ($aaBB \times AAbb$) the hybrid offspring are all $AaBb$, and essentially normal for whatever effect *A* and *B* have. But since the mutant recessive alleles of this type are so drastic in their effect, most of these deficiencies are removed by natural selection in all species whether self-fertilized or cross-fertilized. Therefore they have little part in the heterosis that is shown by these organisms when crossed. Furthermore, genes of this type are eliminated when naturally cross-fertilized species, such as maize, are artificially self-pollinated. Yet such inbred strains show the largest amounts of heterosis.

There is evidence, as will be shown later, that there are many genes of this

type having small effects that are not eliminated by natural or artificial selection either in the wild or under domestication, and that these deficiencies or degenerative mutants do have a large part in bringing about reduced growth. Before presenting this evidence, there are other types of gene action that should be considered.

CHROMOSOMAL DELETIONS

In addition to the recessive mutant alleles that are deficient as compared to their normal alleles, there are also chromosomal deletions which result in the complete elimination of the normal locus. Large deletions are usually lethal and are quickly eliminated. Small deletions that cannot be detected cytologically are haplo-viable, and may persist indefinitely if they are closely linked with essential loci. Changes of this type have been demonstrated by McClintock (1931) and by Stadler (1933). They cannot be readily distinguished from recessive mutants of the *A-less* type. In fact there may be no difference. In practically all cases they show varying amounts of germ cell abortion, and do not mutate back to normal. Deletions of this type are designated *A_o*.

DOMINANT UNFAVORABLE GENES

In many cases of deletion the heterozygote, or the hemizygote, is visibly and unfavorably altered from normal, in which event the genes involved are listed as dominant, and if partially viable they can bring about negative heterosis or hybrid weakness. It is not known whether all dominant unfavorable genes are deletions of this type, but as far as their effect on heterosis is concerned it makes little difference whether or not they are. An illustration of this type of gene action may be seen in a cross of Ragged and Knotted maize plants. Both of these genes result in a marked reduction in growth in the heterozygous condition. They are not completely lethal in the homozygous dominant condition, but seldom produce seed or pollen. When both dominant genes are present together in the heterozygous condition, there is a marked reduction in size, rate of growth, and reproductive ability as compared with either parental type.

Tunicate, teopod, and corn grass are also dominant genes that reduce grain yields in both the homozygous and heterozygous condition. They are probably reversions to a primitive condition which in suitable genetic combinations may be favorable to survival in the wild. Dunn and Caspari (1945) describe many structural abnormalities in mice that seem to be due to deletions having a dominant effect in the hemizygote. Some of these counteract each other and tend to restore a more normal condition, while others accumulate unfavorable effects. A similar situation has been reported in *Drosophila* by Stern (1948).

In addition to recessive deletions with a dominant effect in the heterozygote, there are also dominant inhibitors that have no indication of being

deletions, but do prevent other genes from having their usual expression. Most of these inhibitors control color characters and are usually not involved in heterosis. If they were, there would be more negative heterosis than actually is found.

GENES WITHOUT DOMINANCE

Unlike the visible Mendelizing genes with their clear-cut dominance and unfavorable action of one or the other allele, there are many genes that differentiate size or number of parts, time of flowering and maturing. These are the genes usually involved in normal variation. They are the ones the plant and animal breeder are mainly concerned with and could expect to have a major effect on heterosis. Since neither member of an allelic pair can be considered abnormal or deficient, both are designated with a capital letter with some prefix to differentiate them, as for example A and A^1 .

Genes of this type usually have simple additive effects such as the Y endosperm color gene in maize, in which each allele adds a definite increment in total carotene content. Such additive genes without dominance are used to interpret the inheritance of quantitative characters which have been shown to segregate and recombine in a Mendelian manner.

No clear distinction can be made between the Aa and AA^1 types of genes and this has led to much confusion. The first class shows complete or nearly complete dominance. The second shows no dominance or very little dominance, but one type integrates into the other. The principal question at issue is whether either type shows over-dominance, or in other words, an interaction between alleles such that $Aa > AA$ or aa or $AA^1 > AA$ or A^1A^1 . Before considering the evidence for or against over-dominance, two remaining types of genes should be considered.

CHROMOSOMAL REARRANGEMENTS

By chromosomal rearrangements such as inversions and translocations, genes without alteration are placed in different spatial relations with other genes. In their altered position they have different effects. Dobzhansky and his associates have studied many geographical races of *Drosophila* that differ by chromosomal rearrangements. Crosses between these chromosomal types from the same region exhibit heterosis, whereas the same chromosomal type from different regions do not show such a high degree of heterosis. This seems not to be a position effect, but is the result of an accumulation of gene differences that are protected from random distribution by the prevention of crossing over in hybrids of different chromosomal types.

COMPOUND GENES AND GENES WITH MULTIPLE EFFECTS

In many organisms, loci are known which have different effects on different parts of the organism. In maize the A , P , and R genes have been studied in considerable detail by Stadler and his co-workers. These loci each have a

series of alleles that produce characteristic color patterns and intensities of colors in different parts of the plant such as culm, leaf sheath, leaf blade, glumes, anthers, silks, cob and pericarp, and endosperm. They may be considered either as genes located so closely together that they never show crossing over, or compound genes with multiple effects. Without going into the evidence for or against these two hypotheses, it is obvious that compound genes can have an important part in heterosis if they control growth processes. More information is needed on the specific effect of compound genes.

In *Godetia* a series of multiple alleles has been described by Hiorth (1940) that is often cited as an illustration of an interaction between alleles producing an effect analogous to heterosis. Actually these are color determiners that control pigment production in different parts of the flower quite similar to the *A*, *P*, and *R* loci in maize. Each allele has a different manifestation, and all tend to accumulate color in the heterozygotes.

The familiar notation of a chromosome as a linear arrangement of loci, each of which is the site of a single gene with one effect function, is probably an oversimplification of the actual condition. It is difficult to see how an organism could have originated in this way. It is more likely that a chromosome is an association of primitive organisms of varying types and functions. These primitive organisms found it to be an advantage in the evolutionary process to become associated in some such process as the colonial organisms now exhibit. This association has undergone very great modification and ramifications, but the compound genes may be vestigial structures of such an association, differing greatly in size, arrangement, and function. Many of them still retain some independence, and when removed from their normal position in the chromosome could function as plasmagene or viroid bodies.

These compound genes may undergo mutation and possibly recombination or reorganization within themselves, but crossing over takes place for the most part only between these compound structures. Compound genes also arise by unequal crossing over and duplication of loci are shown by the Bar eye gene in *Drosophila* and others of similar type.

In addition to compound or multiple genes, there are single genes with multiple effects. Many of these are important in growth processes and are illustrated by chlorophyll production in maize studied by H. L. Everett (1949). One major gene is essential for the production of carotene. In the recessive condition the seeds are pale yellow in color, in a normal, dark yellow seeded variety. The young seedlings grown from these pale yellow seeds are devoid of chlorophyll. The recessive allele is therefore lethal. By using the pale yellow endosperm as a convenient marker and crossing with a number of standard field corn inbreds, it has been found that these inbreds differ widely in their normal chlorophyll mechanism. Many of them have genes that can restore normal chlorophyll production without restoring the production of carotene in the seed. Other genes restore chlorophyll production only partial-

ly (see Table 14.1). Hybrid combinations that bring these genes together are appreciably more efficient in chlorophyll production than combinations that lack some of them. However one of these dominant alleles has a suppressing effect on chlorophyll development. The combination of all of these chlorophyll genes so far studied is not the most productive. There are many genes of this type that block chemical syntheses, that are not lethal in the usual genetic assembly, but which combine to give a cumulative efficiency in most cases.

Lethal genes which show complete dominance of the normal allele would have no effect on heterosis other than to reduce the number of offspring. Such

TABLE 14.1
GENES CONTROLLING CHLOROPHYLL PRO-
DUCTION IN MAIZE*

Cl ₁	Cl ₂	Cl ₃	Seed Color	Chlor. Grade	Viability
—	—	—	Pale	Albino	Lethal
—	+	—	Pale	Virescent	Lethal
—	—	+	Pale	Light green	Normal
—	+	+	Pale	Light green	Normal
+	—	—	Yellow	Light green	Normal
+	+	—	Yellow	Med. green	Normal
+	+	+	Yellow	Dark green	Normal
+	—	+	Yellow	?	Normal

* Data from H. L. Everett.

genes would be just as effective in the homozygous as the heterozygous condition. Genes that have any part in the type of heterosis that is manifested in increased growth must be viable and have some degree of dominance. In other words, Aa must be greater than $\frac{1}{2} AA$. Aa may even be greater in its effect than AA or aa in which case theoretically there is over-dominance, but very little specific evidence is available to show that such a situation actually exists.

I can see no way in which it is possible to separate over-dominance from a stimulus of heterozygosis. They seem to be different ways of saying the same thing. The essential point at issue at the present time is whether or not over-dominance actually occurs, and if so, how important this is in the total amount of heterosis in addition to the known accumulation of favorable dominant effects.

INTERACTION BETWEEN ALLELES

Evidence has been presented from many sources bearing on the problem of over-dominance and interaction between different alleles. Much of the argument is based on mathematical treatment of data that require many assumptions. What is needed is more specific evidence where the effect of

multiple genes can be ruled out. Very few specific examples of single gene action are available.

In one case studied by the writer there is clear evidence for an interaction between alleles (Jones, 1921). A mutation in a variety of normally self-fertilized tobacco changed a determinate plant into an indeterminate, non-flowering variation. It was a change in the normal response to the summer day length period. The mutant plants failed to flower in the normal growing season and continued in a vegetative condition. Reciprocal crosses between the mutant and normal types both grew at the same rate as the normal plants showing complete dominance of the normal growth rate. The heterozygous plants continued their vegetative growth longer and produced taller plants with more leaves and flowers than the normal homozygous plants. This result I consider not to be heterosis, since there was no increase in growth rate. It is merely an interaction between alleles to produce a result that is different from either parent. There are undoubtedly many allelic interactions of this type. Whether or not they can be considered to contribute to heterosis is largely a matter of opinion.

Other cases in corn where heterosis resulted from degenerative changes (Jones, 1945) were at first assumed to be single allelic differences, since they originated as mutations in inbred and highly homozygous families. The degenerate alterations were expressed as narrow leaves, dwarf plants, crooked stalks, reduced chlorophyll, and late flowering. All of these mutant variations gave larger amount of growth in a shorter period of time and clearly showed heterosis.

The further study of this material has not been completed, but the results to date indicate that the differences involved are not single genes. Both the extracted homozygous recessives and the extracted homozygous dominants from these crosses are larger than the corresponding plants that originally went into the crosses.

This indicates quite clearly that the visible changes were accompanied or preceded by other changes with no noticeable effects, but which are expressed in growth rates. A more complete summary of these results will have to wait until all of the evidence is at hand. It is a simple matter to extract the homozygous recessives from these crosses, but it is difficult to extract the homozygous dominants. Many of the self-fertilized plants proved to be heterozygous.

GENES CONTROLLING GROWTH

Additional evidence that there are a large number of genes having small effects on growth without visible morphological changes is becoming clearly apparent from a backcrossing experiment now in progress. Several long inbred lines of corn, one of which is now in the forty-first generation of continuous self-fertilization, were outcrossed to unrelated inbred lines having dominant gene markers which could be easily selected. The markers—red

cob, yellow endosperm, and non-glossy seedlings—were chosen because they had little or no effect on growth of the plant.

The first generation outcrosses showed the usual large increases in size of plant, time of flowering, and yield of grain that is expected in crosses of unrelated inbred strains of corn. The hybrid plants were backcrossed as seed parents with pollen from the inbred with the recessive gene marker. In every generation, plants with the dominant gene marker were selected for backcrossing. These plants have now been backcrossed six successive times. Many progenies have been grown. They are all heterozygous for the gene marker plus whatever neighboring regions on the same original chromosome from the non-recurrent parent that have not been lost by crossing over.

The plan is to continue the backcrossing until no measurable differences remain between the backcrossed plants and the recurrent parent, or between the two classes of backcrossed individuals in the same backcrossed progeny, those with the dominant marker and those with the recessive marker. When the point is reached where no differences can be detected, the plan is to compare successive earlier generations from remnant seed to pick up whatever single gene differences there might be that could be measured and detected by their segregation.

So far both classes of backcrossed plants in nearly all progenies are taller and flower earlier, showing that they have not been completely converged to the parental type (see Table 14.2). The differences are small and not statistically significant in the tests so far made, but are nearly all in the direction of a heterotic effect. As yet there are not sufficient data to base final conclusions. It is hoped that the comparison of the two classes of backcrossed progeny with the original recessive parent will permit a distinction between the favorable action of dominant genes and an interaction between heterozygous alleles. Also that it may be possible to show whether or not there is any residual cytoplasmic effect, since some of the outcrossed plants have the same cytoplasm as the dominant gene marker and some do not.

Important facts do stand out clearly from this experiment. Since heterosis still remains after these many generations of backcrossing, it shows clearly that these three chromosome regions selected as samples have an appreciable effect on growth. Since the gene markers themselves have no effect on growth, as far as this can be determined in other material, these three regions are random selections for growth effects. This indicates quite clearly that there are genes in all parts of the chromosomes that contribute to normal growth and development. While the evidence so far available does not permit a clear separation between the effects of an accumulation of favorable genes as contrasted to an interaction between alleles, or between genes and cytoplasm, the results show that there are many loci involved in the heterotic effect in addition to the dominant gene markers.

• This follows from the evidence at hand. If the heterosis now remaining

were due solely to the interaction between the dominant and recessive markers, there would have been a rapid approach to the level of vigor now remaining. If it were due to a larger number of genes distributed rather evenly along the chromosome, the reduction in heterosis would be gradual, as it has proved to be. Small amounts of heterosis may persist for a long time until all of the genes contributing to it are removed by crossing over.

A recent experiment by Stringfield (1950) shows a difference in productiveness between an F_2 selfed generation and a backcross having the same parentage. The amount of heterozygosis as measured by the number of allelic pairs is the same in both lots. In the backcross there are more individuals in the intermediate classes with respect to the number of dominant

TABLE 14.2
INCREASE IN HEIGHT OF PLANT IN SUCCESSIVE BACKCROSSED GENERATIONS HETEROZYGOUS FOR A DOMINANT GENE MARKER

NUMBER OF GENERATIONS BACKCROSSED→	PER CENT INCREASE IN HEIGHT		
	4	5	6
20y×243 Y.....	6.7	2.2	1.5
20y×P8 Y.....	1.9	2.3	1.2
20p×243 P.....	6.6	3.0	1.1
243gl×20Gl.....			-1.3

genes. This indicates a complementary action of favorable dominant genes.

Gowen *et al.* (1946) compared the differences in egg yield in *Drosophila* between random matings, 47 generations of sib mating, and homozygous matings by outcrossing with marker genes. The differences are significant, and indicate a large number of genes having dominant effects on the reproductive rate.

INTERACTION OF GENES AND CYTOPLASM

The suggestion has been made many times that heterosis may result from an interaction between genes and cytoplasm. Within the species, differences in reciprocal crosses are rare. In commercial corn hybrids, reciprocal differences are so small that they can usually be ignored. Evidence is accumulating that there are transmissible differences associated with the cytoplasm, and that these must be considered in a study of heterosis. Small maternal effects are difficult to distinguish from nutritional and other influences determined by the genotype of the mother and carried over to the next generation.

The cross of the two different flowering types of tobacco previously cited shows a maternal effect. The first generation cross of the indeterminate or

non-flowering type as seed parent grows taller than the reciprocal combination, and flowers later. These differences are statistically significant.

Reciprocal crosses between inbred California Rice pop, having the smallest seeds known in corn, with inbred Indiana Wf9 having large embryos and endosperms, show differences in early seedling growth and in tillering. Inbred Wf9 produces no tillers. California Rice, also inbred, produces an average of 4.1 tillers per plant. The first generation cross of Rice pop \times Wf9 averages 1.0 tillers, while the reciprocal combination under the same conditions produced 2.2 tillers per stalk. In this case the non-tillering variety, when used as the seed parent, produces more than twice as many tillers. This seems to be a carry-over effect of the large seed. Tillering is largely determined by early seedling vigor. Anything that induces rapid development in the early stages of growth tends to promote tillering.

PLASMAGENES AND CHROMOGENES

In addition to these transitory effects there are many cases of cytoplasmic inheritance. Caspari (1948) has reviewed the evidence from fungi, mosses, the higher plants, and from *Paramecium*, insects, and mammals to show that many differences do occur in reciprocal crosses and that they persist into later backcrossed generations. Reciprocal differences in the amount of heterosis have been demonstrated in *Epilobium* (Michaelis, 1939) and in mice (Marshak, 1936).

Cytoplasmic pollen sterility has been found in *Oenothera*, *Streptocarpus*, *Epilobium*, flax, maize, onions, sugar beets, and carrots. In every case that has been adequately studied, the basic sterility remains the same in repeated generations of backcrossing, but the amount of pollen produced varies in different genotypes. There is an interrelation between plasmagenes and chromogenes determining the final result (Jones, 1950).

In maize the amount of pollen produced ranges from 0 to 100 per cent. Only by suitable tests can these cases of full fertility be recognized as having any cytoplasmic basis. Interest in this problem now centers on the effect of these cytoplasmic differences on heterosis.

A series of standard inbreds have been converted by crossing these onto suitable sterilizer stocks, and backcrossing a sufficient number of generations to re-establish completely the inbred, and maintaining the inbred in a sterile condition by continuous backcrossing. It has been found necessary to select both the cytoplasmic sterile seed parent individuals and the individual fertile pollen parents for their ability to maintain complete sterility both in inbreds and in crosses. In some lines it has proved to be impossible to establish complete sterility, but the majority are easily sterilized and maintained in that condition.

A comparison of fertile and sterile progenies in inbreds, in single crosses of two inbreds, and multiple crosses of three and four inbreds, shows that this

cytoplasmic difference has no appreciable effect on size of plant as measured by height at the end of the season, in days to silking, or in yield of grain. The results are given in Table 14.3. With respect to pollen sterility-fertility, the cytoplasm has no effect on heterosis.

In the conversion of standard inbreds to the cytoplasmic sterile pollen condition, it has been found that many of these long inbred strains, presumably highly homozygous, are segregating for chromogenes that have the ability to restore pollen fertility. In normally fertile plants these genes have no way of expressing themselves. They are not selected for or against unless they contribute in some way to normal pollen production. It is one more

TABLE 14.3
A COMPARISON OF FERTILE AND STERILE MAIZE PLANTS

	Fertile	Sterile	
5 Inbreds.....	72.3	70.1	Height of stalk
7 Crosses of two inbreds...	102.6	97.7	Height of stalk
7 Crosses of two inbreds...	58.5	58.3	Days to first silk
3 Crosses of three inbreds...	111.7	108.9	Yield, bushels per acre
1 Cross of three inbreds...	99.1	103.3	Yield, bushels per acre
3 Crosses of four inbreds...	123.9	119.0	Yield, bushels per acre
5 Crosses of four inbreds...	61.1	64.5	Yield, bushels per acre
2 Crosses of four inbreds...	115.8	117.3	Yield, bushels per acre
14 Crosses, average yield....	102.8	102.6	Yield, bushels per acre

source of evidence to show that there is a considerable amount of enforced heterozygosity in maize. Even highly inbred families remain heterozygous. This has been shown to be true for other species of plants and animals.

SUMMARY

Specific evidence from a study of chlorophyll production in maize and from similar studies in *Neurospora*, *Drosophila*, and other plant and animal species proves conclusively that there are numerous mutant genes that reduce the ability of the organism to grow and to survive. Such genes exist in naturally self-fertilized and cross-fertilized organisms and in artificially inbred families such as maize. The normal alleles of these mutant genes show either complete or partial dominance, and any crossbred individual contains a larger number of these dominant, favorable alleles than any inbred individual.

Evidence from *Nicotiana* shows that there is an interaction between divergent alleles at the same locus such that the heterozygote produces a larger amount of growth and a higher reproductive rate than either homozygote. There is no increase in growth rate and this instance is considered not to be heterosis. The assumption of an increased growth rate, or true heterosis, in such allelic interactions is not supported by specific evidence that cannot be

interpreted in other ways. The experimental evidence to date does not distinguish clearly between a general physiological interaction and a specific contribution from favorable dominant effects. More evidence on this point is needed.

Backcrossing experiments in maize, where dominant gene markers are maintained in a heterozygous condition, show heterosis continuing to the sixth generation. The approach to the level of growth activity of the recurrent inbred parent is so slow as to indicate that every region of the chromosomes, divisible by crossing over, has an effect on growth.

The growth rate in these backcrossed generations is maintained at a level appreciably above the proportional number of heterozygous allelic pairs. This effect can be interpreted in a number of ways other than a general physiological interaction, such as enforced heterozygosity, and the complementary action of dominant genes at different loci.

There is no way known at the present time to distinguish clearly between the accumulation of favorable dominant effects of compound or multiple genes at the same loci and a general physiological interaction or overdominance.

Reciprocal crosses differ in many species, resulting in appreciable divergence in the amount of growth, and these differences have a cytoplasmic basis. The evidence from maize, however, shows clearly that cytoplasmic pollen sterility has no effect on size of plant, time of flowering, or productiveness.