THE GENETIC BASIS OF HETEROSIS IN MAIZE

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THE literature on heterosis and the reduction of vigor after inbreeding has been reviewed recently from all angles by several authors (SPRAGUE 1946, RICHEY 1946, WHALEY 1944) and thus it is not necessary to enter into a detailed discussion, especially since I shall deal exclusively with the genetical aspect of the problem.

Up to now the phenomenon of heterosis in corn has been explained mainly by one of two alternatives: the hypothesis of dominance and that of heterozygosis. But in spite of continued discussion extending over a period of almost forty years, no conclusive evidence has been presented in favor of one or the other of these mendelian hypotheses.

The dominance hypothesis, first proposed apparently by BRUCE (1910)[2] and by KEEBLE and PELLEW (1910), postulates that the increase of vigor after crossing results from the combination of different dominant alleles, contributed by each parent. On the other hand, inbreeding again produces homozygosis for recessive alleles, causing a loss of vigor.

The heterozygosis theory, first formulated by SHULL (1911a, b,) and EAST and HAYES (1912) in rather vague physiological language, has been put in terms of modern genetic terminology by EAST (1936). According to this concept different alleles, when combined in heterozygotes, exert a complementary physiological action, resulting in an increased vigor limited to such heterozygotes. HULL'S (1945, 1946, 1948) concept of overdominance seems, from the theoretical point of view, to be essentially the same as that of heterotic gene interaction.

As additional hypotheses we might mention other suggestions. BRIEGER (1930) pointed out that the nature of the inter-allelic interaction may be of the nature of dominant complementary factors. RASMUSSON (1933) made detailed references to the nature of quantitative gene interaction in producing heterosis.

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[2] There has been evidently some doubt about the priority in proposing the dominance hypothesis, and RICHEY (1945), reproducing BRUCE'S original paper, points out that undoubtedly priority should be given to him. However, when reading carefully BRUCE'S paper, it becomes quite evident that he missed the essential point of the dominance hypothesis, namely the covering up, in hybrids, of all or most recessive genes by their respective dominant alleles, contributed in part by one or by the other parent. The formulae given refer to a hybrid population obtained by crossing two parent populations which contain identical genes in different proportions, and it is shown that the mean frequency of heterozygotes in the hybrid population is inferior to the combined mean of the two parent populations. It would however be required that the hybrid population contains less homozygous recessives than either parent population individually, if we want to explain heterosis.

There cannot be any doubt that both of the two main hypotheses furnish satisfactory explanations of a large number of the facts. In order to overcome certain difficulties, JONES (1917) formulated his hypothesis of linked dominant factors, assuming that on the whole, dominant stimulating genes should be closely linked with recessive vigor-reducing factors. As a practical matter, this hypothesis occupies a somewhat intermediate position between the dominance and heterozygosis theories. If linkage between dominant and recessive genes became so close that practically no crossing over occurred, it would be difficult to distinguish experimentally between the linkage and the heterozygosis theory. We would be dealing simply with heterozygosis of chromosome regions, instead of that of simple genes. If on the other hand, linkage were rather weak, the difference between the simple dominance and the linkage hypothesis would practically disappear.

The main arguments mentioned in the literature against the dominance hypothesis are the following two:

a) A segregation of a simple system of dominant vigor producing factors should theoretically result in an asymmetrical segregation in the first inbred generation and this is not in accordance with the facts observed. COLLINS (1921) already pointed out that this asymmetry becomes practically obliterated if the number of segregating factor pairs is large and we may also refer to some curves given by BRIEGER (1930) which were calculated under the assumption that non-allelic interaction is simply cumulative, omitting the effect of phenotypic variability. With some 20 factor pairs in segregation, it would be practically impossible to verify statistically the asymmetrical nature of the distribution.

b) The second argument seems to me more serious. It is generally assumed under the dominance hypothesis that individuals exist, completely homozygous dominant for all loci, but that they are so rare that they cannot be discovered. If we start from an individual heterozygote for N loci, it is evident that in the nth selfed generation, without selection, there should be only $(\frac{1}{2})^n$ homozygous dominant descendants. If we attribute sufficiently high values to n, we can keep the value of this frequency so low that the absence of the homozygous dominants may appear fully explained. However, we have actually started from a wrong assumption. We should start from a completely homozygous dominant individual and ask, whether it is possible that a number of recessive alleles may be accumulated during phylogenetic development, sufficient to explain the appearance of highly heterozygous individuals and the complete elimination of the original homozygous dominant type. As I shall explain later in this paper, it seems impossible to obtain this result.

The main reason for the reluctance to accept the heterozygosis hypothesis seems to have been the scarcity of recognized cases of such a type of interaction, while today a considerable number of cases have been described. We may cite in the first place several examples of plants where the flower color in monofactorial heterozygotes is more intense than in either parent: *Silene Armeria*, pink×white gives red (CORRENS 1920), *Phlox Drummondi*, any cross between pink, rose or salmon gives scarlet, any shade of red crossed to blue gives purple, etc. (KOBAL unpublished). The F_2 segregation gives in these cases a simple 1:2:1 ratio. WRIGHT (1947) described a case in the guinea pig where a monofactorial heterozygote had a deeper brown coat color than either homozygote. DUNN and CASPARI (1945) found that heterozygotes for the alleles t^0 and t^1 are normal, while t^0t^0 and t^1t^1 homozygotes are lethal genotypes. KERR (1948) explained the fertility of the queens in Melipona as being due to their heterozygosis for two or for three pairs of factors, the number of fac-

tors varying in different species. STERN and his collaborators (1943, 1946, 1948a, b) described cases in *Drosophila melanogaster*, where heterozygotes in the series of position alleles at the cubitus interuptus locus show a higher degree of abnormality in wing venation than either homozygotes or hemizygotes.

Several cases are known of monofactorial and even of bifactorial segregations where the heterozygotes have a higher survival value than either homozygotes: barley (GUSTAFSON 1946, 1947), Antirrhinum (STUBBE and PIRSCHLE 1940), and we may include also here the cases reported by SINGLETON (1943a, b) and JONES (1944, 1945) though the latter cases have not been carefully analyzed.

Finally, a number of cases are now known in Drosophila where monofactorial heterozygotes (L'HERITIER and TEISSIER 1933, 1934, 1937a, b; TEISSIER 1942, 1944, 1947a, b; KALMUS 1945) or heterozygous inversions (Dobzhansky 1943 to 1947, WRIGHT and DOBZHANSKY 1946) have a higher selective value than either homozygotes.

It is only very recently that the question of heterosis has been discussed from the point of view of population genetics. Simultaneously CROW and BRIEGER presented papers to the Genetics Society of America and the Societade Brasileira Biologica in 1947 (CROW 1948, BRIEGER 1948a and b). As we shall see later, both the discussions lend support to the heterozygosis hypothesis.

The behavior of individual maize plants on crossing or selfing is in general agreement with either the dominance or heterozygosis hypothesis. But results that I obtained when studying indigenous South American maize which have been cultivated for a very long time without the aid of modern genetical methods, led me to study the evolutionary origin of heterotic gene systems, under the effects of natural or artificial selection. The result of this study was a new approach to the heterosis problem, which in turn led to a new breeding procedure that has already been used in the production of the new Brazilian sweet corn (BRIEGER 1948b).

The evolutionary or phylogenetic aspect of heterosis seems to have been largely overlooked. COLLINS (1918) postulating that corn was derived from a hybrid between Euchlaena and some wild Andropogoneae, assumed that the latter already possessed the heterotic gene system. In considering the extreme reduction in vigor following selfing of maize BRIEGER (1944) suggested that it might be explained by recessive lethals having become established as balanced lethals in an original interspecific hybrid, owing to a mutual incompatability of these genes of either species when homozygous.

The main object of the present paper is twofold. First, to determine the method by which a genic system responsible for heterosis could arise by normal evolutionary processes, and secondly, to decide whether or not the reduction of vigor following inbreeding is in accord with known principles of population genetics.

DEFINITION OF THE TERM HETEROSIS

Before entering into a more detailed discussion it is necessary to define "heterosis." When SHULL introduced it in a lecture given in 1914, but pub-

lished for the first time in 1922, his intention was to follow JOHANNSEN'S method when the latter defined the gene, that is: to propose a term for the phenomenon of hybrid vigor free from any previous theoretical concept (1948).

To begin with, we may state that heterosis is generally considered to be present when the mean of any character or characters in a hybrid exceeds the mean of its descendants obtained by any system of close inbreeding.

From a purely formal point of view, we may expect that the transgression of the mean of heterozygotes, beyond the range of the means of homozygotes, may be in either direction, that is, in what we may call a positive or negative direction. But at least for the time being it seems to me unnecessary to distinguish between positive and negative heterosis. From a biological point of view, it is immaterial where we locate the zero point and which direction we choose to call positive or negative. The cases reported by STERN and his collaborators (1943, 1946, 1948a, b) may be considered examples of negative heterosis since heterozygotes are less normal or, if we prefer to say, more abnormal than the homozygotes. In selecting for height of plants in maize, we may take our measurements of height with the zero point at the basis of the plant or with the zero point at a value which corresponds to the mean height of the plant. If we want to obtain smaller plants, as is frequently the case in South American varieties, we may indicate deviations in the undesirable direction, that is, an actual increase of height, by a negative sign, while decrease in height in the desired direction would be considered as a positive result and thus characterized by a positive sign.

In order to avoid the impression that there are two fundamentally different kinds of heterosis, a positive and a negative one, one should select the scale of measurements in such a way that the transgression of the mean of heterozygotes appears positive. However, a blind application of this principle may lead to absurd conclusions, for instance, the increased vigor of many interspecific hybrids may be justly considered as positive heterosis, but a reduction of vigor or fertility which also occurs in interspecific crosses quite frequently is an entirely different problem and cannot be considered as "negative heterosis."

There are some additional considerations which we have to make:

A) Heterosis must refer to the comparison between characters of hybrids and their descendants, obtained through inbreeding.

Since the phenotype of hybrids depends largely upon the complicated interaction between alleles at the same or at different loci, the presence of heterotic gene action in hybrids may frequently become quite obscure. If we cross two inbred lines of different varieties, the effect of heterosis may become much more pronounced than the result of gene interaction in general, but if instead, we use two hybrids between inbred lines of either variety for obtaining the varietal hybrid, then frequently, the latter will be no more vigorous than its parents, which were themselves hybrids. In other words, the mean values for quantitative characters of an intervarietal hybrid may not be superior to the means of the corresponding intravarietal hybrids, but they will show the presence of heterosis when compared with the means of inbred

lines. It is thus evident that the comparison between hybrids and their parents will not always yield a reliable criterion on which to base a definition of heterosis.

If, however, we compare hybrids with their offspring a reduction in vigor will always be observed whenever the hybrids were heterotic. This reduction in vigor is generally quite pronounced in the first inbred generation of intravarietal crosses. In intervarietal crosses, ordinary mendelian segregation for quantitative differences may obscure the reduction in vigor in the first or the first two generations, but it will always appear in subsequent generations.

Thus we may formulate the following definition: Heterosis is characterized by an increase of the mean value for quantitative character differences, when comparing hybrid means with those of their offspring obtained by selfing or any other method resulting in close inbreeding. Heterosis may also cause an increase of means of hybrids over their parents. The main and most important feature of heterosis is the impossibility of maintaining hybrid vigor in the offspring obtained by any kind of inbreeding.

B) Heterosis does not affect the individuals as a whole, but the expression of each character, as a rule, independently of that of other characters. Thus it is not correct to expect a plant on the whole to be heterotic.

Heterosis in maize affects mainly the following characters: plant height, position of the ear, size of leaves, intensity of chlorophyll formation, size and strength of root system, resistance to disease, pests and unfavorable conditions, size and number of kernels, width and length of ear, size and branching of tassel, and amount of pollen shed. Inbred plants may grow somewhat slower than the corresponding hybrids, but earliness and lateness is an ordinary quantitative and not heterotic character, with earliness being completely or partially dominant. Row number in the ear is not affected by heterosis, heterozygotes having in general an intermediate row number. Plant and kernel color show a typical mendelian qualitative or quantitative behavior. The degree of heterosis may be quite different in individual cases. For instance, South American indigenous corn shows generally a very pronounced reduction in ear size of the first inbred generation, while the plant height may not be affected to the same degree.

Recently there has been a tendency to use the terms heterosis and high adaptive value as identical. The relative adaptive value of any character depends evidently upon the type of selection applied. Thus a new criterion is introduced which in itself is independent of the nature of the heterotic character itself. For instance, plant height is a typical heterotic character, using the ordinary definition. If maize has to compete in badly cultivated fields with weeds and the brush coming up from roots of burned forest trees, then an excessive height of the plants will represent a high adaptive value. But if we keep the corn fields clean, the excessive plant height, with ears far from the ground, may produce an extreme degree of lodging, and thus would be an undesirable character. Taking the degree of adaptation to natural or artificial conditions as a decisive criterion, the second case could no more be called heterotic. We may cite another example: the Indians in the Choco, a region

of Colombia, plant their maize broadcast, and under such conditions plants which are too vigorous would tend to be eliminated. But under ordinary conditions of a mixed maize-bean planting, vigorous stalks which can support the weight of the bean vines, are selected. Thus we see that an ordinary heterotic character such as plant height will sometimes have a positive and sometimes a negative adaptive value.

In conclusion, I may thus say that it seems to be best to avoid any subjective criteria and to consider heterosis to be present in those cases in which any quantitative character shows a higher mean in the hybrid generation than in any of the subsequent generations, obtained by any form of close inbreeding.

PRINCIPLES OF POPULATION GENETICS, SELECTION AND MUTATION

In a population which contains the three genotypes AA, Aa and aa, their relative frequency will depend mainly upon three factors: a) their constancy, that is, the rate of mutation, b) the reproductive system, whether random mating, preferential mating or selfing, and c) the relative survival values of the three genotypes. First we shall discuss the latter in some detail.

In order to obtain index values for fitness we must accept one of these three genotypes as a standard with a survival value as unity. We find in the literature numerous notations. Thus WRIGHT (1931) sometimes uses the letter s to denote the selective value of a recessive lethal or a partially lethal mutant, and $h \cdot s$ for the selective value of intermediate heterozygotes. In the case of heterosis he uses the letters s and t for the selective values of both homozygotes (1931, 1947). The standard value is represented in the first two cases by the viability and fertility of the homozygous dominant individuals and in the third case by that of the heterozygote. The corresponding survival values will then be $(1-s)$, $(1-h \cdot s)$, etc. HALDANE used a similar procedure (1926) for heterotic cases, using however different letters of the alphabet. I shall use here, as in some previous publications, still other letters, in order to avoid confusion since there are some apparent differences in the biological definition given by the authors mentioned. If we admit that survival indexes may take any positive value and may be larger or smaller than unity, then there is no need to use special letters for individual genetical cases. I proposed to use (1948a) uniformly the letter R to denote the individuals or gametes remaining free to function in a population after all selective processes have had played their part, accepting as standard the survival value of the monofactorial heterozygotes. The survival values of the homozygotes are calculated as indexes in reference to this standard or unit value. If the heterozygote should be less viable than one of the homozygotes, the survival values of the latter would be larger than one, and if there should be heterosis for survival values, the indexes for both homozygotes will be smaller than one.

The survival values, as just defined, may actually be determined in special experiments. Suppose we plant equal numbers of individuals of all three genotypes and are able to count the total number of gametes produced by all surviving individuals of each genotype, we may obtain numbers which we may indicate by the letters x, y and z. Dividing the triple proportion of genotypes

after selection by the value y, we obtain a mathematical definition of survival values.

Initial phase:

$$1\,(AA):1\,(Aa):1\,(aa).$$

After all selective processes:

$$x\,(AA):y(Aa):z(aa)$$

or finally

$$\left\{\frac{x}{y} = R_A(AA)\right\}:\left\{\frac{y}{y} = 1(Aa)\right\}:\left\{\frac{z}{y} = R_a(aa)\right\}.$$

If the survival indexes of the two homozygotes are different, we shall use the capital letter A as a subscript to denote the higher index and the letter a to denote the lower of the two indexes.

These total survival indexes R are compound values, if studied biologically in some detail. If we take the whole vegetative phase, only a certain fraction of individuals will reach reproductive age, and this fraction may be denoted by the symbols R_{AV} and R_{aV}. Furthermore only part of the gametes of the surviving plants may function and we shall denote the remainder, after reproduction selection by the letters R_{AR} and R_{aR}. Mathematically these two partial survival values are related by a very simple equation:

$$R_A = R_{AV} \cdot R_{AR};\ R = R_{aV} \cdot R_{aR} \cdots \qquad (1)$$

The partial survival values may be still further subdivided, if necessary. We may, for instance, determine successively the frequency or the percentage of seeds with good embryos, of good seeds which germinate, of plants surviving seedling stage and of plants reaching flowering stage, etc. If we design these successive fractions using numbers as subscripts, we obtain the following subdivision of vegetative survival indexes:

$$R_{V1} \cdot R_{V2} \cdot R_{V3} \cdot R_{V4} \cdots R_{Vn} = R_V.$$

Furthermore, we may determine the causes of elimination at each of the above mentioned stages, for instance, how many seedlings are not albinos, but green, how many of the green seedlings are dwarfs, etc. Using letters as subscripts, it is easy to see that these partial survival values are additive:

$$R_{V2a} + R_{V2b} + \cdots = R_{V2}.$$

In order to give a better idea of the importance of the subdivision of the total survival indexes we shall give some special cases. For instance, it is quite common that inbred lines of maize show a reduction in the percentage of germinating kernels, and that ears and tassels and the number of ovules and of pollen grains is considerably reduced. A vegetative survival value of .75 caused by imperfect germination, and a reproductive survival index of .5 in consequence of the reduction in size of ear and tassel, could not be considered as very low, but when combined, they result in a rather low total survival

index: $.75 \times .5 = .375$. In plants such as Capsicum inbreeding results also in a loss of vigor and here we may readily obtain a percentage of germination of about .7, a slight reduction of the flower formation of the order of .7 and a further reduction in the number of seeds in the fruits formed of about .6, always compared with the same characters in hybrids. Thus, we obtain a low total survival value of $.7 \times .7 \times .6 = .294$.

In numerous discussions on the effect of selection, very low selective values and thus very high survival values have been used when calculating demonstrative curves, and the corresponding survival values were actually not very different from one. The two examples given above show that even when the individual partial survival values are quite high, the total survival value turns out to be rather low.

Next we shall briefly discuss *mutation rate*. Though there are few data at our disposal about the actual mutation rate in maize at different loci, a rate of the order of 10^{-4} to 10^{-6}, with a most probable average of 10^{-5}, seems to be acceptable as a preliminary estimate.

The number of mutable loci is another point to which we shall refer repeatedly. Though there may be a total of the order of 5,000 loci present in maize, it seems to me dubious if we can follow CROW (1948) and suppose that all of them may mutate to one type of allele, i.e., to recessive lethals or subviables. We may accept as a general rule that new mutant genes are recessive in their most conspicuous phenotypic effect. We cannot, however, expect that all mutations will simply reduce survival.

The different phenotypic effects which may cause a loss of vigor can be attributed to subviable mutant genes, such as deficient seed formation, deficient germination, deficiency in chlorophyll formation, etc. Thus we can subdivide the total of subviable mutants into groups. There are hardly more than a few hundred loci in maize responsible for any one of these different ontogenetic phases.

With regard to the *reproductive system* we only have to distinguish in the present publication between the most extreme possibilities. A population of maize plants may be considered as a random-mating breeding unit, where the pollen of any plant has equal chances to reach the silks of any individual in the population, including that of the same plant. Actually this assumption is not quite correct for two reasons: The chances for natural self-pollination are somewhat reduced. That is, there is less chance for self-pollination than for cross-pollination because there is frequently a slight difference in flowering time between tassel and ear of one individual and furthermore the pollen rarely falls in a vertical direction. Also the chances of cross-pollination are not strictly random and decrease with increasing distance between plants. But for the present discussion both these limitations may be disregarded. In a lot of several thousand plants, the chance of self-pollination is insignificantly low anyway, and the effects of the variation in distance between plants will be without importance if all different genotypes in a field of maize are distributed at random.

Self-pollination on the other hand must be carried out artificially in maize

and this selfing can be done in such a way that we avoid all experimental errors which may lead to contamination and to crossing which would result in an incomplete system of inbreeding.

POPULATION EQUILIBRIUM IN A RANDOM MATING POPULATION

The formula for determining the proportion of two alleles at equilibrium in a population can be derived by a simple process. The gametic frequencies at equilibrium in generation n must be the same as that in the following generation $(n+1)$, in spite of the occurrence of new mutations and of all selective processes. Let us assume that the final frequency of two alleles A and a in generation n was equal to p and q respectively. Following the HARDY-WEINBERG law we should have in the next generation the following proportion of phenotypes:

$$p^2(AA) + 2pq(Aa) + q^2(aa).$$

After all selective processes have played their part again, and without the occurrence of any mutation this sum reduces to:

$$p^2 \cdot R_A(AA) + 2pq(Aa) + q^2 \cdot R_a(aa).$$

Furthermore if a mutation occurs from A to a at the rate u, and without any selection, the original HARDY-WEINBERG formula suffers the following alteration:

$$(p - pu)^2(AA) + 2(p - pu)(q + qu)(Aa) + (q + pu)^2(aa).$$

On the whole we are justified not to consider the occurrence of recurrent mutations from a to A, its frequency v being considerably smaller than u. If however we want to include this phenomenon in the formula, we must add the term qv to the gametic frequency of p and subtract it from that of q.

If we want to combine both processes, mutation and selection, we encounter certain difficulties. If a mutation occurs in a homozygote (AA), giving rise to a heterotic heterozygote (Aa), this would cause an alteration of survival value. The homozygote underwent only part of the total selection before undergoing mutation, and thus we should use in the formula for those homozygotes, which will mutate eventually, a partial survival value R_A' which is larger than R_A. On the other hand, if a mutation should occur in a heterozygote (Aa) resulting in the appearance of a homozygote (aa), this in turn would have again an effect on survival. The new homozygote will have again a partial survival value larger than the usual survival value R_a of homozygotes (aa). We may, however, assume that on the whole both these partial survival values will be so near to the value one, that we may disregard them.

Thus, combining the two formulas given above, and remembering that after meiosis the heterozygotes segregate into equal proportions of gametes (A) and (a), we may write the formula at equilibrium, with selection and mutation:

$$\frac{p}{q} = \frac{p^2R_A + pq - p \cdot u}{q^2R_a + pq + p \cdot u}. \qquad (2)$$

By simple algebraic transformation this proportion reduces to:

$$\frac{p}{q} = \frac{(1 - R_a) - u/q^2}{(1 - R_A)} \tag{3a}$$

or, if we prefer, we may write also:

$$u = (1 - R_a) \cdot q^2 - (1 - R_A) \cdot q \cdot (1 - q). \tag{3b}$$

We may use these formulas to decide whether the dominance hypothesis or the hypothesis of a heterotic gene interaction is correct. It is generally agreed that the heterotic mechanism in maize has been established long ago. Thus we must assume that such mutations, either to recessive genes, which reduce viability and which we may call subviables, or to heterotic genes, have been slowly accumulated and have reached a population equilibrium, governed by the formulas given above. It it should be shown that such a phylogenetic accumulation is impossible for certain assumed values of R, then the hypothesis used in estimating these values of R must be rejected.

We shall start with a discussion of the *dominance hypothesis*. Here the survival value of the homozygous dominant individuals (R_A) is equal to that of the heterozygotes (*Aa*), both being unity. The homozygous recessive subviable mutants will have a survival value R_a, smaller than 1. Under these conditions the second term in formulae (3b) becomes equal to zero and vanishes, and we come actually to HALDANE'S formula for recessive subviable mutants:

$$u = (1 - R_a) \cdot q^2 \quad \text{or} \quad q = \sqrt{\frac{u}{(1 - R_a)}}. \tag{4}$$

The limiting extreme values are:

Recessive lethal mutants:	Recessive neutral mutants:
R_a = zero	R_a = unity
$q = \sqrt{u}$	q = infinite
$p = 1 - q = 1 - \sqrt{u}$	p = zero

The frequency of the different homozygous and heterozygous genotypes per locus in a population at equilibrium can be easily determined, and the mean frequency for any number of mutable loci can be obtained by multiplying the individual frequencies per locus by the number of loci involved. Table 1 gives the individual frequency of monofactorial recessives and heterozygotes, for a series of mutation rates for four different types of subviable mutant genes.

The next step must consist in establishing limiting conditions which enable us to decide whether the dominance hypothesis is in accord with the facts known. We can establish the following requirements: (a) no homozygous dominants should occur in the balanced random mating population; (b) the inevitable reduction of vigor after selfing requires that every individual of the

population should be heterozygous for at least one or more loci; (c) the absence of an asymmetrical distribution requires that every individual of the population is heterozygous for numerous loci.

All these requirements will be fulfilled if we can be sure that any individual obtained by selfing will contain at least one or more subviable mutant genes in the homozygous recessive condition. Thus we have to solve the question: which Poisson series does not reach, within reasonable probability limits, the value zero. The answer is a series with mean 7 where 99 percent of the

TABLE 1

Frequencies of recessive alleles and of heterozygotes at different mutation rates for four different types of subviable mutant genes of one locus.

MUTATION RATE	GAMETIC FREQUENCY OF REC. ALLELE	ZYGOTIC. FREQUENCY OF HETEROZYGOTE
	$R_a = .9$	
10^{-4}	.0316	.0612
10^{-5}	.0100	.0198
10^{-6}	.0032	.0063
	$R_a = .75$	
10^{-4}	.0200	.0392
10^{-5}	.0063	.0125
10^{-6}	.0020	.0040
	$R_a = .5$	
10^{-4}	.0141	.0280
10^{-5}	.0040	.0080
10^{-6}	.0014	.0028
	$R_a = 0$	
10^{-4}	.0100	.0188
10^{-5}	.0032	.0063
10^{-6}	.0010	.0020

total distribution lies between the limits 1 and 15. Thus we must require that any plant of the original population is heterozygous for at least 4 times 7 or 28 loci.

This requirement leads us to the next argument: We must now find the mean of another Poisson series which does not go below the value 28 within reasonable probability limits.

Next we must decide what we should accept as such a probability limit. I am using in the first part of the discussion the limits of 10^{-2} and 10^{-4}. Any genotype expected with frequencies higher than these limits is either very likely or at least reasonably probable to occur. Before stating final conclusions, however, these limits must be defined in a still more rigorous way. Everybody agrees that no homozygous dominant individual of maize has yet been found, and it is thus an essential question to estimate how rare such individuals must be in order to have escaped notice—if they should really exist.

Personally I have probably seen, in my experiments, offspring from only about 7,000 selfed individuals. If one allows similar or higher figures for every geneticist or breeder of the last 30 years, the total number of selfed offspring studied would correspond to more than a few hundred thousand selfed individuals. Thus, I think, we are justified in stating that any constitution expected with a frequency of more than once in a million had a good chance to come under observation. We should further add to the evidence studies on the indigenous corn, though it is hard to make a safe estimate as to numbers. I have studied during the last 13 years about 1,000 families from indigenous selfed material without finding any homozygous dominants.

The argument is thus divided into parts: first using the lower and preliminary limits and then passing to the high limit of probability.

I shall consider three types of mutants: Lethals, medium subviable mutants with R = .5 (s = .5), and weak subviable mutants with R equal to .9 (s equal .1) (table 2) and a mutation rate of 10^{-5}.

TABLE 2

TOTAL OF MUTABLE LOCI	NUMBER OF HETEROZYGOUS LOCI PER INDIVIDUAL AT EQUILIBRIUM		
	MEAN	EXTREMES AT 1/100 PROBABILITY LEVEL	EXTREMES AT 1/10,000 PROBABILITY LIMIT
	Lethal mutants: R=0		
1,000	6.3	1 — 13	0 — 15
3,000	18.6	8 — 28	4 — 36
5,000	31.5	18 — 45	12 — 55
	Subviable Mutants: R=.5		
1,000	9	2 — 17	0 — 23
3,000	27	14 — 40	9 — 49
5,000	45	25 — 68	21 — 73
	Subviable Mutants: R=.9		
1,000	20	8 — 28	4 — 35
2,000	40	25 — 56	18 — 67
2,500	50	33 — 68	25 — 79
3,000	59	41 — 79	32 — 96

From table 2 we can conclude that considerably more than 5,000 loci, mutable to subviable genes with survival index R equal or smaller than .5, are necessary if we want to reach a sufficient degree of heterozygosis of at least 28 heterozygous loci.

We might have reached the same conclusion in a shorter way, but it may have been interesting for those, not familiar with Poisson series, to have first individual limits. But we may have simply asked what are the means for Poisson distributions which do just reach, at different probability levels, the value 28, and calculate then the total number of loci which correspond to these Poisson means (table 3).

TABLE 3

PROBABILITY LEVEL	MEAN OF POISSON SERIES	CORRESPONDING TOTAL NUMBER OF MUTABLE LOCI		
		$R_a = .9$	$R_a = .8$	$R_a = .7$
10^{-6}	63	3.150	4.500	5.530
10^{-5}	57	2.850	4.070	5.000
10^{-4}	54	2.700	3.860	4.740
10^{-3}	49	2.450	3.500	4.300
10^{-2}	42	2.100	3.000	3.680

Thus we require from 3,000 to 5,000 loci changing, with a mutation ratio of 10^{-5}, to recessive, with survival index above .7, and more loci still if the survival index should be below .7, in order to fulfill our minimum requirement or with other words, we need almost all loci present.

I do not think we can dispose of all loci in this way. We know that a considerable number of loci yield mutants with much smaller survival values (defective seeds, albino and luteus seedlings, barren stalk, silkless, etc.). Furthermore we know that any maize plant contains a large number of modifier genes with no appreciable effect on survival values, as can be shown easily by selection experiments for shifting phenotypic expressions of any character. I stated above that heterosis is a complex phenomenon, forcing us to distinguish and to treat to some extent independently the heterosis in mean plant height, in mean ear size, in mean kernel weight, etc. There are evidently not enough mutable loci in maize, sufficient in number to build up the necessary genic system with only recessive subviable mutations.

Thus I think we have to reject the dominance hypothesis as an exclusive or general explanation of heterosis in maize. There seems to exist no possibility of accumulating a number of heterozygous factor pairs, sufficient for the phenomena observed and which we set out to explain.

On the other hand, the data seem to me in accordance with the estimated actual frequency of recessive subviable mutations which appear in South American maize after inbreeding, if we assume that there exist only a few hundred or less mutable loci for any phenotypic group of mutations. The frequency with which cytological aberrations appear in inbred lines is also of the same low order. These abnormalities cannot be the cause of heterosis or of the reduction of vigor after inbreeding as was supposed by MÜNTZING (1946), but rather the consequence of the breeding system.

CROW (1948) has reached a somewhat different conclusion. Since the frequency of homozygous subviable recessives is equal, at equilibrium, to u/s, one may say that the fraction lost, due to the presence of the homozygous subviable mutations, is equal to $s \cdot u/s$ or u. Assuming that there are N mutable loci, the total loss would be equal to $N \cdot u$, which gives for a mutation rate of 10^{-5} and 5,000 mutable loci a total average loss of .05. From this CROW concludes that the dominance hypothesis would be a sufficient explanation of heterosis, if ordinary random mating populations would yield, at the most,

TABLE 4

SURVIVAL INDEXES		ZYGOTIC RATIOS									GAMETIC FREQUENCY
		REPRODUCTIVE ELIMINATION			REPR. AND VEGT. ELIMINATION			VEGETATIVE ELIMINATION			
R_A	R_a	*AA*	*Aa*	*aa*	*AA*	*Aa*	*aa*	*AA*	*Aa*	*aa*	*A*
		Frequencies at equilibrium in random mating populations									
.8	.8	44.44	50.00	25.00	23.68	52.64	23.68	22.22	56.56	22.22	50.00
	.6	44.44	44.44	11.11	42.86	47.62	9.52	41.03	51.26	7.69	66.67
	.4	58.25	37.50	5.25	55.10	40.82	4.08	52.94	44.12	2.94	75.00
	.2	64.00	32.00	4.00	63.16	35.09	1.75	60.95	38.10	.95	80.00
	.0	69.44	27.78	2.75	—	—	—	66.67	33.33	—	83.33
.6	.6	25.00	50.00	25.00	22.22	55.56	22.22	18.75	62.50	18.75	50.00
	.4	36.00	48.00	16.00	33.33	55.56	11.11	28.42	63.16	8.42	60.00
	.2	44.44	44.44	11.11	42.11	52.63	5.26	36.36	60.61	3.03	66.67
	.0	51.02	40.82	8.16	—	—	—	42.85	57.15	—	71.43
.4	.4	25.00	50.00	25.00	18.75	62.50	18.75	14.29	71.43	14.29	50.00
	.2	32.65	48.98	18.37	25.80	64.52	9.68	19.88	74.54	5.59	57.14
	.0	39.06	46.88	14.06	—	—	—	24.99	75.01	—	62.50
.2	.2	25.00	50.00	25.00	14.29	71.43	14.29	8.33	83.33	8.33	50.00
	.1	28.02	49.83	22.15	16.76	73.30	9.93	9.72	86.43	3.84	52.94
	.0	30.86	49.38	19.75	—	—	—	11.11	88.89	—	55.56
.1	.1	25.00	50.00	25.00	11.54	76.92	11.54	4.55	90.90	4.55	50.00
	.05	26.37	49.96	23.67	12.63	79.81	7.56	4.90	92.90	2.20	51.35
	.00	27.70	49.86	22.44	—	—	—	5.26	94.74	—	52.63
		Frequencies at equilibrium in selfed populations									
.5	.5	50.00	—	50.00	50.00	—	50.00	50.00	—	50.00	50.00
	.4	100.00	—	—	100.00	—	—	100.00	—	—	100.00
.4	.4	41.67	16.67	41.67	37.50	25.00	37.50	33.33	33.33	33.33	50.00
	.2	57.69	23.08	19.23	52.94	35.30	11.76	46.15	46.15	7.69	69.21
	.2	62.50	25.00	12.50	—	—	—	50.00	50.00	—	75.00
.3	.3	35.71	28.57	35.71	27.78	44.45	27.78	21.43	57.14	21.43	50.00
	.2	40.54	32.43	27.03	31.92	51.06	17.02	24.32	64.86	10.82	56.76
	.0	45.45	36.36	18.18	—	—	—	27.27	72.73	—	63.63
.2	.2	31.25	37.50	31.25	20.00	60.00	20.00	12.50	75.00	12.50	50.00
	.0	35.76	42.83	21.43	—	—	—	14.31	85.69	—	57.17
.1	.1	27.78	44.44	27.78	13.64	72.72	13.64	5.56	88.89	5.56	50.00
	.0	29.41	47.06	23.53	—	—	—	5.88	94.12	—	52.94

only five percent less than the maximum possible in a hybrid population not containing any homozygous recessive individuals. I think one may safely say that most populations of open pollinated field corn yield considerably less than 95 percent of maximum yield possible. If it were not so, it seems most unlikely that breeders would have resorted to the complicated and rather expensive process of producing hybrid corn which certanly would not be an economic proposition unless the gain was considerably more than five percent.

Next we shall turn back to formula (3a) and study the situation for *heterotic mutants*, i.e., mutation to genes which cause the heterozygotes to have a higher survival value than either the original or normal genes or the new subviable mutants. Since the term (u/q^2) can now be considered as generally very small when compared with the usual values of R_A and R_a, the formula may be simplified and written as given by several authors:

$$\frac{p}{q} = \frac{1 - R_a}{1 - R_A} \cdot \tag{5}$$

If we want to calculate the zygotic frequencies, we cannot apply the HARDY-WEINBERG rule in its simple form. We have to take into consideration that generally individuals are observed after they have undergone at least part of the vegetative selection. Thus we must write (BRIEGER 1948a) the triple proportion:

$$\begin{array}{c} AA \\ \hline Aa \\ \hline aa \end{array} \quad \begin{array}{c} (1 - R_a)^2 \cdot R_{Av} \\ \hline 2(1 - R_a)(1 - R_A) \\ \hline (1 - R_A)^2 \cdot R_{av} \end{array} \cdot \tag{6}$$

Formulas (5) and (6) show that population equilibrium will be reached independently of the original gene frequencies or the mutation rate. Table 4, upper part, repeated after BRIEGER (1948a) contains the gametic and zygotic proportions obtained at equilibrium. It is evident from this table that for survival values of homozygotes smaller than .6, we have about 50 per cent or more of monofactorial heterozygotes in the population at equilibrium. If a plant should be heterozygous for one locus only, one half of its descendents after selfing will maintain the vigor and the other half will be less vigorous than the parent. If we should require that practically no descendent should have the same vigor as the parent individual, it would be sufficient to assume the presence of ten heterotic loci.

It should also be noted that equilibrium will generally be reached rather rapidly. From tables and figures of the publication already mentioned (BRIEGER 1948) we can take the following example.

If mutations to a heterotic mutant gene occur at a rate of 10^{-4} with survival values of $R_A = .6$; $R_a = .4$, it only takes about 9 generations for this mutant to reach a frequency of 10^{-2} or one percent. In another 10 generations equilibrium will be reached where the population contains 60 per cent of original genes and 40 percent of the mutant gene. If the mutation rate was only

10^{-6}, it will require about 18 generations to reach a one percent level for the new mutant gene and another 10 generations to reach equilibrium.

The superiority of a system of heterotic mutants as compared to a system of dominant recessive genes can be shown more directly in the following way:

Figure 1 illustrates the frequencies of heterozygotes at equilibrium, (a) in a population in which 20 loci have mutated to heterotic allelic pairs, with average

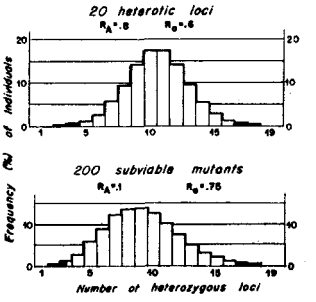

FIGURE 1

survival values of $R_A = .80$, $R_a = .60$ and (b) in a population in which 200 loci have mutated at a rate of 10^{-4} to subviable recessives, with an average of $R_A = .1$, $R_a = .75$. The two distributions are approximately equal, the mean number of heterozygous loci being 9.4 in the case of heterotic loci and 8.0 in the case of recessive subviable loci. We may conclude that it would require about 200 loci

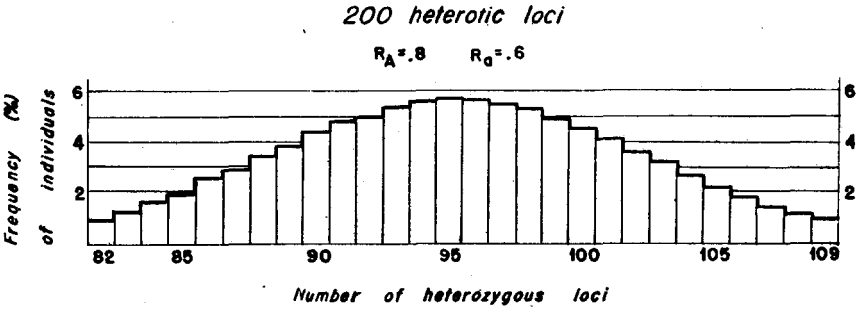

FIGURE 2

which are capable of mutating to recessive subviable alleles, to give about the same degree of efficiency as 20 loci which had mutated to heterotic subviable alleles. Figure 2 shows the frequency distribution, calculated for 200 loci, containing subviable heterotic mutations. On the average, an individual contains 95 heterozygous and 105 homozygous loci.

Finally the following summary shows in a very decisive manner the greater efficiency of heterotic gene pairs over subviable or lethal recessive mutants. (The numbers given refer to the total of mutable loci necessary in order to obtain, at equilibrium, 50 or 100 heterozygous loci.)

TYPE OF MUTANT	NUMBER OF MUTABLE LOCI	
	50 HETEROZYGOUS LOCI	100 HETEROZYGOUS LOCI
Recessive lethal mutants ($R_A=1$; $R_a=0$) $u=10^{-5}$	8,300	16,000
Recessive subviable mutants ($R_A=1$; $R_a=.9$) $\mu=10^{-5}$	2,500	5,000
Heterotic gene pairs:		
$R_A=.8$; $R_a=.6$	98	196
$R_A=.4$; $R_a=.2$	67	135

Thus it becomes evident that in order to build up an efficient heterotic mechanism by means of mutation to subviable recessive alleles one would require mutation at practically more than every locus in the corn plant.

On the other hand, the hypothesis of the heterotic subviable loci does not involve any such difficulty since a very small fraction of the total of loci in maize are sufficient to explain heterosis of any individual character.

THE RESULTS OF INBREEDING UNDER THE TWO ALTERNATIVE SYSTEMS

We have to establish a formula which will allow us to calculate the genotypic frequencies in populations that are subjected to close inbreeding. The results, to be expected when individuals carrying recessive lethals are selfed, have been discussed in great detail during the last forty years, but in addition we require a new and special formula which will permit us to extend the discussion and to include both subviable recessive genes and heterotic gene pairs. The necessary formulas have been developed by BRIEGER (1948a, 1948b). To simplify the question we shall take only into consideration the initial frequencies of the three genotypes AA, Aa and aa and the survival values R_A and R_a of the two homozygotes, while the survival value of the heterozygotes is taken as equal to one and used as basic value for comparison. We may omit the possibility of any new mutation, since with the small mutation rates ordinarily found only insignificant changes can occur. The formula is valid only for populations, but not for selected groups of inbred pedigree lines.

Starting from the initial frequencies u, v and w for the three genotypes we obtain in the mth generation of selfing, the following frequencies:

$$\begin{aligned} AA:&\quad 4\cdot u\cdot R_A^m + v\cdot\left\{1 + (2R_A) + (2R_A)^2 + \cdots + (2R_A)^{m-1}\right\}\cdot R_{AV}\\ Aa:&\quad 2v \qquad\qquad (7)\\ aa:&\quad 4\cdot w\cdot R_a^m + v\cdot\left\{1 + (2R_a) + (2R_a)^2 + \cdots + (2R_a)^{m-1}\right\}\cdot R_{aV}. \end{aligned}$$

The first term in the formula for frequencies of homozygotes can be omitted since its value will be comparatively small, except in rare cases where the initial frequency v, of heterozygotes, is much smaller than u and w.

The second term has some very interesting mathematical properties:

If the survival value for any of the homozygotes is equal to or larger than .5,

the exponential series will increase indefinitely and thus all heterozygotes will tend to disappear rapidly from the population. If in this case R_A should be larger than R_a, the frequency of the corresponding homozygote will increase more rapidly and reach infinity before that of the other homozygote. Consequently only the homozygotes with higher survival value will remain in the population. The same will occur and much more rapidly when only one of the survival values is larger and the other smaller than .5.

When both survival values of homozygotes are smaller than .5, all the exponential series will reach a finite value, and thus both heterozygotes and homozygotes will remain present in the population at equilibrium. I repeat from another publication (BRIEGER 1948) data (table 4, lower part) which contain the expected frequencies for all three genotypes at equilibrium. These values are calculated easily since the exponential series in formula (8) may give rather simple values such as: 5 for $R=.4$; 2.5 for $R=.3$; 1.66 for $R=.2$, and 1.25 for $R=1$.

Thus there are two quite different alternatives of population equilibrium under selfing: If one or both survival values of homozygotes are larger than one half, only the homozygotes with the higher survival value will be preserved. If both these survival values are below one half, the population will always contain all three genotypes, the two homozygotes and the heterozygotes (table 4, lower part). The second alternative is of special importance, because it shows that current belief is wrong in as far as selfing does not always lead automatically to complete homozygosis.

The approach to equilibrium will be gradual in selfed populations with large numbers of individuals. In most inbreeding experiments, however, the situation will be different. If we establish pedigree lines no compensation is possible in later generations for the accidental selection of a given individual as parent of the next generation. If this individual should contain more homozygous loci than expected for the mean of its generation, no return to a higher degree of heterozygosis is possible. If the chosen individual was more heterozygotic than expected, this corresponds, on the other hand, to a return to frequencies of a previous generation and thus a slowing down of inbreeding speed. Thus genetic drift in small inbreeding units may either slow down or speed up the process of reaching final equilibrium and of obtaining homozygosis. It may also lead to establishing genotypic proportions which are quite different from the expected mean. Selfed pedigree lines will tend on the whole to establish a higher degree of homozygosis more quickly than would be expected in large inbreeding populations where different pedigree lines are not kept separated.

We are now in a position to discuss whether or not our two alternative explanations of heterosis, the dominance hypothesis and the hypothesis of heterotic gene interaction, are in accord with theoretical expectation. In order to simplify matters we shall limit the discussion by assuming that the individuals are at the beginning completely heterozygous for ten pairs of factors. This is a considerable simplification since normally the number of loci involved in corn will be much higher.

We shall begin with the *dominance hypothesis*. The frequency of hetero-

zygotes expected in the first, third and sixth generation of an inbred population are illustrated in figure 3, and it is evident that the survival value of the recessive homozygotes has very little influence on the final results. In the first inbred generation, the individuals contain on the average more than half of their loci in a heterozygous condition, and hardly any individual contains less than three or more than eight or nine heterozygous loci of the ten loci in consideration. However, in the sixth generation, between 70 and 80 percent of individuals are completely homozygous, and almost no individual should contain more than two out of the ten loci in the heterozygous state.

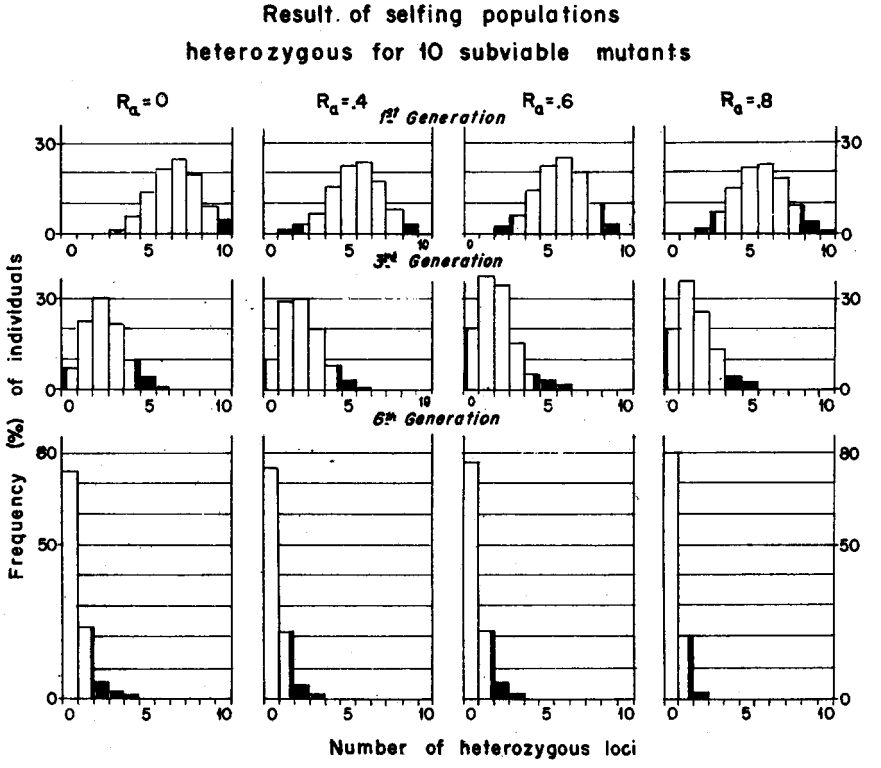

FIGURE 3

The influence of survival values becomes somewhat more pronounced if we take into consideration the number of dominant or recessive homozygous loci per individual plant. In the third inbred generation we still expect a considerable number of homozygous subviables while after the sixth generation they tend to disappear except when survival indexes are quite near the value one.

If we are dealing with inbred pedigree lines instead of populations, it is clear that on the whole they also should tend to become homozygous and contain at least some subviable recessive characters, unless we are always able to select the most vigorous of the inbred lines in such a way that they contain almost only dominant factors for normal viability and fertility.

All the authors who have studied the problem of inbreeding during the last forty years, have already stated that the dominance hypothesis leads us to postulate that inbred families should be nearly homozygous from about the sixth selfing onward, and we have just shown that this rule holds true whether we are dealing with lethal genes or with subviable mutants causing only a rather slight reduction of survival values. However, it seems to me that it has been taken too frequently for granted that such a high degree of homozygosis is really attained. Uniformity or homogeneity in a population or an inbred line is not necessarily a proof for homozygosis, since balanced heterozygous popula-

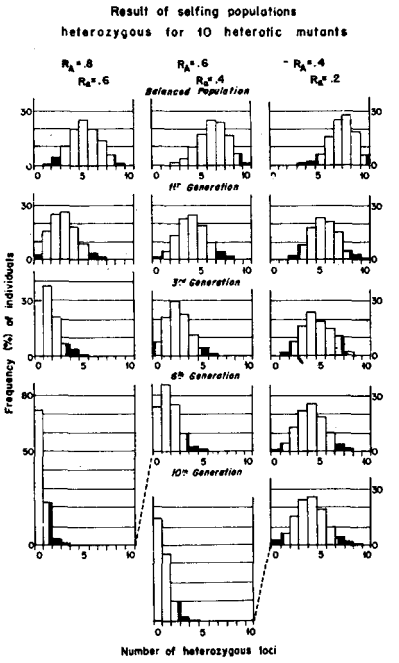

FIGURE 4

tions can easily also be quite uniform. We shall come back to this important point again later on.

Now we shall discuss the behavior of inbred populations carrying *heterotic gene pairs*. Figure 4 shows the existence of pronounced different results in accordance with the values of survival indexes. If the survival values of both homozygotes are rather high the situation does not differ very much from that just described for subviable recessives. In the sixth inbred generation about 70 percent of all individuals should be completely homozygous and very few individuals should be heterozygous for more than one locus. If both survival values are close to .5, with at least one above this limit, the final result will again be the same, except that, many more generations will be required, and even in the tenth inbred generation we will have only about 50 percent homozygous individuals, while the other half of the population will still be heterozygous for one or two of ten loci. Finally, if both survival indexes are below .5,

the picture changes completely, and no complete homozygotes are expected even in the tenth inbred generation. The mean number of heterozygous loci will be about four out of the ten loci present and the maximum may be seven out of ten loci.

Finally, the frequency of loci which should become homozygous for the less viable alleles is considerably higher than the values for homozygous subviable recessives. In the three cases of heterotic genes under discussion only about 10 to 20 percent of all individuals may become completely homozygous for the genes with a higher survival value.

In short, we may say that factor pairs with a heterotic gene interaction: (a) will tend to maintain a higher degree of heterozygosis for a longer period, and (b) do not favor the appearance of individuals which are completely homozygous for all the genes with the higher survival value. Inbreeding in pedigree lines instead of in populations will somewhat alter the final results, without changing anything fundamentally.

The hypothesis of heterotic gene interaction is thus also in accord with the facts generally observed, and we now have to decide which of the two alternative hypotheses gives a better and more satisfactory explanation of all facts.

In the first instance, I want to repeat my doubt in the current belief of attaining complete homozygosity by inbreeding, as required only under the dominant hypothesis. In fact, it seems to me far from being proved experimentally that inbreeding yields an almost complete homozygosis in about six generations, and moreover there are some clear indications that this is at least not always the case. For instance, several years ago we received from DR. M. M. RHOADES a highly inbred line of "good pachytene" maize and expected it to be highly homozygous. The line when first grown in Brazil was very weak, but by using the best ears obtained from selfing we were able to improve the climatic adaptation in about three generations. Such an adaptive change is, of course, only possible, if there still existed a sufficient number of heterozygous loci, and thus our observations prove that the inbred line still contained "hidden" heterozygosity. By growing the inbred line in a completely different climate, we evidently changed the adaptive value of many genes and thus established a new and different level for final equilibrium. We started a new selective trend which could only have been effective if sufficient heterozygosity was still present.

Next we shall consider a surprising situation present in indigenous maize varieties. Since the Indians have grown maize many generations in small plots or populations, we should expect a high degree of inbreeding. Under the dominance hypothesis a high degree of homozygosis should have been attained, while the theory of heterotic gene interaction hypothesis would permit the maintenance of a considerable degree of heterozygosis, if survival values for homozygotes should be rather low. My extensive studies on South-American indigenous maize have definitely shown that the latter alternative is correct. The degree of heterozygosis must be quite considerable, since marked changes may be produced by a few generations of selection. Furthermore, the reduction of vigor even in the first inbred generation is so pronounced

that numerous indigenous types cannot be maintained after two or three selfings, because no ears with good kernels are produced. We must assume that the Indian plant breeder, by always selecting the best ears and the strongest plants succeeded in attaining an extreme degree of heterozygosity at heterotic loci. At the same time, it may be mentioned that by selecting typical ears of their preferred varieties, they also achieved a genetic drift in favor of uniformity of type and a high degree of homozygosis at certain other loci, than those responsible for "vigor."

I may also mention that by a special breeding technique I have been able to select a new type of sweet corn adapted to the Brazilian subtropical conditions. It represents a balanced population, in the same way as the indigenous varieties: maintaining automatically both its vigor and heterozygosity for heterotic gene pairs, and uniformity for type. When a plant of such a population is selfed there is pronounced reduction of vigor in the first generation.

The residual heterozygosity of inbred lines, the existence of balanced heterotic populations in indigenous South-American maize and the possibility of actually obtaining such balanced populations by breeding are facts which are definitely not in accord with the dominance hypothesis, but can be readily explained by the alternative hypothesis of heterotic gene interaction. Thus again we reach the same conclusion as in the preceding chapter: only the second of the two hypotheses is completely in accord with the observed facts.

REFERENCES TO BREEDING PROCEDURE

There is no doubt that the method called "hybrid corn" has given excellent results in the United States, but the results obtained by this method elsewhere are far from satisfactory. There is at least one rather simple explanation for this divergence of results. The number of research workers, Experiment Stations and farmers engaged in the breeding program in the United States is in excess of and not even comparable to the very low intensity of the work carried out in other countries. There, isolated geneticists try to achieve practical results with limited numbers of inbred lines, obtained from limited material from a few sources only. However, it may well be that other and more fundamental reasons are involved as well. It is a known fact that the North American dent corns were not directly derived from any existing populations but from variety hybrids. In the original hybrids between the tall southern dent or "gourd seed" varieties and the smaller northeastern little flints, a large amount of factor interaction between size factors must have been involved. Therefore, the attempts to select again by simple mass selection in crossbred populations for a uniform dent type may have created a situation quite different from that existing in old indigenous balanced populations. It seems to me to be rather difficult to judge the results of unscientific mass selection, carried out between about 1830 and 1910. Anderson (1945) reports that most of the modern lines of inbred dent corn in the United States can be traced back to a few original selections, mainly to Reid's Yellow Dent and to Lancaster Sure-cropper. The vast majority of the practical breeders of the last century have thus failed in producing a good new variety. At the same

time, it seems to me very doubtful that these selections may have again established balanced heterotic populations, which are characterized by their sensitivity to any degree of inbreeding. The basic technique in modern corn breeding is to select lines which can stand a good deal of inbreeding and which, therefore, require high values for the survival index of homozygotes. Thus the basic genetic element which enables us to establish a balanced heterotic population is intentionally eliminated from the material by the maintenance of only such inbred lines that still remain rather productive.

Since the US-dent varieties are to a large extent derived from intravarietal hybridization, the hybrid corn technique is probably quite appropriate for them, and represents the best and most rapid practical solution for the North American plant breeder. However, it seems to me very doubtful whether the same method will give equally good results in other countries, such as the South-American ones, where we have at our disposal local or indigenous balanced populations, containing a large number of genes with a low survival index when homozygous. In fact, it may be more expedient instead to establish new and improved balanced populations and to study in detail to what extent balanced populations of maize may not be only the first but also the final solution of the breeding problem, at least in South and Central America where we have generally the basic gene material at our disposal.

The breeding procedure in establishing such balanced populations differs in several points from the ordinary technique. We must inbreed for about three generations in order to eliminate recessive undesirable characters and to increase homogeneity of non-heterotic loci. During this inbreeding phase, preference should be given to lines which are less resistant to inbreeding since they will contain heterotic alleles for low survival values in homozygotes necessary for building up the balanced population. In the second phase of the work, we must carry out repeated and multiple crosses eliminating all lines with low combining ability. After another period of about three generations of continuing crossing in selecting, the remaining multiple hybrids should be thrown together to form now one breeding unit. If the procedure has been carried out successfully, a new balanced population will be obtained.

SUMMARY

1. The use of the term heterosis is discussed. It is suggested that one should apply this term whenever the mean of hybrids for any quantitative character is higher than that of descendants, obtained by any form of inbreeding. Hybrids which are heterotic in this sense, may be superior, equal to, or inferior to their own parents. Furthermore, heterosis does not affect an individual as a whole, but only its separate characters.

2. Two fundamentally different genetic formulas have been proposed during the last forty years in explanation of heterosis: The dominance hypothesis is based on the assumption that heterotic hybrids are heterozygous for recessive subviable mutant genes, while the alternative explanation postulates the existence of loci containing special "heterotic" alleles which when heterozygous increase vigor. The linkage hypothesis represents an intermediary solution

between these alternatives and was intended to overcome certain difficulties. The evidence presented in the literature up to now gives no conclusive proof in favor of either of the hypotheses.

3. Applying the principles of population genetics to the problem of the evolution of heterotic gene mechanisms, we were able to show that there exists no effective breeding system which would tend to accumulate a sufficient number of recessive subviable or lethal mutants, as required by the dominance hypothesis. But there will be a rapid accumulation of heterotic mutations, which does not depend upon the mutation rate.

4. Using the number of heterozygous loci present in a population as a measure for the efficiency of a genic system, it is evident that heterotic gene systems are much superior to systems based on recessive subviable or lethal mutations. In order to accumulate an average of 100 heterozygous loci per individual in a population, we require (a) from 100 to 200 mutable loci, producing mutations to heterotic gene pairs, (b) 5,000 loci which may mutate at a rate of 10^{-5} to recessive subviable genes with survival values of the high order of .9 or finally (c) over 15,000 loci which may mutate at the rate of 10^{-5} to lethal mutant genes.

5. Facts are cited to show that the dominance hypothesis is not sufficient to explain all results obtained by inbreeding. The hypothesis of heterotic gene interaction on the other hand is again capable of explaining in a satisfactory manner all known facts.

6. A certain number of recessive subviable or lethal mutations tend to accumulate in any random mating population, and thus the appearance of the corresponding homozygotes after inbreeding is not a cause, but rather a consequence of the breeding system.

7. Two alternative methods of maize breeding are mentioned. They may be characterized by the terms "hybrid corn technique" and "balanced population technique." Their advantages and disadvantages are briefly discussed.

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