AN EXPERIMENTAL STUDY OF INTERACTION BETWEEN GENETIC DRIFT AND NATURAL SELECTION

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INTRODUCTION

The role of random genetic drift in the evolutionary process has, for about two decades, been one of the controversial issues in population genetics. Some authors have appealed to "drift" as a convenient explanation of the origin of differences among organisms for which no other explanations seemed to be available. But one's inability to discover the adaptive significance of a trait does not mean that it has none (cf. Dobzhansky, 1956). The hypothesis of random genetic drift should not be used as a loophole; to be accepted it requires a firmer basis than suspicion. Other authors seem to think that drift and natural selection are alternatives. As soon as a gene is shown to have any effect whatever on fitness, the conclusion is drawn that its distribution in populations must be determined solely by selection and cannot be influenced by random drift. But this is a logical non-sequitur. The important work of Aird *et al.* (1954) and of Clarke *et al.* (1956) disclosed that the incidence of certain types of gastro-intestinal ulceration is significantly different in persons with different blood groups. This is, however, far from a convincing demonstration that the observed diversity in the frequencies of the blood group genes in human populations is governed wholly, or even partially, by selection for resistance to ulcers. To make such a conclusion tenable it would have to be demonstrated that the environments in which human racial differences have evolved actually favored greater resistance in certain parts of the

world and lesser resistance in certain other parts. Thus far no evidence has been adduced to substantiate any such claim.

As defined by Wright (1949) random genetic drift includes all variations in gene frequencies which are indeterminate in direction. Such variations are caused by accidents in gene sampling in populations of finite genetically effective size, as well as by fluctuations in the intensity or in the direction of selection, mutation and gene exchange between populations. Wright (1932, 1948, 1948, 1951) as well as the present writer (Dobzhansky, 1937–1941–1951) have stressed that random drift by itself is not likely to bring about important evolutionary progress. Indeed, variations in gene frequencies induced by random drift in small isolated populations are apt to be inadaptive, and hence likely to result in extinction of such populations. However, random drift may be important in conjunction with systematic pressures on the gene frequencies, particularly with natural selection. What is most necessary, then, is the type of experimental evidence that would permit analysis of the interactions between random drift and selection. Such evidence, although difficult to obtain, should be within the range of what is possible. Kerr and Wright (1954) and Wright and Kerr (1954) studied models of Drosophila populations in which the number of the progenitors in every generation was fixed arbitrarily, and in which classical laboratory mutants were used as traits subject to drift and to selection. In the experimental Drosophila populations described in the following pages naturally occurring genetic variants, inversions in the third

chromosomes of *Drosophila pseudoobscura* were used. Severe limitation of the population sizes was introduced in some of the populations in only a single generation, at the beginning of the experiments. Experiments so conducted may to some extent reproduce genetic events which occur in natural populations.

PRELIMINARY EXPERIMENTS

It has been shown (see Dobzhansky, 1949 and 1954, for reviews) that heterozygotes of *Drosophila pseudoobscura* which carry two third chromosomes with different gene arrangements derived from the same locality are, as a rule, superior in Darwinian fitness to the corresponding homozygotes. The situation is more complex when flies of different geographic origins are hybridized. Chromosomal heterozygotes which carry two third chromosomes derived from different geographic regions may or may not exhibit heterosis. Experimental populations, bred in the laboratory in so-called population cages, behave differently depending upon whether the foundation stock of the population consists of flies of geographically uniform or of geographically mixed origin. In the former case, the chromosomes with different gene arrangements usually reach certain equilibrium frequencies. Replicate experiments, conducted with reasonable precautions to make the environments uniform, give results repeatable within the limits of sampling errors. With geographically mixed populations the results do not obey simple rules. The course of natural selection in such populations is often erratic; equilibrium may or may not be reached, or may be reached and then lost; replicate experiments do not give uniform results; heterosis may or may not be present at the start of the experiments, and may or may not develop in the course of selection in the experimental populations.

The indeterminacy observed in the populations of geographically mixed origin is however understandable (Dobzhansky and Pavlovsky, 1953; Dobzhansky, 1954).

Race hybridization releases a flood of genetic variability; the number of potentially possible gene combinations far exceeds the number of the flies in the experimental populations; natural selection perpetuates the genotypes which possess high adaptive values under experimental conditions, but it is a matter of chance which of the possible adaptive genotypes will be formed first in a given population. In some populations these genotypes will happen to be structural heterozygotes, and in others homozygotes.

We have tested about thirty experimental populations of mixed geographic origins, using different combinations of flies from diverse localities (Dobzhansky and Pavlovsky, 1953, and much unpublished data). Among them were two replicate populations, Nos. 119 and 120, which are relevant here. They were started on February 8, 1954, in wood-and-glass population cages used in our laboratory and described previously. The foundation stocks consisted of F_1 hybrids between 12 strains derived from flies collected near Austin, Texas in 1953 and 10 strains derived from Mather, California, in 1947. The Texas strains were homozygous for the Pikes Peak (PP) gene arrangement, and the California strains for the Arrowhead (AR) gene arrangement in their third chromosomes. In each of the two cages 2,395 flies of both sexes, taken from the same F_1 culture bottles of Texas by California crosses, were introduced. The populations were kept in an incubator at 25° C., samples of eggs deposited in the population cages were taken at desired intervals, larvae hatching from these eggs were grown under optimal conditions in regular culture bottles, and their salivary glands were dissected and stained in acetic orcein.

The course of the events in the populations Nos. 119 and 120 is shown in table 1 and figure 1. The percentage frequencies of PP chromosomes are given in this table, the frequencies of AR chromosomes are the balance to 100 per cent. Each sample is based on determination of the

TABLE 1. *Changes in the frequencies (in per cent) of PP chromosomes in two replicate experimental populations of* Drosophila pseudoobscura *of mixed geographic origin (Texas PP by California AR)*

Days from start	Population No. 119	Population No. 120	Chi-Square	P
0	50.0	50.0	—	–
35	49.3	48.7	0.02	0.90
70	39.0	40.7	0.08	0.75
105	42.3	36.7	1.01	0.35
250	30.0	43.7	6.01	0.01
300	29.0	40.7	4.50	0.03
365	26.3	42.0	15.60	0.001
425	25.0	41.7	9.37	0.002

gene arrangement in 300 third chromosomes (150 larvae, taken in 6 subsamples on 6 successive days). The first samples, 35 days from the start, showed little change from the original frequencies, 50 per cent, of the chromosomes. At 70 and 105 days the frequencies of PP diminished, about equally in both populations, as shown by the low chi-square (each chi-square has one degree of freedom). But at 250 days the frequency of PP diminished in the population No. 119, while it failed to change, or even increased, in No. 120. This situation persisted until April 9, 1955, about 425 days from the start, when the last samples were taken and the populations were discarded. The chi-squares shown in table 1 attest that the outcomes of natural selection in these two experimental populations were clearly unlike. It should be noted that the magnitude of the divergence between the replicate populations Nos. 119 and 120 is not exceptionally great for the type of experiments in which flies from geographically remote localities are involved.

MAIN EXPERIMENTS

Certain consequences should follow from the above interpretation of the indeterminacy observed in populations of geographically mixed parentage. The indeterminacy should be a function of the genetic variability in the foundation stock
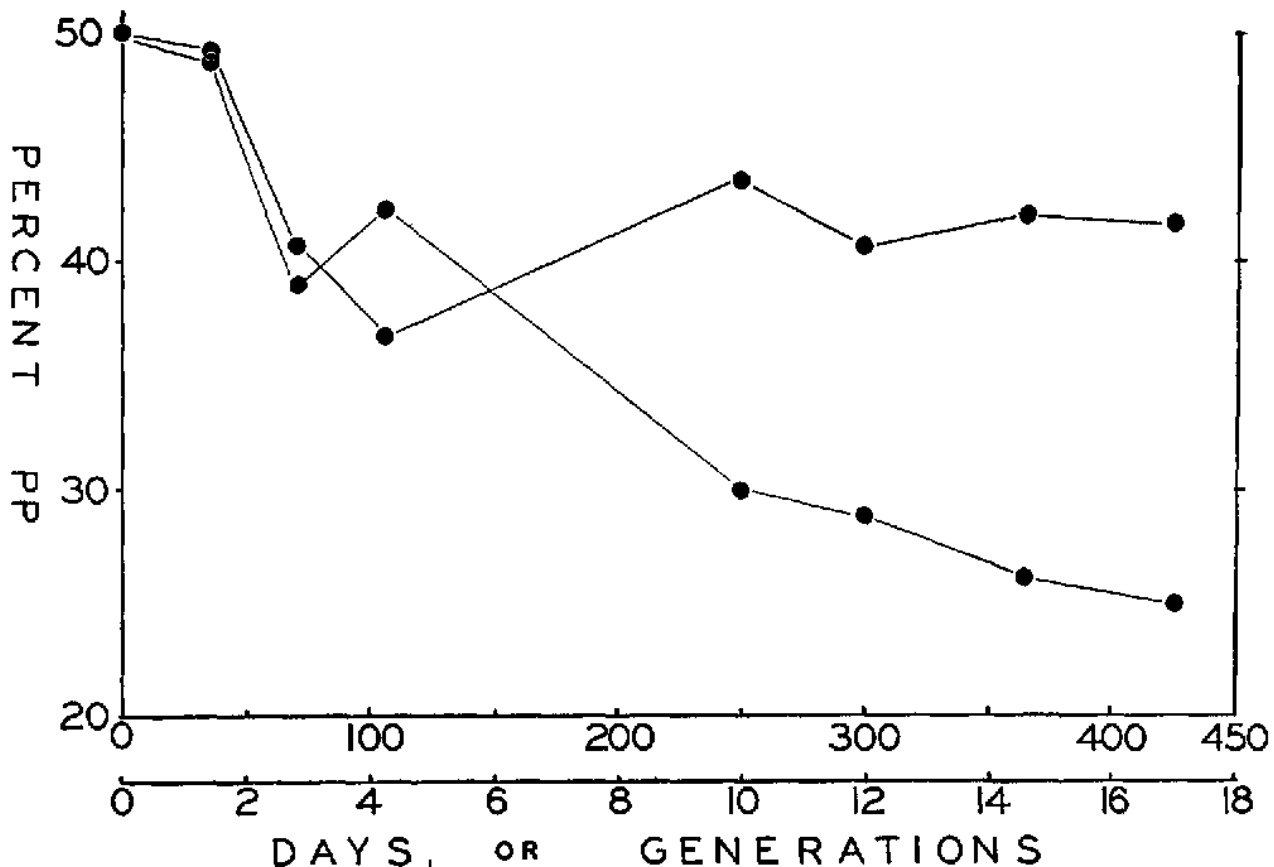

FIG. 1. Changes in the frequencies of PP chromosomes in two replicate experimental populations of mixed geographic origin (Texas by California).

of the populations. Chromosomes with PP and AR gene arrangements are recognizable under the microscope; their frequencies are made uniform in the foundation stock of all populations, and we observe changes in their frequencies as the experiment progresses. However, we infer that, apart from this overt variability in the frequency of the gene arrangements, there must exist also a large amount of genic variability released owing to gene recombination in the F_2 and later generations of interracial hybrids. Although there is no way of telling by how many genes the races differ, the number of the possible gene combinations must be several to many orders of magnitude greater than the number actually realized. The outcome of selection in the experimental populations should, then, be more variable in small than in large populations.

This working hypothesis is open to experimental test, but the experimental technique must be carefully thought through. One could make some experimental populations smaller than others by keeping them in cages of different sizes and with different amounts of food. The drawback of this would be that the environments of the populations of different sizes would be dissimilar. Therefore, we have chosen to vary the sizes of the foundation stocks of our populations, but to permit them to expand to equal size,

which, because of the high fecundity of the flies, they do within a little more than a single generation.

The same 12 Texas PP and 10 California AR strains were used in the main as in the preliminary experiments (see above). F_1 hybrids between them, which were necessarily heterozygous PP/AR, were raised in regular culture bottles, and so were the F_2 hybrids. In June 1955, 4,000 F_2 flies, about equal numbers being females and males and derived equally from the different crosses, were placed in a population cage. Between June 15 and 27, 15 cups with yeasted culture medium were inserted in the cage daily. The flies covered the medium with eggs overnight. The cups with the eggs were then withdrawn and placed in another population cage containing no adult flies. In this manner ten population cages, Nos. 145–154, were obtained on ten successive days. They were descended, then, from the same foundation stock of 4,000 F_2 interlocality hybrids. The frequencies of PP and AR chromosomes in the foundation stock are evidently 50–50. These are the "large" populations.

Ten groups of 20 F_2 flies each, 10 ♀♀ and 10 ♂♂, were taken from the same F_2 cultures which served as the source of the foundation stock for the "large" populations, care being taken to include in each group flies from all the F_2 cultures.

TABLE 2. *Frequencies (in per cent) of PP chromosomes in the experimental populations*

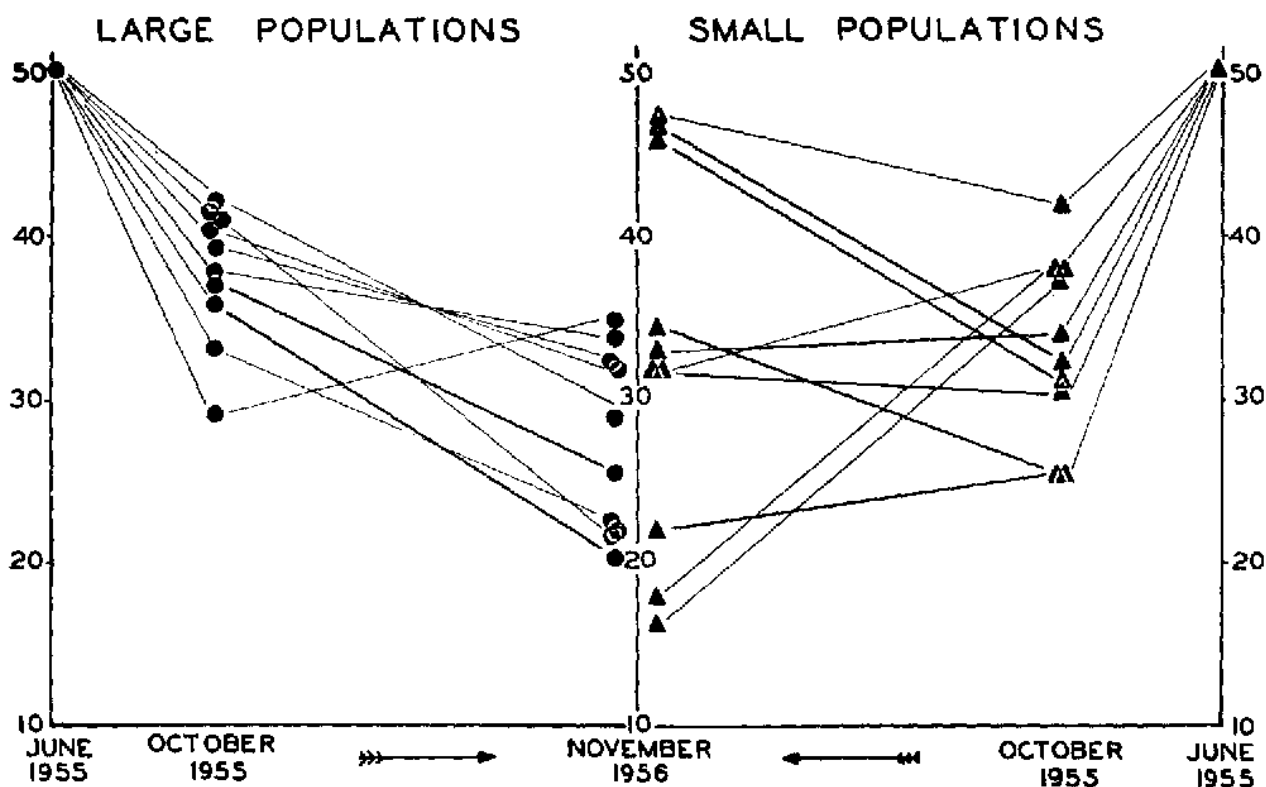| Large populations			Small populations		
No.	Oct. '55	Nov. '56	No.	Oct. '55	Nov. '56
145	39.3	31.7	155	37.7	18.0
146	42.3	29.0	156	30.7	32.0
147	29.3	34.7	157	31.0	46.0
148	38.0	34.0	158	32.3	46.7
149	33.3	22.7	159	34.3	32.7
150	36.0	20.3	160	41.7	47.3
151	40.3	32.0	161	37.3	16.3
152	41.0	22.3	162	25.3	34.3
153	37.0	25.7	163	37.7	32.0
154	42.0	22.0	164	25.3	22.0
Mean	37.85	27.44	Mean	33.33	32.73
Variance	15.30	26.96	Variance	26.73	118.91

FIG. 2. The frequencies (in percent) of PP chromosomes in twenty replicate experimental populations of mixed geographic origin (Texas by California).

These groups of F_2 flies were placed in regular culture bottles and allowed to produce progenies. Each progeny was then transferred to population cages of the same type as those used for the "large" populations. Ten population cages, Nos. 155–164, were thus obtained. They are the "small" populations. It should be reiterated that the "large" and the "small" differed only in the foundation stocks, these being 4,000 and 20 flies respectively. All populations were kept at 25° C. and treated similarly in every way.

In October and November 1955, about 4 generations after the start, the populations were examined; egg samples were taken and the chromosomes in the salivary gland cells of the larvae that hatched from these eggs were studied. The gene arrangements in the chromosomes were determined and scored by Dr. Louis Levine. The results are summarized in table 2 and figure 2. As usual, each sample consisted of 300 chromosomes. The frequencies of PP varied from 29.3% to 42.3% in the "large" populations, and from 25.3% to 41.7% in the "small" ones.

The heterogeneity is significant in both; the chi-square for the "large" is 19.5 and for the "small" ones 35.9, which correspond to probabilities of about 0.02 and of much less than 0.001 respectively. The heterogeneity among the replicate experiments is, of course, not surprising in view of the outcome of the preliminary experiments (table 1), although in these latter a significant heterogeneity first appeared after somewhat more than 4 generations from the start. It may be noted (table 2) that the variance for the "small" populations (26.7) is ostensibly greater than for the "large" ones (15.3), but the F value is not significant. It may also be noted that the frequencies of PP chromosomes have declined from the 50% value in the foundation stock, the decline being somewhat greater in the "small" (33.3%) than in the "large" (37.8%) populations.

The next, and the final, test of the populations was made in November 1956, i.e., more than a year after the first test and about 19 generations after the populations were placed in the population cages. The preliminary experiments

(table 1) show that the populations reach equilibria in the frequencies of PP and AR chromosomes within less than a year from the start. The samples, 300 chromosomes per cage, were taken in the usual manner and scored by one of us (Th. D). The results are reported in table 2 and figure 2.

It may be noted that the mean frequency of PP in the "large" populations is now 27.4% and in the "small" ones 32.7%. These means are not significantly different from each other, but the 1956 mean for the "large" populations is significantly lower than the 1955 mean. The outcomes of natural selection in the "large" and the "small" populations are, then, similar on the average. It is otherwise when the outcomes in the individual populations are considered. As shown in table 2 and figure 2, the frequencies of PP in the "large" populations range from 20.3% to 34.7%, and in the "small" ones from 16.3% to 47.3%. In both instances the heterogeneity is highly significant (the chi-square for the "large" population is 40.4 which, for 9 degrees of freedom, has a negligible probability of being due to chance. Both in the "large" and especially in the "small" populations the variance has increased during the year intervening between the two tests (1955–1956).

Most important of all is, however, that the "small" populations show a heterogeneity significantly greater than the "large" ones. The variances, 118.9 and 27.0, now give an F ratio of 4.4 which is significant at between the 0.025 and 0.010 levels. The greater heterogeneity is evidently due to the different magnitudes of the foundation stocks in these populations. This heterogeneity was indicated already by the tests in October 1955, but it has become significant as the selection continued during the year between the two tests. Finally, it may be pointed out that there appears to be no significant correlation between the status of a given population in 1955 and 1956. For example, No. 160 had the highest frequency of PP in both tests (Table 2), but No. 161 which had

the lowest frequency in 1956 had an above average frequency in 1955.

DISCUSSION

The results of the present investigation can be stated very simply: Although the trait studied (the gene arrangement in the third chromosome) is subject to powerful selection pressure, the outcome of the selection in the experimental populations is conditioned by random genetic drift. The either-selection-or-drift point of view is a fallacy.

In our experiments, the heterozygotes which carry two third chromosomes with different gene arrangements are heterotic; natural selection in the experimental populations establishes equilibrium states at which both gene arrangements occur with certain frequencies; these frequencies are determined by the relative fitness of the homozygotes and heterozygotes. Now, the environments being reasonably uniform in all experimental populations, the outcome of the selection processes in the replicate experiments should also be uniform. And so it is, in experimental populations of geographically uniform origin. But it is not so in geographically mixed populations. In the latter, the selective fates of the chromosomal gene arrangements become dependent upon the polygenic genetic background, which is highly complex and variable because of the gene recombination that is bound to occur in populations descended from race hybrids. Here random drift becomes operative and important. It becomes important despite the populations being small only at the beginning of the experiments, because the foundation stocks in some populations consisted of small numbers of individuals. Thereafter, all the populations expand to equal sizes, fluctuating roughly between 1,000 and 4,000 adult individuals. Such populations can be regarded as small only in relation to the number of gene recombinations which are possible in populations of hybrid origin.

For reasons that are not far to seek, geneticists visualize the evolutionary proc-

ess usually in terms of the destinies of single genes. With the notable exception of the contributions of Wright (1932 and subsequent work), this is the frame of reference of most of the mathematical theory of population genetics. This makes manageable an otherwise impossibly complex topic, and yet the oversimplified models usually suffice for understanding of microevolutionary processes. But as we move into the realm of mesoevolution (Dobzhansky, 1954), not to speak of macroevolution, it becomes indispensable to consider not only the destinies of single genes but also of integrated genotypes, and finally of the gene pool of Mendelian populations. In our experiments, the foundation stock of the populations consisted of F_2 hybrids between rather remote geographic races; a highly variable gene pool arose owing to the hybridization; random drift caused different segments of this gene pool to be included in the foundation stocks of each population, especially in the small ones; natural selection then produced divergent results in different populations, especially again amongst the small ones.

It is now logical to inquire whether the events observed in our experimental populations resemble situations which occur in nature. The excellent work of Dowdeswell and Ford (1952, 1953) and Ford (1954) has disclosed a most suggestive case. Populations of the butterfly *Maniola jurtina* are rather uniform throughout southern England, despite some obvious environmental diversity in different parts of this territory. In contrast to this, the populations of the same species show quite appreciable divergence on the islands of the Scilly archipelago, although these islands are within only a few miles of each other and their environments appear rather uniform. Especially remarkable is the divergence observed between the populations of certain small islands, while larger islands have more nearly similar populations. The small islands happen, however, to be situated between the larger ones. The investigators have estimated

that the populations of the small islands consist of numbers of individuals of the order of 15,000 and that the populations of the large islands must be considerably greater. The authors conclude that the genetic divergence between these populations must be produced entirely by selection, random drift being inconsequential. The evidence is, however, weighed in favor of the view that the genetic divergence was initiated by the island populations being derived from small numbers of immigrants from the mainland or from other islands. These immigrants introduced somewhat different sets of genes on each island, whereupon natural selection built different genetic systems in the different populations.

The divergence between island and mainland populations has been studied also by Kramer and Mertens (1938) and by Eisentraut (1950) in lizards, and by Lowe (1955) in mammals and reptiles. Most data agree in showing that the divergence is greater on smaller than on larger islands, and greater on islands more remote from the mainland than on those which are apt to receive immigrants most frequently. Many authors, including this writer (Dobzhansky, 1937, 1941), interpreted these situations as arising through random drift in populations of continuously small size, or frequently passing through narrow "bottlenecks." This interpretation need no longer be sustained. It is more probable, especially in the light of the experiments described in the present article, that in the island populations we are observing the emergence of novel genetic systems moulded by interaction of random drift with natural selection.

Mayr (1954) has pointed out that conspicuous divergence of peripherally isolated populations of a species is a fairly general phenomenon, well known to systematists. He rightly concludes that this divergence cannot be due entirely to random drift "in the ordinary sense," i.e., to fluctuations of the gene frequencies in populations of persistently small size. Indeed, some of the peripheral populations

consist of thousands or even millions of individuals. Mayr's interpretation can be stated best in his own words: "Isolating a few individuals (the 'founders'.) from a variable population which is situated in the midst of a stream of genes which flows ceaselessly through every widespread species will produce a sudden change of the genetic environment of most loci. This change, in fact, is the most drastic genetic change (except for polyploidy and hybridization) which may occur in a population, since it may effect all loci at once. Indeed, it may have the character of a veritable 'genetic revolution.' Furthermore, this 'genetic revolution.' released by the isolation of the founder population, may well have the character of a chain reaction. Changes in any locus will in turn affect the selective values at many other loci, until finally the system has reached a new state of equilibrium." The outcome of our experiments described above may, in a sense, be regarded as experimental verification of Mayr's hypothesis.

SUMMARY

Twenty replicate experimental populations of *Drosophila pseudoobscura* were kept in a uniform environment for approximately 18 months. The foundation stocks of all the populations consisted of F_2 hybrids between flies of Texas origin which had the PP gene arrangement in their third chromosomes, and flies of California origin with the AR gene arrangement in the same chromosome. In ten of the populations the founders numbered 4,000 individuals; the other ten populations descended from only 20 founders each.

The frequencies of PP and AR chromosomes in all the populations were originally 50 per cent. Eighteen months later, the frequencies of PP varied from about 20 to 35 per cent in the populations descended from the large numbers of founders, and from 16 to 47 per cent in those descended from small numbers of the founders. The heterogeneity of these frequencies of PP chromosomes observed in the replicate populations is statistically highly significant. More important still, the heterogeneity is significantly greater in the populations descended from small numbers of founders than in those descended from large numbers of founders.

Heterozygotes which carry a PP and an AR third chromosome are superior in adaptive value to the PP and AR homozygotes. Therefore, the frequencies of PP and AR chromosomes in the experimental populations are controlled by natural selection. However, the heterogeneity of the results in the replicate populations is conditioned by random genetic drift.

Only some of the possible combinations of the genes of the Texas and California genomes are actually realized in the populations. The segments of the gene pool which arise from race hybridization are smaller, and therefore less uniform, in the populations descended from small than in those descended from large numbers of founders. It may reasonably be inferred that evolutionary changes involving interactions of natural selection and random drift of the kind observed in our experiments are not infrequent in nature.

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