HETEROSIS

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INTRODUCTION

THE fact that vigorous growth so frequently accompanies hybridization has puzzled biologists for nearly two centuries. The phenomenon was given a purely formal solution some twenty-five years ago when a series of analyses of the effects of inbreeding in maize, supplemented by studies of the results obtained when these inbred lines were crossed, made it possible to regard heterosis as something incident to the operation of genetic laws. Nevertheless, the picture of these laws in action was extremely vague. Genetic knowledge, at the time, was so meagre that it seemed necessary to assume that vigor is promoted when the genes at certain loci are unlike,—an assumption for which there was no proof, and which was not illuminating as a dynamic interpretation.

Somewhat later JONES (1917) suggested that this postulate was unnecessary. Owing to the establishment of MORGAN'S theory of linkage, it became possible to see how heterosis could result from normal gene action and yet be a phenomenon accompanying hybridity. The essential points of the argument are as follows. Genes affecting growth are found at numerous loci and have cumulative action. These genes mutate frequently to more efficient (dominant) allelomorphs, and to less efficient (recessive) allelomorphs. Since any given chromosome may contain x dominant genes and y recessive genes distributed more or less at random, it follows that heterosis will be manifested very frequently in F_1 hybrids, owing to the apposition of dominant to recessive at various loci, and that the recovery of homozygotes having the characteristics of the individuals of the F_1 generation will be virtually impossible, owing to the rarity with which the crossovers required to produce homozygosis may be expected to occur. BRUCE, and KEEBLE and PELLEW (1910) are sometimes given prior credit for this conception, but not upon just grounds. The earlier authors based their theory upon independent segregation at a time when linkage was not understood, and their scheme did not and could not fit even the then known facts. The particular use made of the notion that the chromosomes are strings of genes obeying special laws of transfer was what made JONES' theory acceptable, not simply the employment of the words *dominant* and *recessive*.

The explanation of heterosis offered by JONES was so probable that it was generally accepted, at least until 1930 (ASHBY), in spite of the fact that there was no direct proof for it. This was not altogether fortunate.

Even though there is, as I believe, much critical evidence in favor of the essential features of JONES' ideas, the subject should not be regarded as a closed issue. Certain ramifications are still puzzling.

THE PHYSIOLOGICAL THEORY OF ASHBY

Numerous items of fact on heterosis have been published since 1918, but the only attack on the theoretical aspects of the problem has been that of ASHBY (1930, 1932).

The inspiration of ASHBY's investigation was a statement by V. H. BLACKMAN that the two major factors determining the amount of tissue produced by a plant are the size of the seed (or embryo) and the efficiency index (that is, the angle of slope of the logarithmic curve of growth). ASHBY divides the second factor into two components,—the length of time that the efficiency index remains constant, and the form of the growth curve after that time. This declaration, which is a restatement of the problem rather than an explanation, ASHBY regards as an analysis. He says that the next step is to discover the way in which all these factors are inherited. Undeterred by its length or the obstacles involved, ASHBY proposed to take this step.

Maize was selected as the experimental material. Three ears were obtained from the Bureau of Plant Industry, viz., inbred P_w, inbred P_b, and the F_1 from $P_w \times P_b$. The seedlings were thinned to 1 per hill, and 10-plant samples were gathered fortnightly during 16 weeks, from which to calculate leaf area and dry weight. From these data, growth curves were constructed by plotting the logarithms of the figures against time. The curves proved to be straight lines; and the curve for the F_1 was approximately parallel with that for P_b, though higher. In addition, the average dry weight of the embryos was determined to be .038 gm., .0125 gm., and .045 gm., respectively, for P_w, P_b, and F_1; while the dry weights of the seeds were .368 gm., .163 gm., and .405 gm., in the same order. Cell size was also examined.

ASHBY concluded: (a) that the hybrid does not differ in the least from its more vigorous parent as regards relative growth rate, or from either parent as regards cell size, photosynthetic efficiency of leaves, or the time of flattening of the sigmoid growth curve; (b) that the only physiological differences observed are an increased percentage of germination of the hybrid seeds and a greater initial weight of embryo which gives an advantage that is maintained throughout the "grand" period of growth; and (c) that the relative growth rate is apparently inherited in the manner of a dominant Mendelian factor.

One of the major omissions in this first work was the test of reciprocal crosses. It was rectified in ASHBY's second paper. This later contribution

also contained calculations of respiration rate. The conclusions of the previous paper were thought to have been corroborated. Reciprocal crosses had the same efficiency index but showed different degrees of hybrid vigor that were attributed to variation in embryo weight. Thus it follows, according to ASHBY, that "hybrid vigour in these strains is nothing more than the maintenance of an initial advantage in embryo size." And he believes that the notion that the higher of the two parental efficiency indices behaves as a "factor" dominant over the other, is also corroborated.

It was hardly to be expected that geneticists should share ASHBY's complete confidence in his solution of such an ancient and involved problem. Nevertheless, in spite of the meagre evidence that he has presented, his obvious lack of acquaintance with manifestations of heterosis, his failure to consider adequately the pertinent published evidence on the subject, and his unorthodox expressions when dealing with genetic concepts, ASHBY's papers should not be overlooked, for one group of his observations is very much worth while.

Let us examine first the question of the influence of the size of the embryo—or of the endosperm—on heterosis. Presumably no one would argue that there is any high degree of correlation between seed size and rapidity of growth when different groups of plants are compared, since numerous small-seeded plants produce tissue at a faster rate than do seedlings from large seeds like those of *Cocos nucifera*. But within the species, and often within the genus, the size of the seed is undoubtedly a growth factor. The phenomenon has been noted many times. In maize alone HAYES and GARBER (1927) cite five authors who have considered the matter.

It is commonly assumed that the F_1 seeds in maize hybrids are larger than those of the maternal parent, and that both the endosperm and the embryo are increased in size. In fact, ASHBY's own data show that this is true, despite his emphasis on embryo only. EAST and JONES (1920, table 11) reported the weights of seeds in a series of reciprocal crosses, using the same parental individuals. The average seed weight of parents from A stock was 24.2 cg., and from B stock was 24.4 cg. The average weight of the seeds from the A×B crosses was 28.0 cg, while that from the B×A crosses was 30.2 cg. Thus there was an increase over the seed parent of 15.3 percent in the first instance, and of 24.2 percent in the second. A smaller series (5 reciprocals) was submitted (table 15) where embryos, pericarps, and endosperms were isolated and weighed separately. The embryos were materially larger than those of the larger parent in only one case, but the endosperms were more than one-fourth larger in every case. There are also a few data on the yields from reciprocal crosses made from the same stocks used in determining the effect of crossing on seed size

(table 25). The great increases observed in each case seem hardly attributable to seed size alone.

There are a number of similar observations on other species in the literature, though there is none, so far as I am aware, where the component parts of the seed have been separated. The evidence is contradictory. Some times the F_1 seeds are larger than the seed parent; sometimes they are not larger than the arithmetic mean or the geometric mean between them. MALINOWSKI (1935), for example, crossed two varieties of the common bean weighing, on the average, .54 gm and .32 gm, respectively. The F_1 average weight was .42 gm, which is slightly closer to the geometric mean (.416) than to the arithmetic mean (.43). The plants themselves exhibited marked heterosis. Some years ago, I crossed two varieties of peas together that had seeds of practically the same size. The F_1 seeds were not significantly different, yet the plants showed a high degree of heterosis. Again, I have crossed *Nicotiana Tabacum* and *N. rustica*, obtaining plants showing more heterosis than any other crosses I have ever observed; yet no difference in seed weight could be detected.

I am very much inclined to believe, therefore, that there is no general rule about increase of size in either the endosperm or the embryo in the seeds of crosses, although heterosis is so generally to be noted in the plants themselves. Certainly MALINOWSKI's and EAST's observations on legume seeds, where endosperm tissue is virtually non-existent, do not favor the idea that F_1 increases in embryo are necessarily common or important. And in the experience of EAST and JONES on maize, the increase in the endosperm is often materially greater than the increase in the embryo; while in the experience of the wheat breeders, certain crosses between tetraploid and hexaploid types exhibit noticeable increases in vigor, even though the endosperms are shrivelled and the embryos are no larger than those of the parents. It seems reasonable to conclude, therefore, that increase in endosperm or embryo size is itself a phenomenon of heterosis detectable, at times, in early stages of ontogeny. Moreover, where considerable increase in size of endosperm appears, as in certain crosses of maize, and the amount of difference between reciprocals is significant, the latter may be a factor of some account to the end of the life cycle, thus helping to explain the differences in yield of reciprocals sometimes observed.

There is other evidence that hybrid vigor can not be "nothing more than the maintenance of an initial advantage in embryo size." JONES (1918, fig. 3) studied the growth curves of maize as exemplified by height, and their end points as illustrated by yield of grain, for parental stocks, F_1 populations, and F_2 populations. The data for the inbred parental stocks and for the F_1 generation may be taken as representative of any

given plant, for the dispersion coefficient is small. The data for the F_2 population express an average where the dispersion coefficient is large. The plants of the inbred parental stocks show less vigor than the plants of the F_1 or F_2 populations. The plants of the F_1 generation, owing partly, no doubt, to the larger seeds from which they come, grow faster than either parent, reach sexual maturity earlier in most cases, and gain a greater total height and weight. The F_2 seeds borne on the F_1 plants, however, are *larger* than the F_1 seeds borne on the parental plants; yet the F_2 seedlings continue to grow faster than the F_1 seedlings, through their initial advantage, *only* up to about 70 days from planting; after which they grow more slowly and fail to reach the height of the preceding generation.

Two direct tests of this point have been made since the publication of ASHBY'S papers, moreover, and the results have failed to sustain his position. In COLLINS' laboratory (RICHEY 1935) it was found that the larger hybrid seeds of maize had a significantly higher growth rate for only two weeks, after which no difference was detectable. In Miss PASSMORE'S (1934) experiments on reciprocal cucurbit crosses it was found that, while the cross having the larger seeds reaches its ultimate size before the reciprocal, the reciprocal catches up by having a longer period of growth.

These experiments are sufficient, in themselves, to show that ASHBY failed to get to the heart of this part of the problem; and they are corroborated, as far as I can learn, by the more casual observations of other workers on a variety of species. At all events, they are supported by my own observations. I have seen several Nicotiana hybrids, for example, that were double the height and bulk of either parent, although there was no detectable difference in the size (weight) of selfed and of crossed seeds on the maternal parent. The conclusions indicated, it seems to me, are: (1) that seed size is a nutritional advantage, other things being equal; and (2) that it may be one of the manifestations of heterosis or may be the result of other causes, but that seed size, or the size of any part of the seed, can not be the true cause of heterosis.

There is also much other evidence, of a somewhat different character, destructive to this particular thesis; so much, in fact, that one hardly knows what to select. One might cite the direct test of the hypothesis made by LINDSTROM (1935) on maize, where the handicap of the F_1 plants in "initial capital" was reduced by cutting back the seedlings. The F_1 plants gave much higher yields of grain than the inbred parents did, despite the "initial capital" reduction and the mutilation. Since animal crosses also show heterosis, one might cite the experiment of ROBERTS and LAIBLE (1925), where a Duroc Jersey sow was double mated to both Duroc Jersey and Poland China boars. Ten pigs were born. Six were pure Duroc Jersey and four were crossbred. They were distinguishable by color char-

acters. The crossbreds averaged half a pound more than the purebreds at birth; while at six months the two purebreds still alive weighed 183 and 188 pounds, respectively, and the crossbreds weighed 224, 225, 231, and 261 pounds, respectively. Other experiments with crossbred animals are not quite so critical as this one,—for example LIVESAY (1930) on rats; but in many of them marked heterosis is noted and recorded. Just how cross-fertilized animal eggs could show more "initial capital" than line-fertilized eggs is difficult to imagine. And beyond the special experimental data on the matter, there is a body of general information that appears to destroy ASHBY'S hypothesis utterly. Nearly all our asexually propagated horticultural plants derive the vigor that has made them useful from heterosis, since they invariably lose much of this vigor when inbred. Among them may be mentioned potatoes, apples, pears, peaches, grapes, strawberries, raspberries, etc. *And it is well known that these plants hold their heterosis not only through a single life cycle, but also through a series of life cycles.* Deterioration of asexually propagated forms does sometimes occur, but it has been established that this phenomenon is due to disease and not to asexual propagation in itself or to loss of heterosis. This is shown by the fact that most clones of our various asexually propagated fruits and vegetables retain their characteristics over long periods, though a few may degenerate. Disease is demonstrable in many of the degenerate stocks.

Heterosis, therefore, is not simply a manifestation of an initial advantage in embryo size or the size of any other part of the seed or the propagating piece. It is a genetic effect on the organism as a whole, as has been suggested many times, and as even ASHBY'S mathematical treatment of his results has demonstrated; but it is an effect controlled by many genes and is not interpretable as a simple Mendelian dominant. The last statement is a fact well known to all plant breeders who have had occasion to cross a series of strains on one particular strain, and it needs no extended defense. What I shall endeavor to do in the remainder of this paper is to show that JONES' hypothesis is not a dominance hypothesis, as it is usually called, but is instead a linkage hypothesis that is strongly supported by the evidence from amphidiploids. At the same time I shall hope to show that a modernized conception of gene action leads us back closer to the original heterozygosis theory.

THE DIRECT EVIDENCE ON THE LINKED GENE THEORY

The recent work on the salivary gland chromosomes of Drosophila and other dipterans indicates that these organisms possess several thousand genes. Presumably the number in most other organisms is greater rather than less. It need not follow that the number of *different* allelomorphs in a

population of any given strain is high; but more and more evidence accrues daily that these also are to be numbered in the hundreds. It can readily be seen, therefore, that if developmental vigor is dependent upon any very large number of genes existing in various allelomorphic conditions, the chance of obtaining homozygous forms like those appearing in the F_1 generation is very low, even with free recombination. With a relatively small number of linkage groups and such distribution of essential genes as is actually found (see LINDSTROM 1920, for chlorophyll development in maize), the chance would ordinarily approach zero. Yet a significant degree of heterosis might occasionally be dependent on such a small number of genes that approach to the F_1 type could be obtained in a homozygous strain. Success in such a system as RICHEY has proposed in his Convergent System of Improvement in Maize is, for this reason, not wholly idealistic. I have had at least two populations in tobacco where the theoretical expectancy appeared to have been realized; and MÜNTZING (1931) had one in a cross between *Galeopsis Tetrahit*×*G. bifida*. I do not agree with MÜNTZING, however, that such cases can be relied upon as proof of the linked gene theory of heterosis.

On the other hand, a substantial amount of critical evidence in favor of the theory is to be found among polyploid plants. To weigh this evidence properly, it is necessary to discuss briefly the general effects of polyploidy.

It is ordinarily supposed that any increase in the number of complete genoms is accompanied by increase in size and vigor of the organism. But is this supposition sound? I believe that it is unsound. Some dozen or so haploids have been described in genetic literature. Probably hundreds could have been described had it been thought desirable. At this laboratory alone, at least ten have been studied. They are invariably smaller and less vigorous than diploids. Thus there does seem to be some reason why the common somatic condition, persistent so long under natural selection, is a better running physiological machine than the haploid condition. But it does not follow that higher polyploids are still larger and more vigorous than diploids.

The idea that doubling the chromosomes of a species produces a giant form arose from the use of the term *gigas* to describe the first autotetraploid of *Oenothera Lamarckiana*. The name clung when similar traits were frequently found to characterize the autotetraploids of Datura (BLAKESLEE, BELLING, and FARNHAM 1923), Solanum (JORGENSEN 1928, LINDSTROM and KOOS 1931), and of other species. But the plants are not giant forms. Their distinguishing features are stouter and stockier stems, somewhat larger leaves and flowers, and, in particular, very rugose leaves. And these characters are by no means designating traits for all autotetraploids or higher autopolyploids. So far as I am aware, few monocotyledonous

autopolyploids are distinguishable from the diploids from which they arose. As evidence on this point there are the tetraploid maize plants produced through heat treatment by RANDOLPH (unpublished), the numerous tetraploid Tradescantia species (ANDERSON and DIEHL 1932, ANDERSON and WOODSON 1935), a tetraploid Rhoeo (SAX, unpublished), several Iris species (ANDERSON, unpublished; these are difficult to distinguish from diploids), and several types of Phleum (GREGOR and SANSOME 1930). In fact, the only giant forms that appear to be autotetraploids are *Iris mesopotamica*, a bearded iris, and *Iris Sintenisii*, a beardless iris (SIMONET 1934). In the dicotyledons, autopolyploids are not always distinguishable. BLACKBURN (1927, 1928) has reported them in Silene. I have seen them in Petunia and Lycopersicum (distinguishable with difficulty). And apparently, from the descriptions, it is not uncommon to find difficultly distinguishable tetraploids in Datura, Solanum, and other members of the Solanaceae. I suggest that *gigas* forms are special cases found only in certain groups, probably where the epidermis is soft. It seems likely that more indistinguishable autotetraploids will be found when more chromosome determinations are made at random, as in ANDERSON'S work. Hitherto, *gigas* forms have had more attention from cytogeneticists than ordinary types.

This matter has been stressed because it gives one a clearer idea as to how much, or how little, vigor should be allowed for when comparing autotetraploids and allotetraploids. These terms are convenient, but they are not alternative descriptions. On the contrary, they form a continuous series. The best cytological criterion for separating them is whether the chromosomes form bivalents (auto), or trivalents and quadrivalents (allo) at meiosis. Now, most polyploid series of natural species are assumed to have been derived from hybrids by chromosome increase; yet there is scarcely any evidence that the vigor of members of a polyploid series rises with increase in chromosome number. Only in Erophila has it been maintained that length of leaf increases with chromosome number (WINGE 1933). If this is not the rule, then either amphidiploids show no hybrid vigor, or the natural polyploid series do not originate from hybrids. We shall show that many artificially produced amphidiploids do exhibit hybrid vigor comparable to their diploid parents, thus indicating some validity to the linked gene hypothesis and also indicating that hybridization has less to do with natural polyploidy than is generally assumed.

It would hardly be defensible to try to diminish the value of hybridization too vociferously. HUSKINS (1930) has shown that there is high probability that *Spartina Townsendii* is an amphidiploid arising from a cross between *S. alternifolia* and *S. stricta;* and it is so vigorous a type that it has displaced the parental forms where in contact, spread all over southern

England, and passed over to France, since 1870. But if one thinks a moment, it is clear that hybridization followed by amphidiploidy has been overemphasized as an evolutionary factor. Species hybridization under natural conditions is very rare. Amphidiploidy is rare. The second event must wait on the first. Thus it seems that the resultant new species must have to have extraordinarily favorable characteristics and must meet extremely favorable environmental conditions in order to persist. On the other hand, autopolyploidy is extremely common. It can be produced at will, at least in certain groups, by temperature extremes and by mutilation. All that is needed for persistence under competition is a slight selective advantage in form and function and genetic changes (known to occur) bringing about normal pairing at meiosis. In fact, chiasma frequency in most plants is so low that polyvalent association is not a material obstacle. We know little about the actualities of such a scheme. But ANDERSON'S and WOODSON'S survey (1935) of the Tradescantias in the United States, probably the most careful and extensive investigation of its type, is very suggestive. Tetraploid forms appear to be increasing more rapidly than diploid forms around the periphery of the distribution of several species.

About forty amphidiploids arising from hybrid parentage are known. From a careful study of the somewhat fragmentary breeding records, I believe it is clear that, unless additional variables are present, these amphidiploids possess the characteristics of the diploids and breed true to them. This evidence definitely proves the hereditary basis of heterosis and is consistent with the linked gene theory. It fails to give us a clear picture of the activity of the genes concerned; but shows that the problem of heterosis is the problem of the inheritance of quantitative characters.

Any analysis of the amphidiploid evidence in relation to heterosis is rational and consistent only if the action of certain extraneous variables is kept in mind. The more important of these complications are as follows. First, it must be remembered that, though normally heterosis increases roughly with genetic dissimilarity within a genus, different genera can not be compared easily because each genus has its own scale of manifestation. Again, it often happens that when rather distantly related species are crossed, there is a disharmonious relation between the components that results in dwarf forms; and these dwarf forms frequently show their nature by monstrous developments in particular organs. Second, one must not forget that the amphidiploids used in genetic research are types that have not been made into smoothly running physiological entities by long periods of selection. Numerous cytological aberrations appear—aneuploidy translocation, inversion, deletion, and the like—in addition to other disturbing internal factors, like secondary association, and to disturbing external factors. The frequency and type of the cytological disturbances

appear to show relationship (1) with mode of origin of the hybrid, whether by somatic doubling or by polyploid gametic unions, and (2) with mode of origin of the components, whether polyploid or not, in the genetic rather than the cytological sense. Little is known about the possibility of external disturbances, but ANDERSON'S investigations indicate that an autotetraploid may react to conditions in a somewhat different manner than the diploid from which it came. Third, any possible changes due to chromosome doubling itself must be discounted.

The only amphidiploid known to have arisen by somatic doubling is *Primula kewensis*, which came from amphidiploid buds on a chance cross between *P. floribunda* and *P. verticillata* (NEWTON and PELLEW 1929). As shown by artificial hybrids made by COUTTS, the hybrid exhibits definite heterosis, particularly in the leaves and flowers. In *P. kewensis*, these manifestations are slightly exaggerated, as if a little of the *gigas* condition had been added by the chromosomes' doubling. The species breeds true in the horticultural sense of the term, though not strictly true in the genetic sense. Of 287 plants raised at John Innes Horticultural Institution, 261 bred true, and 26 plainly showed variation from type. Several variants studied showed chromosomal aberrations.

The remaining amphidiploids, though some of them may have arisen by somatic doubling, appear to involve diploid gametic unions. They derive either from $2N \times 2N$, or from $(2N \times N) \times N$ by backcrossing a triploid. Some few were too sterile to afford any data on the matter in hand, as in Crepis (BABCOCK and NAVASHIN 1930); and some have not been described sufficiently for a satisfactory judgment, as the Pimpinellifolia-villosa hexaploid rose (BLACKBURN and HARRISON 1924); but most of them afford useful information on the point under consideration. The greatest amount of work has been done on the wheat relatives, the tobaccos, and the cabbage-radish hybrids. The other examples are more scattered.

The amphidiploids among the wheat relatives ordinarily show a considerable degree of segregation due to chromosome aberrations or to polyvalent chromosome unions, as is to be expected where at least one of the parents is nearly always a natural polyploid. Moreover, the components of these hybrids are often too different genetically to show heterosis because of physiological disharmony. But apart from the interference of chromosomal aberrations, the double diploids breed true to the characters of the diploid hybrids, vigor included. The constant wheat-rye hybrids of Rimpau (LINDSCHAU and OEHLER 1935) are intermediates without noticeable heterosis; the constant wheat-rye hybrids of MEISTER (1928, LEVITSKY and BENETZKAIA 1931, LEBEDEFF 1934) show marked vigor in many of the combinations made. The $(7 \times 14) \times 2$ chromosome hybrids are not infrequently weak types (KIHARA and KATAYAMA 1931, TAYLOR and

LEIGHTY 1931, OEHLER 1934); but some of them are quite vigorous (PERCIVAL 1930, TSCHERMAK and BLEIER 1926, TSCHERMAK 1929, 1929a, 1930, 1934).

Within the cabbage tribe, two notable amphidiploids have been produced. FRANDSEN and WINGE (1931) made crosses between the turnip and the swede (10+18), which manifested marked heterosis; but the fertile amphidiploid which appeared was still more vigorous, and its progeny apparently retained this vigor. The new species, *Brassica napocampestris*, was intermediate between the parental species in its general traits. There was also considerable heterosis in the true breeding balanced forms of Brassicoraphanus originated by KARPETSCHENKO (1927, 1927a, 1928. 1929) from crosses between *Raphanus sativus* (9)×*Brassica oleracea* (9). The unbalanced forms usually showed their condition by aberration, including dwarfing. Another constant Brassicoraphanus amphidiploid has been isolated by TERASAWA (1932). *B. chinensis* (n · 10) was the cabbage used. Nothing is said about heterosis. It is unfortunate that the varieties in the crosses of KARPETSCHENKO—and apparently in those of TERASAWA —did not produce hybrids of such formidable vigor as those obtained by GRAVETT (1914) and SAX (unpublished).

A number of amphidiploids have been obtained from Nicotiana hybrids. In most cases one component was already a polyploid, *N. Tabacum*, *N. rustica*, and *N. Bigelovii*. This introduced some cytological irregularity. Some, moreover, were combinations which are ordinarily rather weak—*N. glutinosa*×*N. Tabacum*, and *N. suaveolens*×*N. Bigelovii*. At least one, *N. Tabacum*×*N. glauca* (TERNOVSKY 1935), showed so much unbalance that a constant race was not obtained. But, in general, the amphidiploids bred true to the characteristics of the hybrid; and where heterosis was present, it was continued. *N. glutinosa*×*N. Tabacum* was, in CLAUSEN and GOODSPEED'S case (1925), not markedly vigorous, and in my experience the hybrid has been dwarfish; but in TERNOVSKY'S case it exhibited "völlige Lebensfähigkeit und eine energische kräftige Entwickelung."

The amphidiploid of *N. Tabacum*×*N. rustica* (EGHIS 1927, RYBIN 1927), which came from a triploid backcrossed to *N. rustica*, was exceptional in that it showed less heterosis than the hybrid diploids. Nevertheless, it was notably vigorous and bred fairly true to its characteristics. Here the new species was really an octoploid derived from two tetraploid components.

The fertile double diploid hybrid between *N. Tabacum* and *N. sylvestris* (EGHIS 1930, RYBIN 1929) is again a special case in that *N. sylvestris* is a component of *N. Tabacum*. The new form showed heterosis; but I judge that the particular combination of varieties made gave somewhat less vigorous hybrids than are usually obtained.

The amphidiploids from *N. rustica*×*N. paniculata* (LAMMERTS 1931,

SINGLETON 1932) are again an instance of adding similar genes, for *N. paniculata* is a component of *N. rustica*. The hybrids are always intermediate in size, but show vigor in rapidity of growth and early maturity. LAMMERTS obtained both dwarf and giant races. SINGLETON obtained plants much like those of the F_1 generation.

The amphidiploid between *N. glutinosa* and *N. tomentosa* (GOODSPEED 1933, ELVERS 1934) had great vigor, even more than the diploid. The *N. suaveolens*×*N. Bigelovii* amphidiploid has not been described (GOODSPEED 1933).

The constant amphidiploid arising from a hybrid between *Digitalis ambigua* and *D. purpurea* (n = ca 112) described by BUXTON and NEWTON (1928) had a notable degree of heterosis in the leaves and apparently in the flowers.

CRANE and DARLINGTON (1927) indicate that the Loganberry, presumably an amphidiploid of unknown parentage, exhibits a high degree of vigor; and my own observations corroborate this view. Its progeny are somewhat variable, as is to be expected from the cytological complexity of Rubus.

HIORTH (1934) describes a polyploid hybrid between *Collinsia bicolor* and *C. bartsiaefolia* which has been constant for five generations. Heterosis is apparently marked by "thick leaves, broad with rounded tip," and large flowers. HUSKINS' (1930) description of the Spartina case has already been mentioned. SKOVSTED (1929) describes a constant amphidiploid showing marked heterosis arising from a cross between *Aesculus pavia* (20 small chromosomes) and *A. Hipposcastaneum* (20 large chromosomes). WINGE (1933) describes a constant amphidiploid arising from two types of *Erophila verna*, where n equals 15 and 32 respectively. There is a fair degree of variability in this form; but the amphidiploid is much more robust than either of the parents, at least in the rosette stage. SKALINSKA (1935) describes a peculiar amphidiploid coming from *Aquilegia chrysantha* (n = 7) and *A. flabellata nana* (n = 7). The plants of the first species are 70–80 cm in height, while those of the second species are only 30–40 cm in height. The F_1 individuals are much larger than either parent, ranging from 100 cm to 110 cm. The tetraploids are only about 70 cm in height. They show marked heterosis in size of rosette and in number and stockiness of the stems produced, but they certainly are less vigorous than the diploids. The variability of the forms indicates additional variables at work.

Finally, MÜNTZING'S (1931, 1932) admirable work in synthesizing *Galeopsis Tetrahit* from *G. pubescens* crossed with *G. speciosa* should be emphasized. It is the most complete description of any amphidiploid. The plants, except for two dwarf segregates, were generally larger and more vigorous than the parental species.

On the other hand, certain crosses exhibit no noticeable heterosis either as diploids or amphidiploids. This is apparently the case in MÜNTZING'S (1935) example in Phleum and in NILSSON'S (1935) example in Festuca. Such instances are to be expected.

THE DEPENDENCE OF HETEROSIS UPON GENETIC DISPARITY IN THE PARENTAL STOCKS

In 1912 EAST and HAYES expressed the opinion, based on much too meagre evidence, that the amount of heterosis expressed in an F_1 plant is roughly proportional to the genic differentiation in the two parental stocks, provided normal ontogeny is possible. Since that date, I have had the opportunity of studying a variety of crosses in the following genera: Antirrhinum, Aquilegia, Begonia, Beta, Capsicum, Campanula, Catalpa, Chelidonium, Crepis, Cucumis, Cucurbita, Datura, Delphinium, Digitalis, Eschscholtzia, Euchlaena, Fragaria, Glycine, Gloxinia, Impatiens, Linaria, Linum, Lycopersicum, Lythrum, three genera of the Malvaceae (Althaea, Lavatera, and Malva), Nicotiana, Oxalis, Pelargonium, Petunia, Phaseolus, Pisum, Primula, Salpiglossis, Solanum, and Zea. In addition, about a score of different natural hybrids have been examined, these being mostly arboreal types. And the bearing of the data collected on the problems of heterosis has never been forgotten.

The conclusion given in the preceding paragraph has been confirmed. Heterosis does increase as the genetic disparity of the parental stocks increases, on the average. Crosses between pedigreed inbred stocks exhibit diminishing heterosis as genetic relationship increases. Unrelated autogamous varieties show more heterosis when crossed than do related varieties. Crosses in heterogamous stocks show less heterosis than crosses in autogamous stocks. Increase in heterosis is noticed when heterogamous stocks have been successively selfed before crossing. This is particularly apparent in self-sterile species (certain Nicotianas, Lythrum, Oxalis) that can be inbred by special technique. Interspecific crosses show more heterosis than intraspecific crosses. This is true, however, only when the genic differences "nick," as they say in stock-breeding, and there is no interference with developmental processes; but if the genic differences fail to "nick," and there is disharmony, then dwarfs are obtained. In such cases, heterosis may be very marked in a whole series of interspecific crosses; yet other apparently similar combinations give dwarfs; and there are no intermediates between the two types. Of course, one can not say with certainty that the dwarf hybrids would have been vigorous hybrids had it not been for the failure of the genic combinations to work together harmoniously, since dwarfing may come about for other reasons (single genes are known to produce it); but this is the indication. In Nicotiana and Fragaria, two

genera with which I have worked intensively, hybrids between species belonging to different subgenera show more heterosis than hybrids between species belonging to the same subgenera; but the former also show more dwarf combinations than the latter. The end of the series, theoretically, should be intergeneric hybrids, since interfamilial hybrids have not been obtained; and there is some evidence that this is so. It is difficult to obtain intergeneric hybrids that are not abnormal; yet the greatest amount of heterosis ever noted is the Raphanus-Brassica combination.

On the other hand, our more recent experiences indicate that the earlier conclusion must be somewhat modified. Particular genes, or perhaps particular combinations of a small number of genes, have special effects. Different maize varieties, and different tobacco varieties, that are not particularly distinct genetically, as is shown by the behavior of a series of crosses among them, show markedly different degrees of heterosis when crossed with more diverse varieties or species. This experience appears to indicate that certain genes can exert greater effects than other genes, and also that a given gene can have greater effects in some combinations than in others.

Again, the effects of heterosis can not be compared in different genera. Each genus must be considered by itself. In other words, the genetic evidence indicates that certain generic groups are varying more than others, and in ways peculiar to the group concerned. This is not to say merely that one genus may show greater diversity than another, or even that one genus may show greater speciation than another. These are truisms; though it should not be assumed that speciation is a perfect measure of genetic diversity. What I really have in mind is that present-day genetic variability (that is, current or recent mutation rates) is very low in some groups and very high in others.

THE CHARACTER OF THE MANIFESTATIONS OF HETEROSIS

A second point, not so obvious as it may seem, is that heterosis is most aptly described by the old term *hybrid vigor*. In considering the various hybrids with which I have had to deal, a special effort has been made to determine just what characters are affected. Roots, stems, hairs, leaves, flowers, fruit, and seeds have been studied with care. As a result, it can be said that hybrid vigor is something which almost invariably concerns the plant—or the animal—as a whole. Its effect is comparable to the effect on a plant of the addition of a balanced fertilizer to the soil, or to feeding a more adequate and more chemically complete diet to the animal.

In plants the root system is increased, the branching is more profuse, the leaves are larger and more abundant; growth takes place faster—at least in the early stages— and often retains its pace longer before showing the characteristic sigmoid curve that indicates approaching maturity. I have

not studied the anatomical changes; but according to my colleague, Professor I. W. BAILEY, the wood of the very vigorous hybrid poplars (SCHREINER and STOUT 1934, SCHREINER 1935), when compared to that of their parents, shows just those changes that occur when tree species are grown under "good" rather than under "poor" conditions. Generally speaking, cell division is more noticeably influenced than cell size.

Hybrid vigor may be shown by an early maturity or by a late maturity. It all depends on the type of the genetic union. It is exhibited early. It is expecially marked in seedlings and the rosettes of species that form rosettes; but it is also apparent at late periods in the vegetative part of the life cycle. It is not marked in fruits, however, and is seldom noticeable in flowers I take it that this is partly because the general vegetative impetus has, in a sense, lost its force by the time the plant has reached sexual maturity. It also appears that the phenomena attending the reproductive process stand somewhat apart from ordinary vegetative growth. At the same time, it should be noted that *preparation* for reproduction is vegetative; hence, heterosis is frequently shown in the *profusion* of flowers and fruit.

In this connection I have studied the inheritance of a large number of characters which might be expected to show heterosis from *a priori* reasoning, but which do not show it. They are characters determined by a small number of genes and affecting particular organ systems. They do not, as far as can be judged, affect the general efficacy of the whole physiological machine. I will mention two examples,—leaf number on the main stem of the tobacco plant, and number of rows in the ear of maize. Varieties may sometimes be crossed which differ in the genes which determine these characters, as *AAbbCCdd* and *aaBBccDD*, thus giving a greater number of leaves, or of rows per ear, in the F_1 individuals than either parent possesed. But the segregation of the determinants is relatively simple. In no case have I found the great genetic complexity involved that is so obvious in heterosis, nor can the effect be described as one of increased general metabolic efficiency.

It is evident, then, that heterosis is a resultant exhibited by the organism as an entity and characterized, as CHILD might say, by a change in the metabolic gradient. This is what is shown so nicely by ASHBY'S logarithmic curves. Heterosis is ordinarily manifested by a rise in the "efficiency index," in the sense with which ASHBY used the term. And the unity of its expression might well lead a physiologist to think of it as a "unit character" with the notion which that expression conveyed in the early days of genetics.

These facts clearly teach that, in any given population, more *different* allelomorphs are present among the genes influencing the physiological efficiency than among the genes influencing the morphological pattern. Just why this should be the situation is less obvious. Naturally, the familial

generic, and specific characteristics of a genetic group are retained over long periods; hence, one may assume that the more conservative organ systems, presumably the older systems phylogenetically, are relatively less affected than others by the common run of gene mutations. It is also probable that the genes themselves vary in stability. There is evidence in favor of both propositions. I suggest, therefore, that the clearest and most reasonable interpretation of the facts lies in a combination of these two viewpoints based on the idea that gene mutation rates vary with function rather than with specific identity.

Basic changes in morphology, meristic variation, and even changes in certain ratios of parts (that is, shapes) occur relatively rarely; and when they do occur, the differences between the mutants and the wild types are found to depend upon a small number of genic changes. The changes involved in heterosis, though they are often insignificant phenotypically, are nearly always found to depend upon a large number of gene mutations; and they seem to be superimposed upon the first type. The first type appears to set the character of the reaction, while the second type sets the speed of the reaction. Heterosis, then, is largely concerned with changes in the speed of the various physiological reactions. And the genes controlling the speed of ontogenetic processes have higher mutation rates than the genes controlling the nature of these processes.

THE NATURE OF HETEROSIS

If heterosis is a phenomenon produced when numerous linked "dominant" genes are opposed to homologous "recessive" genes, and if these genes are mainly though not wholly those which influence general vigor, any further insight into the problem can come only from a clearer view of the manner in which these genes behave during development. We may assume that all genes are distributed by the same mechanism, but we must inquire as to whether all genes function similarly during ontogeny. The evidence indicates that genes may be divided into two main groups functioning differently, and that their behavior furnishes us with the solution of the problem. The first group causes breakdowns in physiological processes; the second does not. Naturally, the distinction between the two classes is not always clear cut. But most of the mutations used in linkage experiments may be placed in the first category without serious error. For example, in Drosophila, the lethals, the eye colors, the body colors, the bristle deficiencies, the wing simplifications, and all similar genic effects are to be included. In plants, chlorophyll deficiencies, deficiencies producing dwarfness of the whole or of a part, sexual deficiencies, organ simplifications, and most color changes are to be admitted, though the latter are ordinarily defective only for non-essential developmental processes. These mutations

all tend to be recessive to the "normal" or "wild" type, and it is not hard hard to see why. Most organisms are 2x-cylindered engines, so to speak, but can run on x-cylindered engines. Any defectiveness in one of the paired genes is compensated by normality in the other, and the processes in which these genes are involved go on as usual. The only easily imagined way in which a defect may appear to be dominant is when a defective gene impairs cytoplasmic properties to such an extent that there is a reflected effect on the activity of the normal gene; and such mutations would ordinarily be weeded out rapidly.

Genes of this defective type are the masked deleterious genes that are uncovered by inbreeding. In fact, inbreeding is often described as a process of purification, whereby a strain is purged of its pernicious elements. But I have been driven to conclude that the elimination of deleterious recessives is of little importance in practical breeding and of no consequence whatever in the solution of the problem being considered here.

A huge number of maize stocks have now been inbred through self-fertilization for periods of from ten to thirty years. The invariable experience is this: There is rapid decline in vigor during the early years, becoming less marked until, after approximately eight years, there is no further detectable decline. There is a similar unmasking and elimination of deleterious recessives which gradually diminishes and disappears; and there is segregation into differently characterized biotypes. *But these purified biotypes exhibit as great or greater manifestations of heterosis when combined after they no longer segregate defective recessives as they did earlier.* It follows that heterosis is not concerned with defective genes but rather with the different genic isomers of the physiologically active and more or less normal genes belonging to our second category. It also follows, since heterosis is almost omnipresent in crosses between inbred lines which have had virtually all defective genes eliminated, that non-defective genic isomers greatly outnumber the defective ones.

These conclusions are fairly obvious when one considers the matter. If *A* and *B* are normal genes and *a* and *b* are defectives, it is usually found that *Aa* or *Bb* individuals show a close approach to complete dominance. In other words, *Aa* and *Bb* individuals can not be separated from *AA* and *BB* individuals. How, then, are our so-called "dominants" and recessives to be opposed to each other by crossing, since we do not use *AAbb* and *aaBB* individuals as our pure strain components? No! Heterosis must be interpreted on the basis of the behavior of non-defective allelomorphs. The decline in vigor through successive self-fertilizations must be due to increase in homozygosis of genes belonging to this category. And JONES' hypothesis is a linkage hypothesis rather than a "dominance" hypothesis,

unless "dominance" and "recessiveness" in the classical sense are attributes of non-defective gene behavior.

Since one may say, in short, that the final analysis of the heterosis problem depends on the type of behavior exhibited by the genes affecting quantitative relationships, the question is mainly as to how this behavior is to be described. The literature on the subject is too voluminous to consider in detail here. I can only say that the universal experience is to find "dominance," in the classical sense, virtually absent. I have, in my own papers, presented analyses of numerous studies during a period of 27 years; and I will call attention only to an investigation of species hybrids (EAST 1935), where the behavior of the genes involved indicated that each is an active pattern former. This particular study could not be carried beyond the F_1 generation; but numerous other investigations of frequency distributions from intraspecific crosses show that a simple additive interaction of multiple factors is inconceivable (the first suggestion of this was probably in EAST 1913). RASMUSSON (1933), therefore, has proposed a gene interaction hypothesis to account for the facts actually observed. He assumes that "the effect of each factor on the genotype is dependent upon all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction." Thus A and B, acting alone, may each have an effect equal to 1; but $A+B$ have an effect less than 2. Now, it is unnecessary to accept RASMUSSON'S hypothesis precisely as he has presented it. It may need some modification.[1] But it is safe to say that all plant breeders agree that a non-arithmetical accumulative hypothesis is required. On this basis I wish to make a suggestion that enables us to visualize the heterosis situation with considerable clarity. The suggestion is simply this: *The cumulative action of the non-defective allelomorphs of a given gene approaches the strictly additive as they diverge from each other in function.* It is impossible, at the present time, to give rigorous proof of this theorem. All I can say is that a long experience with this type of gene leads me to believe that it is a close approximation of the truth.

[1] Rasmusson was impressed, as all plant breeders have been, by the fact that many more different genes are present in the average population of plants than is usually assumed. This point is undeniable. Similarly, it can not be denied that most observations on quantitative gene action are uninterpretable on the basis of simple arithmetical increments. Such interpretations have been used, but only as diagrammatic representations. I have used them myself in several early papers, but I was careful to point out, in various places, that this was a simplified representation used only for ease of presentation. There may be gene action at times similar to that suggested by Rasmusson. There is also gene action where the presence of a basic gene is necessary for the expression of modifiers. But in growth phenomena, one is dealing with ratios, not simple additions. It is likely, therefore, that many first approximations, in dealing with quantitative inheritance, must be interpreted on a simple logarithmic basis or by means of higher geometric series. It is not improbable, however, that series of genes show diminishing returns over what they would otherwise be expected to produce in a logarithmic or other geometric basis.

The two types of genes may be assumed to behave as follows. If A_1 is a normal gene and a differs from it only by some degree of defectiveness in the process that A has been capable of performing, then the capacity of a for performing the function of A_1 approaches zero. Yet A_1 in combination with a performs its full function, and A_1a and A_1A_1 individuals are indistinguishable. If, on the other hand, A_1 takes on a series of isomeric conditions $A_2 \ldots A_4$, each having positive active functions diverging further and further from those of A_1, then the combination A_1A_4 may be supposed to have greater physiological efficiency, provided the various functions are harmonious, than A_1A_2. Quantitatively the action of A_1a is virtually A_1+o; while the action of A_1A_1 is not $2A_1$ but $2A_1-\alpha$ and α is virtually equivalent to A. Similarly, the action of A_1A_2 is $A_1+A_2-\beta$, that of A_1A_3 is $A_1+A_3-\gamma$, and that of A_1A_4 is $A_1+A_4-\delta$; and $\alpha>\beta>\gamma>\delta$.

Actually, many critical data are available only on comparisons between A_1+defective a and A_1+the identical allelomorph A_1. But since the frequency distributions where genes at different loci accumulate quantitative effects sometimes demand a diminishing returns interpretation, it is plausible to postulate a similar activity for non-defective allelomorphs at a single locus. Moreover, I have had several populations in my studies of size inheritance where very few gene differences at separate loci seemed to be involved; and the actual segregations obtained appeared to be more easily explained if segregation of pattern effects such as $A_1A_4B_1B_4$ (though probably not so simple) were assumed. There is marked positive skewness to the distributions; yet it appears to be impossible to fix the characteristics of the extreme positive variants, and the difficulty of fixing any positive variants is much greater than that of fixing minus variants. This last statement may seem to contradict an earlier one to the effect that heterosis possibly is fixable in diploids in rare cases. I believe that the two cases are not wholly incompatible but depend upon the type of the allelomorphs involved.

SUMMARY

1. Various lines of evidence are cited which show that ASHBY'S physiological theory of heterosis is unsound in all its essential features.

2. The experimental evidence on amphidiploids is examined for its bearing on heterosis. The data show that heterosis is gene-controlled, since amphidiploids breed true (with certain explainable exceptions) to the vigorous conditions exhibited by the original hybrids. The facts are compatible with a theory of linked gene inheritance; and this is considered to be the fundamental basis of JONES' theory, rather than the dominance idea by which it is usually identified.

3. Reasons are given for thinking that autopolyploidy has been a more

important factor and allopolyploidy a less important factor than is generally assumed in the origin of natural polyploid series.

4. *Gigas* types are found not to characterize all polyploids. They are rare in monocotyledonous species. It is suggested that they occur most frequently when the epidermal tissues are soft.

5. Experimental studies on hybrids in 37 genera, in addition to examination of various natural hybrids, have confirmed an earlier conclusion that heterosis increases with genetic disparity between the parents. It is found that manifestations of heterosis are different in type and extent in different genera, indicating that current mutation rates in certain groups are much greater than in others.

6. Observations on the characters affected under manifestations of hybrid vigor show that heterosis concerns the plant as a whole. ASHBY'S "efficiency index" leads to the same conclusion. It follows that physiological efficiency is governed by numerous genes that have mutated frequently. Structural variations, on the other hand, are less common, though more noticeable, and involve fewer gene differences. It is suggested that structural variations and physiological efficiency variations belong to different categories. The first type is concerned with reaction character, the second with reaction speed. Heterosis is largely a matter of reaction speed.

7. Inbreeding commonly results in a diminution in vigor and an unmasking of deleterious recessives. These recessives are mostly defective gene mutations. Crosses between the "purged" inbred strains manifest a high degree of heterosis if the parental stocks are not too closely related. It follows that the presence of defective genes hidden by normal dominants has little to do with heterosis. It also follows that normal, positive, active genic allelomorphs are much more common than defective genic allelomorphs.

8. A consideration of the known behavior of normal and of defective allelomorphs, such as A and a, also shows that such combinations can not be effective in producing heterosis, for A is usually completely dominant to a. The effect of AA is not substantially greater, therefore, than that of Aa. Thus heterosis must be interpreted through the behavior of "normal" allelomorphic series. The key to heterosis is the inheritance of quantitative characters.

9. Since all experiments on quantitative characters have shown that "dominance," in the classical sense, is virtually absent, and that each gene of a bivalent combination actively affects the end result, a theory is suggested for explaining heterosis which combines the essential features of JONES' hypothesis, of RASMUSSON'S hypothesis concerning the behavior of genes influencing quantitative characters, and of the earlier idea that heterozygosis *per se* is effective.

If numerous non-defective allelomorphs are common in any given species heterosis can be accounted for as follows. The effect of A_1A_1 is not $2A_1$ but $2A_1-\alpha$ where the value of α approaches the value of A_1. But if $A_1 \ldots A_4$ is a series of non-defective allelomorphs of A_1, where their functions depart from those of A_1 on an ascending scale, then the resultant effects may be visualized as A_1A_2 equaling $A_1+A_2-\beta$, A_1A_3 equaling $A_1+A_3-\gamma$, and A_1A_4 equaling $A+A-\delta$, where $\alpha>\beta>\gamma>\delta$.

This theory appears to be compatible with the known facts, especially those accumulated in experiments on the inheritance of quantitative characters.

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