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Chapter 20

Gene Interaction in Heterosis

Sugar cane behaves very much like corn in its reaction toward inbreeding and outcrossing. Although the sugar cane flower is normally provided with both male and female organs, male sterility is not uncommon. Among the varieties that produce an abundance of pollen, many are partially or highly self-sterile. As a consequence, cross-fertilization by wind-borne pollen is the rule in sugar cane, as in corn. When sugar cane is subjected to self-pollination, the usual result is a reduction in seed setting and a marked reduction in the vigor of the offspring.

The sugar cane breeder enjoys one great advantage over the corn breeder: sugar cane can be propagated asexually. Each node on the stalk is provided with a bud and with a number of root primordia. In field practice, stalks of the selected variety of sugar cane are sectioned into cuttings of two or more internodes each. These cuttings are then placed horizontally in furrows and covered lightly with soil. In due course the cutting sends out its roots, the buds develop into shoots, and a new plant is established.

Were it possible to apply this procedure to corn, and thus to perpetuate outstanding individuals from whatever source, it is unlikely that the corn breeder would have felt obliged to resort to the laborious procedures now employed.

When sugar cane varieties are propagated by cuttings, the traits by which we are able to distinguish one variety from another maintain their integrity through many cycles of clonal propagation. This is true not only of morphological traits, but also of physiological traits.

Sugar cane has a number of relatives growing in the wild, some of which may be ancestral to the original cultivated forms. Wild *Saccharums* are widely distributed in the tropical and sub-tropical regions of the Old World, from central Africa through Asia and Malaya, to and including the Indonesian and many of the more westerly Pacific islands. This heterogeneous array of

wild forms has been somewhat arbitrarily classified into two great groups—the *S. spontaneum* group and the *S. robustum* group. Each of these groups comprises a diversity of types which differ among themselves in morphology and in chromosome number. The members of the *spontaneum* group have slender stalks; they are often strongly stoloniferous. The members of the *robustum* group have hard, woody stalks, sometimes of good diameter; stolons, if present, are not strongly developed.

The original cultivated varieties likewise may be classified into two great groups. The first of these comprises a number of slender varieties which appear to be indigenous to India, and which have been lumped together under the name *S. Barberi*. Certain of the *Barberi* varieties bear a striking resemblance to the wild *spontaneums* of that region.

The New Guinea region is the home of a group of large-stalked tropical cultivated varieties of the type which Linnaeus named *S. officinarum*. The wild form most closely resembling *S. officinarum* and possibly ancestral to it is *S. robustum*, which is indigenous to that region.

In the closely related genus *Sorghum*, the difference between varieties having pithy stalks containing but little sugar, and varieties with sweet juicy stalks, has been shown to be determined by a single major gene. In *Saccharum* the change from the dry, pithy, low-sucrose stalks of the wild forms to the juicy, high-sucrose stalks of the cultivated varieties appears to have been brought about by several, but perhaps by no more than three or four major gene changes.

The cultivated and wild forms also differ in genes for stalk size. In crosses between the two, the genes responsible for the slenderness of the wild forms show a high degree of dominance.

A striking feature of this multiform genus is the prevalence of interfertility among its members. Widely divergent forms can be crossed without undue difficulty. The resulting hybrids are rarely completely sterile; they are often highly fertile. The explanation is presumably to be sought in the polyploidy which is characteristic of both the wild and the cultivated forms. They range in chromosome number from 24 to 80 or more pairs. It appears that once the minimum chromosomal complement needed to produce a functional zygote has been supplied, there is considerable latitude in the number and in the assortment of chromosomes that can be added without impairing the viability, or even the fertility of the hybrids.

Since the breeder is as yet unable to create superior genes at will, he is obliged to content himself with developing new combinations of the genes available in whatever breeding material he may be able to assemble. The sugar cane breeder is fortunate in having in the wild relatives of sugar cane a reservoir of genes for disease-resistance and hardiness. Those are traits that had to some degree been lost in the course of domestication. Considerable

use has already been made of the wild forms. The important varieties today are almost without exception complex hybrids that include in their ancestry representatives of both the *S. officinarum* and the *S. Barberi* groups of cultivated varieties, together with representatives of one or both of the wild species.

Thus the sugar cane breeder has been exploiting, to the best of his ability, the advantages that heterosis has to offer. He is, however, acutely aware that a better understanding of the genetic basis of heterosis is prerequisite to its more effective utilization. Since he suffers the disadvantage of isolation from the centers of research, he cherishes such rare opportunities as he may have to peer over the shoulder of the research worker, to whom he must look for new facts that may lead to a better understanding of the mechanism of gene action and thus, of heterosis.

Recently some of us who are engaged in sugar cane breeding in Hawaii formulated a number of postulates with the object of providing a basis for discussing heterosis and related matters. These postulates have been excerpted or inferred from the published literature and from correspondence with workers engaged in genetic research, whose helpful suggestions are gratefully acknowledged.

Although the evidence supporting these postulates is sometimes meager, and sometimes capable of other interpretations, we have deliberately phrased them in a categorical vein in the belief that they might thus better serve their primary purpose—that of provoking a free exchange of ideas.

POSTULATES RELATING TO INCIDENCE OF LESS FAVORABLE ALLELES

1. Naturally self-fertilized populations tend to keep their chromosomes purged of all alleles other than those which in the homozygous condition interact to best advantage with the remainder of the genotype and with the existing environment[1] to promote the result favored by natural selection (or by human selection). This does not imply that any single population will contain all of the best alleles existing in the species. Selection can make a choice only between the alleles present in the population.

2. In addition to their prevailing (normal, plus, or wild type) alleles, cross-fertilized organisms such as corn and sugar cane carry in the heterozygous condition, at many loci, recessive alleles which in the homozygous condition would be inferior in their action to that of their normal or prevailing partners.

3. These less favorable alleles may be thought of as belonging to one of two classes, which, although differing in their past history, may have similar physiological consequences: (*a*) fortuitous, resulting from sporadic mutation, and representing the errors in the "trial and error" of the evolutionary process; or (*b*) relic, representing the residue of what were once the prevailing

1. The term *environment* is here used in a broad sense to mean the sum-total of the external influences acting upon the organism, including its nutrition.

alleles but which, in the course of evolution or under a changed environment, have been displaced, to a greater or lesser degree, by still better alleles.

4. The prevailing allele at a given locus has reached its pre-eminent position through the sifting action of natural selection over many generations. Given a stable environment, further improvement, through mutation, at that locus would long since have materialized if the chances for such improvement were high. It is not strange that random mutation should only rarely be able to produce a superior new allele. Nevertheless, once the possibilities for improvement through recombination of existing genes have been exhausted, further evolutionary progress will be contingent upon just such an event, however rare its occurrence may be.

5. Whether dominant or recessive, and whether in a naturally self-fertilized or naturally cross-fertilized population, a substantially superior mutant, once established in the population, is destined to increase in frequency and to become the prevailing allele in the population.

6. A deleterious dominant is doomed to eventual extinction. In a cross-breeding population of sufficient size a deleterious recessive may persist indefinitely, its incidence, except for random drift, being determined by the balance between its elimination by selection and the rate at which it recurs by mutation.

7. The best allele for one environment may not be best for another environment. The burden of less favorable alleles which cross-fertilized organisms carry along generation after generation is not an unmitigated liability. It serves as a form of insurance by providing a reservoir of adaptability to changing conditions.

ROLE OF LESS FAVORABLE ALLELES

Turning now to the role of these less favorable alleles in the heterosis phenomenon as manifested in naturally cross-fertilized organisms we may formulate a second group of postulates:

1. At many and perhaps at most loci, Aa is as good or nearly as good as AA, and both AA and Aa are better than aa.

2. There may be a few loci where aa is better than AA or Aa. This is particularly likely to be the case for loci affecting traits which are advantageous under domestication, but disadvantageous in the wild under natural selection.

3. There may, for all we know, be occasional loci where AA' is better than AA or $A'A'$ (overdominance).

4. There may be many regions in the chromosomes which *behave as though* AA' were better than AA or $A'A'$. With deleterious recessive alleles in the heterozygous condition at many loci, it seems almost inevitable that some of these will be closely linked in the repulsion phase, as for example Ab/aB, which in the absence of crossing over would behave as a single locus, the

heterozygous condition of which is superior to either homozygote. It is to be expected that such a linkage will eventually be broken. However, there may be regions in the chromosomes, such as the centromere region, for example, where crossing over is reduced, and where a group of genes may act indefinitely as a single gene. We may for convenience designate the effect of such reciprocal apposition of favorable dominants to their less favorable recessives as a pseudo-overdominance effect. It will be noted that such a *balanced defective* situation conforms with the dominance and linkage hypothesis advanced by Jones as an explanation of the heterosis phenomenon.

5. Even in the absence of linkage, an overdominance type of reaction (but resulting from pseudo-overdominance) must assert itself whenever each of the two members of a pair of gametes is able to supply the favorable dominant alleles required to counteract the less favorable recessives carried by the other member of the pair. The likelihood of success in retaining, in successive generations of selfing, all of the favorable dominants heterozygous in F_1, and eliminating all of the less favorable recessives, diminishes exponentially with increasing numbers of loci heterozygous in F_1. It would seem that naturally cross-fertilized organisms which carry, at many loci, deleterious recessives of low per locus frequency in the population could hardly fail to manifest a pseudo-overdominance type of response to inbreeding and outcrossing.

6. From an evolutionary standpoint, it may be important to distinguish between the consequences of (*a*) true overdominance (heterozygosis at the locus level) and (*b*) pseudo-overdominance (heterozygosis at the zygote level resulting from the reciprocal masking of deleterious recessives by their dominant alleles). From the standpoint of the breeder who is of necessity working against time, this distinction may have little practical importance if many loci are involved in the pseudo-overdominance effect. A breeding plan designed to deal efficiently with one of these alternatives should be effective also in dealing with the other.

7. Whether due to true overdominance or to pseudo-overdominance, the widespread if not universal occurrence among naturally cross-fertilized organisms of an overdominance type of response to inbreeding and outcrossing poses a problem which the breeder cannot afford to disregard.

8. Neither overdominance nor pseudo-overdominance can be called upon to explain the differences in vigor between different varieties of wheat, beans, sorghums, and other self-fertilized forms. Such differences are determined by genes in the homozygous state, as are also the differences between homozygous inbred lines of corn.

ROLE OF LIMITING FACTORS

A consideration of the role of limiting factors in quantitative inheritance leads us to a third group of postulates:

1. The adequacy of a diet is determined not by those constituents which are present in ample amounts, but by those which are deficient to the point of acting as limiting factors. Similarly the excellence of a genotype is determined not by its strongest but by its weakest links. The term *weak link* as here employed refers to a gene pair at a particular locus which at some moment in the life of the organism proves so inadequate in performing the task required of that locus as to act as a limiting factor—a bottleneck in an essential physiological process. A bottleneck effect may result from a deficiency of an essential gene product or from an excess of a gene product.

2. At each moment throughout its life the physiological processes of even the most vigorous organism are held down to their prevailing rates by bottlenecks or limiting factors. We are merely rephrasing a genetic axiom when we say that a bottleneck in the physiological reaction system is neither purely genetic nor purely environmental. The physiological bottleneck at any given moment results from the interaction of a particular locus (which we may for convenience refer to as the bottleneck locus) with the remainder of the genotype and with the environment of that moment. When we speak of an environmental bottleneck, we are merely focusing attention upon the environmental component of the genetic-environmental bottleneck. When we speak of a bottleneck gene, we are referring to the genetic component of the genetic-environmental bottleneck.

3. The value of an otherwise perfect diet would be seriously impaired by the omission of a single essential element. Similarly an otherwise superior genotype could be rendered mediocre or worse by a single bottleneck. A potentially superior genotype is unable to manifest its potentialities so long as it is being throttled by a genetic-environmental bottleneck. A breeder looks at the bottleneck and sees the need of a better allele at the bottleneck locus. An agriculturist looks at the same bottleneck and sees the need for correcting its environmental component. Bottlenecks relating to climatic limitations usually can be most economically dealt with by breeding.[2] On the other hand, bottlenecks resulting from nutritional deficiencies can often be advantageously dealt with by correcting the environment.

4. The substitution, at a bottleneck locus, of a better combination of alleles[3] will result in an improvement in yield providing that no other limiting factor, genetic or environmental, asserts itself before an appreciable gain has been realized.

5. The substitution of potentially better alleles at loci *other* than bottleneck loci cannot substantially improve yields any more than the addition of calcium to the diet of a plant or an animal can relieve the effect of a phosphorus deficiency in that diet. We take it for granted that each essential

2. This rule is not without exceptions. For example, a bottleneck resulting from a deficiency of rainfall can sometimes be economically eliminated by irrigation.

3. As already indicated, the best combination of alleles may be AA, Aa, or aa depending upon the particular locus.

chemical element has its specific role to perform in the physiological reaction system. Similarly we accept as well established the thesis that gene action is likewise specific—that a particular gene can perform its particular function, and that function only. Nevertheless we sometimes engage in speculations which ignore these convictions and which appear to assume that genes affecting quantitative characters such as yield are freely interchangeable, one with another, and that one *yield* gene can serve as well as another, regardless of its locus or function.

6. A bottleneck locus may act as such throughout the life of the individual or it may act as a limiting factor only for a short period and under specific conditions, such as drought, nitrogen deficiency, or excessively high or low temperatures. Under a varying environment the bottleneck of one moment may be superseded by a different bottleneck at the next moment.

7. The physiological bottleneck may be ameliorated or removed by correcting the particular feature of the environment contributing to the bottleneck. In the examples cited above this would entail supplying moisture, or nitrogen, or lowering or raising the temperature. Or the bottleneck may be ameliorated or removed by substituting a more effective allele at the bottleneck locus, providing that such an allele is available.

8. As already indicated, the amelioration or removal of a bottleneck, either by improving the environment or by substituting a better allele at the bottleneck locus, will permit a rise in the rate of the essential physiological processes. This rise may be small or it may be large, depending upon the point at which the next ensuing bottleneck begins to make itself felt. The substitution of a more efficient allele at a bottleneck locus in a certain genotype, under a particular environment, may result in a large gain. The substitution of the same allele in a different genotype or under another environment may result in little or no gain. It is not strange that difficulty should be encountered in analyzing the inheritance of genes affecting yield and other quantitative characters which are subject to the influence of a varied and fluctuating array of genetic-environmental bottlenecks.

9. A diet that is low in calcium may supply calcium at an adequate rate so long as growth is being retarded by a lack of phosphorus. But once phosphorus is supplied at an adequate rate, calcium deficiency becomes a bottleneck which limits the rate of growth. Similarly a mediocre gene *m* at one locus may be adequate (not a bottleneck) so long as the rate of physiological activity of the organism is being throttled by environmental limitations or by a bottleneck gene at some other locus. But once the other genetic-environmental limiting factors have been removed, the mediocre gene *m* is unable to handle the increased load and becomes the bottleneck in the reaction system.

10. The maximum vigor or yield possible under a given environment will be attained when the organism is endowed with the best available allele or

combination of alleles at each bottleneck locus. There are presumably many loci that never act as bottlenecks in any part of the reaction system affecting vigor or yield, no matter which allele or combination of alleles happens to occupy such a locus.

11. The difference between the weakest inbred and the most vigorous hybrid is merely one of degree. Each represents an integration of the many genetic-environmental bottleneck effects under which it has labored. The weak inbred has been throttled down by one or more bottlenecks to a low level. The superior hybrid is able to go much further, even attaining what we might concede to be extreme vigor. But both the weak inbred and the vigorous hybrid have throughout their lives been held down to their respective levels by their genetic-environmental bottlenecks.[4]

MISCELLANEA

The fourth and last group of postulates comprise a heterogeneous population randomly listed as separate topics for discussion.

1. If each step in a complex physiological process such as photosynthesis is conditioned by the action of a specific gene, and if each successive step in the chain of reactions is contingent upon the successful completion of the preceding steps, it follows that in attempting a biomathematical analysis of the inheritance of quantitative characters such as yield we may not be justified in assuming, as a basis for our calculations, that each of the genes concerned is independent in its action.

2. Since our efforts to "improve" the genotype are constantly being thwarted by bottleneck genes, we may be tempted to damn all such genes as inventions of the Devil. No doubt there are many defective genes that would have to be classed as liabilities under any normal environment. But certainly there are many bottleneck genes that are indispensable to survival—genes that act as governors in regulating physiological reactions and in fitting the organism to its particular ecological niche. A mouse or a moss can survive and reproduce where larger organisms would perish. And a mouse which, as a result of changes in certain of its adaptive bottleneck genes attained the size of a rat, might find itself at a disadvantage in a community of normal mice.

3. If we are correct in assuming that even a single major bottleneck locus can act as a limiting factor in the development of an otherwise superior geno-

[4] Certain of the foregoing postulates pertaining to the role of bottleneck genes in quantitative inheritance may be guilty of gross over-simplification. So complex is the physiological reaction system of even the simplest organism that we are only now beginning to gain an inkling of the extent of its complexity. These postulates may also be guilty of exaggeration. Because we believe that the action of limiting factors in quantitative inheritance has not received the attention that it deserves, we have intentionally stressed the importance of the bottleneck locus, even at the risk of over-emphasis. Furthermore, we have pictured the limiting factor at a given moment as pertaining to a single bottleneck locus. This may or may not be the rule. It would not be difficult to imagine a bottleneck which pertains to several loci and which could be relieved or eliminated by substituting a more effective allele at any one of these loci.

type, it is hardly to be expected that the phenotype of an inbred line will afford a wholly reliable indication of its breeding potentialities in hybrid combinations.

4. We need to keep in mind the limitations that pertain to a rating for general combining ability. The best "general" combiner thus far discovered in corn is not so general in its combining ability as to be able to combine to advantage with itself or with any other genotype that happens to be afflicted with the same bottleneck genes. At best, a rating for general combining ability can represent nothing more than an average arrived at by lumping a given population of specific combinations. An average derived from a different population of specific combinations could result in quite another rating.

5. If a series of inbreds A, B, etc., be crossed with a tester inbred T, we obtain the hybrids AT, BT, etc. The yield of AT will be determined by the bottleneck genes in the AT genotype. The yield of BT will be determined by the bottleneck genes in the BT genotype. The test cross can tell us which lines combine to best advantage with the tester line, but it cannot reasonably be expected to tell us more than that. It cannot, for example, tell us with certainty what we may expect from $A \times B$. Both A and B may combine to advantage with T, but if A and B each happen to be afflicted with one or more of the same bottleneck genes (not present in T) the yield of the cross AB will suffer.

6. The failure of a cross between two convergently improved lines to equal the cross between the two original lines from which they were derived cannot be taken as critical evidence for the existence of an overdominance mechanism. The benefits which convergent improvement seeks to achieve can be vitiated if a recessive bottleneck gene b, present in only one of the original parent lines, should become homozygous in both convergently "improved" lines. Selection exercised with the object of preventing such an occurrence may be ineffective if b becomes a bottleneck only under the enhanced rate of physiological activity of the $A(B) \times B(A)$ hybrid.

7. During recent years several examples of heterosis reported in the literature have been attributed to the effect of heterozygosity at a single locus. When the amount of heterosis is substantial, it should be possible to verify the validity of the hypothesis by breeding tests. If the two parents are really isogenic, except for the heterosis locus H, and if H_1H_2 individuals are more vigorous than either homozygote, then by selfing only the most vigorous individuals in each generation it should be possible to retain in one-half of the population the original vigor of F_1 even after many generations of selfing, and such a line should continue indefinitely to segregate H_1H_1, H_1H_2, and H_2H_2 individuals in a 1:2:1 ratio.

8. East describes the effect of heterosis as "comparable to the effect on a plant of the addition of a balanced fertilizer to the soil or to the feeding of a more adequate and more chemically complete diet to the animal." The simi-

larities noted by East between the beneficial effects of heterosis and those of improved nutrition are more than coincidental. The first prerequisite for enhanced well-being is the removal of the bottlenecks that stand in the way. This can sometimes be accomplished by improving the nutrition, sometimes by substituting more efficient alleles at the bottleneck loci, and sometimes by both.

9. The term *heterosis* remains ambiguous in spite of the many attempts to define it. It continues to have different meanings for different workers.

10. If heterosis is to be measured by comparing performance of offspring with performance of parents, then the higher the standing of the two parents in the scale of measurement, the lower the degree of heterosis to be expected in their offspring. Conversely the lower the standing of the parents, the greater the heterosis to be expected. (Exceptions to the latter rule will occur when both parents owe their enfeeblement to the same bottleneck genes.)

11. Success in crop and livestock production depends largely upon the skill of the grower in detecting, diagnosing, and correcting the environmental components of the bottlenecks affecting yield. Success in developing higher yielding genotypes depends largely upon the ability of the breeder to substitute more effective alleles at the bottleneck loci, and to accomplish this without establishing new and equally serious bottlenecks at other loci.