

Chapter 9

Heterosis in Population Genetics

Population genetics is the study of the genetic structure of populations. Such a statement may look at first to be a truism, a tautology. The subject matter of our research becomes very intricate, however, as soon as we try to specify what we mean by the above definition. The terms "genetic structure" and "population" may have different meanings according to what we are willing to indicate by such words. It therefore seems convenient to start with an analysis of the terms we are using. Such discussion will give us a chance to see how the problem of heterosis is intimately connected with the general theme of population-genetical studies. A few experimental data will be used to illustrate such points.

Let us consider first what we mean by population. If we take a dictionary definition, we find in Webster's that population is "all the people or inhabitants in a country or section." It means, in this sense, the sum of individuals present at a certain moment over a more or less arbitrarily limited territory. But this definition does not correspond to the requirements of our studies, as I have tried to show elsewhere (Buzzati-Traverso, 1950). Such a definition is a static one, while the population, as considered in the field of population genetics, is a dynamic concept. We are interested not in the number of individuals present at a certain time in a certain place and their morphological and physiological characteristics. Instead, we are concerned with the underlying mechanisms which bring about such characteristics, and the particular size the population reaches at any particular moment. Since such mechanisms depend upon the numerical dynamics of the population and upon heredity, it follows that our concept of population is typically dynamic. On this view, then, *a population is an array of interbreeding individuals, continuous along the time coordinate.*

Consideration of a population as a phenomenon continuously occurring in time makes it impossible for the experimental student of population genetics to get a direct and complete picture of what is occurring within a population at any particular moment. We can attempt to collect data on the population under study only by freezing such flowing processes at particular time intervals. Collecting observations on a population at different times gives us a chance to extrapolate the direction and rate of the processes that have occurred within the population during the time elapsed between two successive sets of observations. If the samples studied are large enough and give an unbiased picture of the total population at the time when the sample is being drawn, this experimental procedure may give us a fairly adequate idea of what is going on within the array of interbreeding individuals continuous along the time coordinate. That sum of individuals at a definite time, which one usually means by *population*, is of interest to the population geneticist only as an index of the particular evolutionary stage reached by the array of interbreeding individuals. Since there are actual breeding and genetic relationships between the individuals of any such array, of any such population, the population can be considered as the natural unit of our studies.

If we consider now what we mean by "genetic structure," our task becomes much more complex. At first we could assume that the genetic structure of a population could be properly described in terms of the gene frequencies present at a certain time within a population. But this is only part of the picture.

For the total description of the genetic structure of a population we have to consider not only the frequencies of existing genes, but how these are fitted within the chromosomes, how these allow the release of variability by means of recombinations, how large is the amount of new variability produced by mutations, and several other factors which we cannot analyze now. In a few words, the study of population genetics aims at the knowledge of the breeding system of populations. This, as we shall see, is a rather difficult task because of the complexity of factors responsible for the origin and evolution of such systems.

EVOLUTIONARY FACTORS INVOLVED

When we take into consideration a species or a natural population at a certain stage, we have to assume that such a natural entity is the product of a series of evolutionary factors that have been at work in previous times and that some, or all of them, are still operating on the population while we are studying it. This means that we should try to explain the genetic structure of the population in terms of such evolutionary factors.

Now, if we are willing to examine the nature of the known evolutionary agencies, we conclude that these can be classified into two types. On one side we find, in sexually reproducing organisms, a limited number of chromo-

somes, linkage between genes, sterility mechanisms, mating discriminations, devices favoring inbreeding, and other conservative forces that aim at the preservation of certain constellations of genes over a large number of generations. On the other side we find mutation pressure, recombination between chromosomes, recombination among genes due to crossing over, outbreeding devices, migration pressure, and other revolutionary forces that aim at the production of genetic novelty.

It seems reasonable to maintain that, at any particular time, a species or a natural population can be considered as a sort of compromise between the two conflicting forces—a compromise that is brought about through the action of natural selection. In other words, the fine adjustment or adaptation of a population to its environment is the expression of such compromise. At any particular time the terms of the compromise between the conflicting forces are always different as compared to other moments, as the compromise itself is a dynamic process.

In order to reach the highest possible level of adaptation with respect to a certain set of environmental conditions, natural selection is discriminating not only for or against a certain individual genetic constitution, but for or against a group of individuals, as well. Sometimes selection acts at the level of the individual, sometimes it operates at some higher level. If we consider a genotype that insures resistance against an infectious disease, present in a certain area of distribution of a species, it will be obvious that an individual carrying it shall directly benefit by it. But if we consider a genotype producing fecundity higher than the average of the population, this will be selected by the mere fact that a larger number of individuals having such genetic constitution will be present in the next generation. These, in their turn, shall have a chance of being represented in the next generation greater than that of individuals having a less fertile genotype. The individual itself, though, obtains no direct advantage from such selection.

The next extreme condition we can consider is the one occurring when the advantage of the individual is in conflict with the advantage of the group. This is the case, for instance, of a genotype that would extend the span of life far beyond the period of sexual activity—or higher fertility linked with antisocial attitudes in the case of man. In both cases, natural selection favoring the preservation of the group will discriminate against the individual. A similar mechanism must have played a great role in various critical periods of organic evolution. When intergroup selective pressure is in the opposite direction from intragroup selection, a sort of compromise has to be reached between the two conflicting tendencies. This can be reached in many different ways that are best illustrated by the great variety of life histories and mating systems to be found in the living world.

Those factors which we have classified as conservative tend to produce genetic homogeneity, or what is technically known as homozygosis. Factors

that we have named revolutionary tend to produce genetic heterogeneity or heterozygosis. Thus we come to the conclusion that the mentioned compromise brought about by selection consists of the pursuit of an optimum level of hybridity with respect to the conditions under which the organism lives. Such a hybridity optimum is the product, not only of the mutation rate and selective value of single genes, but also depends largely upon the genetic system and the mating system—the breeding system—of the considered species or population.

The genetic structure of natural populations cannot be solved only in terms of individual variations observable in the group. Instead, it must be integrated into a unitary research on changes in gene frequencies as related to the underlying breeding systems. This is why we are justified in considering the natural population as a unit, since individual variations must be referred to the genetic balance of the whole aggregate of individuals.

What is that hybridity optimum I was speaking about but heterosis? How else could heterosis be defined in population problems other than that type and amount of heterozygosity that gives the population or the individual the best adaptive value with respect to the conditions in which the organism lives? With this view, then, it becomes feasible to analyze experimentally what morphological and physiological characteristics of the hybrids produce the better adaptation.

MECHANISMS WHICH PROMOTE HYBRIDITY

In studying how heterosis mechanisms are brought about in living creatures, we may attempt a sort of classification of the devices present in plants and animals insuring hybridity. Starting from the most complex and proceeding to the less complex cases, we can distinguish three types of mechanisms: (1) mating systems, (2) chromosome mechanisms, and (3) gene effects.

We will not discuss in detail all the devices insuring hybridity found in plants and animals. We will mention a few, in order to show how many different paths have been followed in evolution to reach the same sort of results.

Under the heading "mating systems" we may mention homo- and heterothally among fungi; monoecism and dioecism, incompatibility mechanisms, and heterostyly among flowering plants. Here, in some cases such as *Primula scotica*, there is close relation between the variability of ecological conditions, and, therefore, of selection pressure and the efficiency of the incompatibility mechanisms. Other species of this genus present in England are characterized by heterostyly and incompatibility devices to insure the occurrence of outcrossing, apparently necessary to meet the requirements of varied ecological conditions. *Primula scotica*, living in a very specialized ecological niche, shows that such a mechanism has broken down. In fact, it looks as if the requirements of a constant environment are met better by populations genetically less diversified.

Among animals, the largest part of which are not sessile and therefore not bound to the ground, the differentiation into two sexes offers the best solution to the problem of insuring a wide range of crossing among different genotypes. But even here we see that special behavior patterns have been developed for this purpose. These may be courtship relationships, sexual selection, dominance relationships among a group of animals, or protandry mechanisms, where the presence of two sexes in hermaphrodites could reduce the amount of outcrossing and therefore endanger the survival of the species. Even among parthenogenetic animals, such as Cladoceran crustacea, the appearance of sexual generations after a long succession of asexual ones seems to depend upon extreme environmental conditions. For its survival, the species must shift over to sexual reproduction in order to obtain a wider range of genetic combinations, some of which might be able to survive under the new set of conditions.

At the level of the chromosome mechanisms, several examples of permanent hybrids are known well enough to be sure that they play an important role for the survival of some flowering plants. In animals, too, some similar mechanism may be present. In a European species of *Drosophila* which we are studying now, *Drosophila subobscura*, one finds that practically every individual found in nature is heterozygous for one or more inversions. It looks as if the species were a permanent hybrid.

Rarely, though, one finds individuals giving progeny with homozygous gene arrangement. Such cases have been observed only three times: once in Sweden, once in Switzerland, once in Italy; and they are very peculiar in one respect. The three homozygous gene arrangements are the same, even though the ecological and climatic conditions of the three original populations were as different as they could be. It looks as if the species could originate only one gene arrangement viable in homozygous condition, and that this may occur sporadically throughout its vast distribution range (Buzzati-Traverso, unpublished).

At this level too is the fine example of heterozygous inversions from the classical studies of Dobzhansky (1943-1947). They have demonstrated that wild populations of *Drosophila pseudoobscura* show different frequencies of inversions at different altitudes or in the same locality at different times of the year. Variation in the frequency of inversions could be reproduced experimentally in population cages by varying environmental factors such as temperature. It is shown in such a case that natural selection controls the increase or decrease of inversions determining an interesting type of balanced polymorphism. Finally, according to the investigations of Mather (1942-1943) on the mechanism of polygenic inheritance, it appears that linkage relationships within one chromosome, even in the absence of heterozygous inversions, tend to maintain a balance of plus and minus loci controlling quantitative characters.

We come then to the third level, that of gene effects. Here it is well known that heterozygotes for a certain locus sometimes show a higher viability or a better adaptation to the environment than either homozygote. The most extreme examples are those of the widespread occurrence of lethals in wild populations of *Drosophila*, noted in the next section.

Every population of plants and animals that has been studied from the genetic viewpoint has proved to be heterozygous for several loci. We have now at our disposal a large series of data showing that the phenomenon of genetic polymorphism is frequent in plants, animals, and man. These offer to the student of evolutionary mechanisms the best opportunities to test his hypotheses concerning the relative importance of selection, mutation pressure, migration, and genetic drift as factors of evolution. Wherever we find a well established example of balanced polymorphism, such as that of blood groups and taste sensitivity in man, it seems safe to assume that this is due to selection in favor of the heterozygote. How this selection actually may produce an increase in the chances of survival of the heterozygote, as compared to both homozygotes, is an open question. When the characters favored by natural or artificial selection are the result of several genes in heterozygous condition, the analysis becomes very difficult indeed, as the experience of plant and animal breeders clearly shows.

EXPERIMENTS WITH HETEROSIS

The importance of the problem of heterosis for population-genetical studies is clearly shown, not only by such general considerations and by the few examples mentioned, but also by the everyday experience of people interested in such lines of work. I have come across problems involving heterosis several times and shall describe some results we have obtained which may be of interest for the problem under discussion, especially at the level of single gene differences.

Several *Drosophila* workers have been able to show the occurrence of heterosis in the fruit flies. L'Heritier and Teissier (1933), Kalmus (1945), and Teissier (1947a, b) have shown that some visible recessive mutants of *Drosophila melanogaster* such as *ebony* and *sepia* have a higher selective value in heterozygous condition than either of the corresponding homozygotes under laboratory conditions. Dobzhansky and collaborators in *Drosophila pseudo-obscura*, Plough, Ives, and Child, as well as other American and Russian workers in *Drosophila melanogaster*, have shown that recessive lethals are widely spread in natural populations. It is generally accepted that such genes are being maintained in the population because the heterozygotes are being selected. Teissier (1942, 1944) has brought similar evidence under laboratory conditions for *Drosophila melanogaster*.

It has been shown in several populations of species of the genus *Drosophila* that heterozygous inversions are being selected, under natural and ex-

perimental conditions. It seems, however, that the study of selection in favor of the heterozygote for single loci deserves more careful analysis. The whole problem of heterosis for several genes affecting quantitative characters will be solved, I think, only when the more simple cases of heterosis where single gene differences are involved shall be cleared up. I have been lucky enough to come across some useful experimental material for the purpose.

For a number of years I have kept about one hundred different wild stocks of *Drosophila melanogaster* coming from different geographical localities. Such stocks were maintained by the usual *Drosophila* technique of transferring about once a month some 30–40 flies from one old vial to a new one with fresh food. About twice a year I look at the flies under the microscope. Since all such stocks were wild type, no change by contamination was expected, as these stocks were phenotypically alike. Contamination by mutants kept in the laboratory could not have produced any appreciable result, owing to the well known fact that both under laboratory and natural conditions mutants are generally less viable than the normal type. To my surprise, however, I happened to observe at two different times, in two different wild stocks, that a fairly large number of the flies showed an eye color much lighter than the normal. These two mutants proved to be indistinguishable recessive alleles at the same locus in the third chromosome. The presence of the homozygotes has been checked at different times over a number of years.

In the summer of 1947 while collecting flies in the wild for other purposes, I found in the neighborhood of Suna, a small village on the western shore of the Lake Maggiore, in Northern Italy, several individuals of both sexes showing the same eye color. From these a homozygous stock for such mutant was obtained. Crossing tests proved that it was another allele of the same locus as the above mentioned. The occurrence of several individuals mutant for an autosomal recessive within a free living population was remarkable enough. But finding that the same gene was concerned as in the laboratory stocks, I suspected that such a mutant might have a positive selective value, both under laboratory and natural conditions.

I began an experiment to check this point. Two populations in numerical equilibrium were started, applying the method previously used by Pearl for the study of population dynamics of *Drosophila*, described in detail elsewhere (1947a). Sixteen light-eyed individuals, eight males and eight females, were put together in one vial with sixteen wild type flies. The gene frequency at the beginning of the experiment was therefore .5. Under the experimental conditions the population reached an equilibrium in respect to the amount of available food at about 700–900 flies per vial. After about twenty generations, assuming that each generation takes 15 days, the frequency of recessive homozygotes was about 40 per cent. Assuming random mating within the population, taking the square root of .40 one gets a gene frequency for the

light-eyed gene of about .63. Since in both parallel populations the gene frequency was similar, one could conclude that selection had favored the mutant type, shifting its frequency from .5 to .63 in the course of about twenty generations.

Such an experiment did prove that the mutant gene had a positive selective value. It was impossible to know whether in the long run it would have eventually eliminated its normal allele from the population. At this stage, I

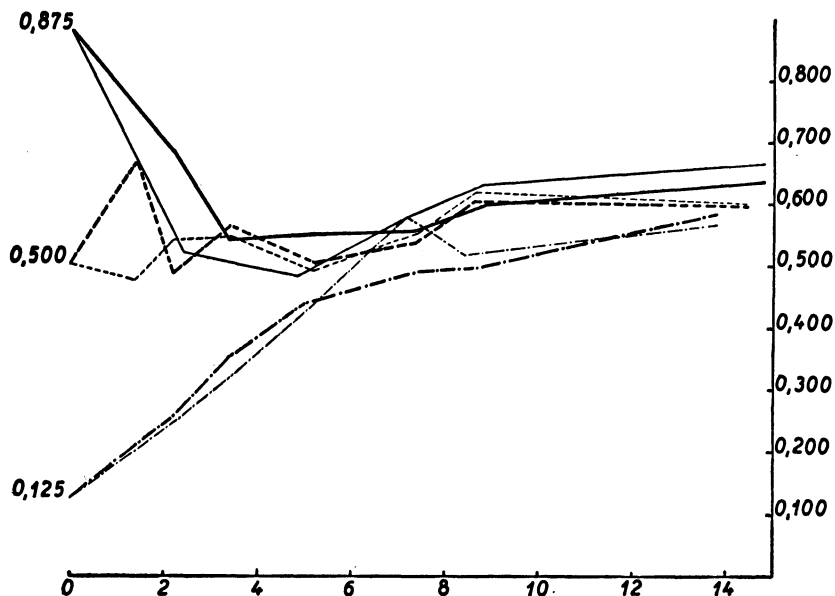


FIG. 9.1—Variation in the frequency of the light-eyed gene in selection experiments. In the abscissae is the number of generations, in the ordinates the gene frequency. Each line represents a single experiment on an artificial population.

have begun a new experiment along the same lines, but with different gene frequencies to start with. Two populations were started with 2 males and 2 females of the mutant type, plus 14 males and 14 females of the normal type. Two populations were started with 16 mutant and 16 wild flies, and two populations with 28 mutant and 4 wild type flies.

I had, therefore, at the beginning of the experiment six populations. Two had a gene frequency of the light-eyed mutant approximately equal to .125. Two had a gene frequency of .5, and two had a gene frequency of .875. Figure 9.1 shows the result of such an experiment after about fifteen generations. Crossings of wild type males, taken from the populations, with homozygous recessive females showed that there was no significant departure from random mating within the population. The gene frequencies indicated on the

ordinates were obtained by taking the square root of the observed frequencies of homozygous recessives.

The following conclusions can be drawn: (1) the three experimental populations, each being run in duplicate, have reached the same gene frequency at about the .579 point; (2) natural selection has been acting on the three populations producing the same end results, irrespective of the initial gene frequency; (3) natural selection has been acting in favor of the heterozygous flies; and (4) the homozygous mutant seems to be slightly superior in its survival value to the homozygous normal allele.

It was of considerable interest to determine whether the intensity of selection operating in the three experiments was the same. Since the three experimental curves (each being the mean of the two duplicate populations) could not be compared directly, Dr. L. L. Cavalli elaborated a mathematical analysis of the problem (Cavalli, 1950). The function of gene frequency linear with time Y , when the heterozygote is at an advantage, is given by:

$$Y = q_e \log p + p_e \log q - \log [p_e - p],$$

where p and q are the gene frequencies at the beginning of the experiment in a random breeding population, and p_e and q_e are the equilibrium frequencies. By means of this function it is possible to transform the experimental curves to linear ones. Results can then be plotted graphically for the three experiments. Fitting straight lines with the method of maximum likelihood, one obtains the following values for the constants of the linear regression equation:

Experiment	Initial Gene Frequency (Observed)	Slope	Position	Initial Gene Frequency (Theoretical)
1.500	.0879	+1.21	.425
2.125	.0631	- .41	.100
3.875	.0726	+ .27	.830

The *position* is the transformed value of the initial gene frequency which is given in the last column, and is in good agreement with the experimental value. If one tests the parallelism of the three regression lines so obtained, one gets a chi square of 4.0 with two degrees of freedom. Parallelism therefore seems to be satisfactory. This implies that the intensity of selection is independent of initial conditions.

If we take these results together with the two independent occurrences of the same mutant gene in different genotypical milieus, it seems safe to maintain that such a gene has a positive selective value with respect to its normal allele, and that selection is acting mainly through a typical heterosis mechanism. It is worth while to stress that this gene was found both in natural and

experimental conditions. The exceptional occurrence of many mutant individuals in a free living population can be accounted for by assuming that they have a higher selective value.

BASIS FOR SUPERIORITY OF THE HETEROZYGOTE

It would be interesting to try to find out how selection discriminates against both normal and mutant homozygotes. I am just beginning to attack this problem.

Dr. E. Caspari has some interesting results on a similar problem, and I wish to thank him for permission to quote them (Caspari, 1950). In free living populations of the moth *Ephesia kühniella*, this author has observed a balanced polymorphism, whereby individuals having brown colored and red colored testes occur in various numbers. The character brown behaves as a complete dominant with respect to red. The polymorphism seems to be determined by a higher selective value of the heterozygote. It has been possible to show that the heterozygote is equal or superior to the homozygous recessive and the latter is superior to the homozygous dominant with respect to viability. It was found that, while the heterozygote is equal or superior to the homozygous dominant, the homozygous brown is superior to the homozygous red with respect to mating activity. The dominance relationships of such two physiological characters are therefore reversed.

There is no decisive evidence for heterosis for any of the characters studied. The recessive for the testis color acts as dominant with respect to viability, and the dominant testis color acts as dominant with respect to mating behavior. The net result is a selective advantage of the heterozygote that can account for the observed polymorphism. This seems a good example of how a heterosis mechanism can be determined by the behavior of two visible alleles in heterozygous condition. It is hoped that similar analyses will be developed for other cases of balanced polymorphism.

The search for clear-cut examples of heterosis depending on single genes seems to me the most promising line of attack on the general problem under discussion. If I could find another gene behaving in a way similar to the one I have studied in *Drosophila melanogaster*, and could study the interaction of the two, it would be possible to go a step further in the analysis of heterosis mechanisms. The evidence derived from such single genes being favored in heterozygous condition is likely to be very useful in more complex conditions where the action of several genes is involved.

When we come to consider the selective advantage of polygenic characters, even in such an easy experimental object as *Drosophila*, the problem becomes very entangled indeed. In recent years I have been studying, for example, a number of quantitative characters being favored by natural selection in artificial populations in numerical equilibrium, such as the ones I have been speaking about. I have set in competition at the beginning of one

experiment two stocks differing for visible mutants. One stock was *white*- and *Bar*-eyed, the other stock was normal for both characters. The two stocks differed, too, in a number of quantitative characters such as fecundity, fertility, rate of development, longevity, and size.

After about thirty generations the two mutant genes had been wiped out. This could have been expected on the basis of previous data of L'Heritier and Teissier on the elimination of such genes in artificial populations. At that time, however, I did not discard the populations, but kept them going for some seventy more generations. All the individuals present in the populations were phenotypically normal. But testing from time to time the values of the above mentioned characters, I could establish that natural selection was continuously operating and favoring higher fecundity, higher fertility, higher longevity, and quicker developmental rate throughout the four years that the experiment lasted. At the end, the flies present in the population were superior by a factor of more than six to the mean of the considered characters in the two original parental stocks. When I measured such values in the F_1 hybrids between the two stocks I could observe values higher than those obtained after more than one hundred generations of selection.

The selection experiment could then be interpreted in two different ways. Either (a) selection had picked up a new genotype made out of a new combination of polygenes derived from the two parental stocks, or (b) selection had just preserved by means of a heterosis mechanism a certain amount of heterozygosity, which was at its highest value at the beginning of the experiment. The fact that in the course of the experiment the factors had been steadily improving seemed to be against hypothesis b, but I could not be sure that was the case.

I then set up a new selection experiment, whereby I put in competition the original stock *white Bar* with the normal type derived from the population which had been subjected to natural selection for more than one hundred generations. The result was clear. The genes *white* and *Bar* were eliminated in this second experiment at a much higher rate than in the first experiment. In the first experiment the gene frequency of the gene *Bar* after ten generations had dropped from .50 to .15. In the second experiment, after as many generations, the *Bar* gene frequency had dropped from .50 to .03. It seems that the genotype produced by a hundred generations of natural selection under constant conditions was so much better adapted to its environment that it could get rid of the competing genes with much greater ease than the original wild type flies. But could it not be that all or at least part of this result could be accounted for by the action of some heterosis effect?

Another example of a similarly puzzling condition is an experiment on artificial populations under way now in my laboratory. I would like to find out whether it is possible to produce so-called small mutations or polygenic

mutations with X-rays, and whether an increase in the mutation rate may speed up the evolutionary rate under selection pressure.

For this purpose I have set up four artificial populations starting from an isogenic stock of *Drosophila melanogaster*. One of these is being kept as control while the other three get, every two weeks, 500, 1000, and 2000 r-units respectively. At the start, and at various intervals, I am measuring fecundity, fertility, and longevity of the flies. The few data so far collected show clearly that in the irradiated populations the percentage of eggs that do not develop is much higher than in the control. This is due to the effect of dominant and recessive lethals. But the startling result is that the fecundity, measured by the number of eggs laid per day by single females of the irradiated populations, is higher than in the control series. Probably X-rays have produced a number of mutations for higher fecundity which have been accumulated by natural selection in the course of the experiment. But, are specific mutations for higher fecundity being produced, or am I dealing with heterosis phenomena dependent upon nonspecific mutants?

These few examples from my own experience with population-genetical studies show, I think, how important the heterosis phenomenon can be in our field of work. Both in natural and artificial populations, heterosis seems to be at work, making our analysis rather difficult, but stimulating as well. Closer contacts between students of selection and heterosis in plant and animal breeding and students of evolutionary problems are to be wished. Let us hope that a higher level of hybridization between various lines of investigation might become permanent, since it surely will make our studies more vigorous and better adapted to the requirements of a rapidly growing science.