

INVERSION GRADIENTS AND NATURAL SELECTION IN ECOLOGICAL RACES OF *DROSOPHILA FUNEBRIS*

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INTRODUCTION

NATURAL populations of *Drosophila funebris*, like those of many other species of *Drosophila*, show variations in the gene arrangement in their chromosomes due to inversion of chromosome sections. DUBININ and TINIAKOV (1945, 1946a, 1946b, and unpublished data) have found that these chromosomal variants are by no means adaptively neutral but, on the contrary, have a profound ecological significance. Some inversions occur relatively more frequently in southern and others in northern localities, thus exhibiting climatic gradients. However, the overall frequencies of inversion heterozygotes found in a population depend on the degree of industrialization of the locality in which this population lives. Thus, the highest recorded frequencies of inversion heterozygotes have been found in the central part of the city of Moscow and in certain other industrial cities of U.S.S.R. In smaller towns, in garden-like cities like Alma Ata in Turkestan, in cities devastated by the war, and in suburban districts of large cities inversion heterozygotes are less frequent. Finally, in rural districts inversion heterozygotes are rare or even absent. Within a locality, the frequencies of inversion heterozygotes need not remain constant with time; changes from month to month connected with the seasonal succession of environments have been recorded. Experiments on hibernation of the flies have shown that some of the gene arrangements survive hibernation more frequently than others, and that the fecundity level of the survivors is higher than the average for the population before hibernation.

The above observations are strikingly paralleled by the quite independently conducted work of DOBZHANSKY (1943) and WRIGHT and DOBZHANSKY (1946) on *Drosophila pseudoobscura*. These authors have also found cyclic changes in the frequencies of inversions in populations of certain localities in California. These changes are induced by natural selection acting differently at some seasons of the year than at others. Furthermore, some of these changes have been reproduced experimentally in especially constructed population cages.

The discovery of the ecological significance of inversions found in natural populations of *Drosophila* opens the possibility of experimental approach to the study of evolutionary changes in free-living populations. It is possible to create in nature populations of a desired composition, and to follow the alterations which occur in such populations under the influence of natural agents. The present paper reports the results of an experiment of this kind conducted in 1945 at the Biological Station of our Institute at Kropotovo, 115 kilometers southwest of Moscow.

MATERIAL AND METHOD

Although several investigators have attempted to introduce into free-living populations certain laboratory mutants and to follow their subsequent fate, no experimental populations containing a genotypic diversity which has gone through a long process of adaptive evolution under natural conditions have ever been created in nature (WRIGHT and DOBZHANSKY (1946) however, have studied such populations in experimental cages in the laboratory).

GORDON (1935, 1939) released individuals of *Drosophila melanogaster* heterozygous for the mutant gene ebony at a certain locality in England. This species is not native to England and is introduced there accidentally by man. In 1934, GORDON placed 36,000 ebony heterozygotes into large jars. On May 15, 1934, these jars, now containing larvae and pupae as well as adults, were exposed in a garden. Flies were collected 128 days after the release at the point at which the flies had been liberated, as well as in a store and in a laboratory located within half a mile from the point of release. Eleven ebony heterozygotes were found among the 57 flies collected. Thus, the frequency of the gene ebony in the released population was 50 percent, and 128 days later it fell to between 10 and 15 percent. GORDON believes that this result is due to complete elimination of ebony homozygotes in every generation elapsed between the dates of the release and of the collecting of the flies. OLENOV and collaborators (1937) released a mixture of wild type and of the dominant mutant Bar of *Drosophila melanogaster* in the basement of a house. As could be expected, the Bar mutant was very soon crowded out by the wild type.

GERSHENSON (1941) released near Kiev some *Drosophila virilis* homozygous for the autosomal recessive veinlet (shortened wing veins). This species does not occur naturally near Kiev. The author is of the opinion that the dispersal capacity of veinlet individuals varies in proportion to the degree of the expression of this mutant. Four flies, all of them having a slight expression of veinlet, were collected 15 months after the release. GERSHENSON drew the conclusion that the veinlet character underwent an evolution in the direction of its suppression by the action of natural selection.

A different type of analysis of artificial free-living mutant populations was inaugurated by the work of the TIMOFEEFF-RESSOVSKYS (1940). These authors released known numbers of individuals of *Drosophila melanogaster* and *Drosophila funebris* marked with suitable mutant characters at the center of an experimental field consisting of traps placed checker-board fashion at ten-meter distances from each other. Fields of 63 traps (60×80 meters), or of 121 traps (100×100 meters), were used. The mutual flies barely reached the boundaries of these fields at the end of the experiments. The authors concluded that the diffusion rates of both species are so low that populations partially isolated from each other by distance may be formed on very small territories.

DOBZHANSKY and WRIGHT (1943) studied the same problem in *Drosophila pseudoobscura*. Experimental fields consisting of two lines of traps intersecting at right angles were built in localities in which the species normally occurs. The

intervals between traps were 20 meters, and the longest files of traps used were 1000 to 1400 meters long. Individuals of the mutant orange (an eye color) were released at the center of the cross-shaped experimental fields. The numbers of orange and of wild type flies that entered the traps one, two, three, etc. days after the release of the mutants were recorded. One day after the release, most orange-eyed flies were found close to the point of the release, although a few individuals had migrated for longer distances. On subsequent days the distribution curves of the orange flies became progressively more flat. An analysis of the data showed that the rate of migration of the flies is a function of temperature. Below 60°F the movements are very slow. At 70-71°F the flies traveled about 130 meters per day. The number of the mutants present on the experimental fields declined by about eight to nine percent per day. The absolute density of the wild flies is estimated to have been about 400 per 10,000 square meters in June, and 800 per 10,000 square meters in July. DOBZHANSKY and WRIGHT conclude that the rates of dispersal of *Drosophila pseudoobscura* are of a higher order of magnitude than those of *Drosophila funebris* and *Drosophila melanogaster*.

The above attempts to analyze the evolution of mutant populations have dealt with laboratory mutants. The drawback of these techniques is that the mutants involved are deleterious to the organism; they have not been subjected to evolutionary trials in nature and have not become a part of the systems of adaptive polymorphism in the species concerned. Such experiments give only one-sided models of the evolutionary changes in the populations. In our opinion, the materials chosen for studies on the interactions of ecological factors and of natural selection in free-living populations ought to come from among the genetic variants that constitute an integral part of the natural populations.

Therefore, we have made use of the inversion II-1 in *Drosophila funebris*. As shown by DUBININ and TINIAKOV (1945, 1946a, 1946b), this inversion forms a constant adaptive element in the populations of this species. It has a definite ecological optimum, and it has undergone a long evolutionary course in the species concerned. The inversion homozygotes, as well as the heterozygotes and the standard homozygotes, can be recognized readily and quite objectively in the salivary gland preparations. The strains containing this inversion were obtained from flies collected in the central part of the city of Moscow, where the II-1 inversion is very common.

EXPERIMENTS

On May 18, 1945, 10,000 *Drosophila funebris* flies known to have been homozygous for the inversion II-1 were brought to the Biological Station at Kropotovo. These parent flies were placed into three large earthenware jars with fly food medium for propagation. The jars were kept in a warm room. Early in June, the flies then present in the jars were permitted to escape outdoors. A total of about 100,000 flies (estimated by counting the puparia in the jars) were thus liberated.

Since the flies released were phenotypically identical with the wild flies of the

same species constituting the local populations, the fate of the released flies had to be followed by means of cytological examinations. The karyotype of the local Kropotovo population was first examined in 1940 by G. G. TINIAKOV. Among the 108 flies then examined, one fly was heterozygous for the inversion II-1. The frequency of heterozygotes was, therefore, 0.92 percent. A more extensive sample of the Kropotovo population was examined in 1944. Nine heterozygotes were found among 1286 flies, which amounts to 0.70 percent. These are about the normal frequencies of inversion II-1 heterozygotes in the rural areas around Moscow. The Kropotovo population is a stable rural race of *Drosophila funebris*.

In July, a sample of 114 flies was collected at the Biological Station, and an analysis of the gene arrangements in them was made. In this sample, 28 flies were inversion homozygotes, 57 flies were heterozygotes, and 29 Standard homozygotes. The gametic frequency of the II-1 inversion, consequently, is 49.5 percent, instead of the 0.35 percent present before the release of the experimental flies. It may be noted that the populations which inhabit the central part of the city of Moscow have about 50 percent frequencies of this inversion. The release of the experimental flies, therefore, has caused the appearance in the neighborhood of the Biological Station near Kropotovo of a population which has a composition typical for a large industrial city. However, this population now exists in a rural environment.

It is important to note that the inversion and Standard homozygotes and heterozygotes in the above sample have frequencies that agree very well with those expected on the basis of the Hardy equilibrium theorem, as follows:

	INVERSION HOMOZYGOTES	HETEROZYGOTES	STANDARD HOMOZYGOTES
Observed	28	57	29
Expected	28.5	57	28.5

This circumstance is very important, because it shows that the crossing of the released flies with wild ones resulted in the formation of a new random breeding population.

The Biological Station is situated close to the western margin of the village of Kropotovo. This village is bisected by a deep ravine overgrown by trees. On the other side of Kropotovo, about one kilometer distant from the Biological Station, there lies the village Besovo, separated by a cemetery and a road flanked by trees. To the west of the Biological Station, and separated by a forest with deep ravines, likewise about one kilometer distant from the Biological Station, is the village Maloe Kropotovo. These localities are inhabited by a group of interconnected micropopulations of *Drosophila funebris*. Other villages and populations of the fly lie relatively far away (5, 8, and 10 kilometers) from the above system of micropopulations. The area occupied by this system is at least 400,000 square meters (2 kilometers long and 200 meters wide).

Population samples were taken at the Biological Station in August, Septem-

ber, and October, in the near portion of Kropotovo in July, August, and September, in the more remote part of Kropotovo in July and August, in Besovo in August and September, and in Maloe Kropotovo in August and September. The results of the analyses of the chromosomes in these samples are summarized in table 1. This table shows the observed numbers of inversion

TABLE 1

Observed and expected numbers of Inversion and Standard homozygotes and heterozygotes found in different collecting localities in different months.

LOCALITY AND MONTH		STANDARD HOMOZY- GOTES	HETERO- ZYGOTES	INVERSION HOMOZY- GOTES	q	FLIES EXAM- INED
Biological Station, July	Obs	29	57	28	49.5	114
	Exp	28.5	57.0	28.5		
Biological Station, August	Obs	70	52	4	23.8	126
	Exp	72.8	46.0	7.2		
Biological Station, September	Obs	144	73	1	17.2	218
	Exp	150.0	61.5	6.4		
Biological Station, October	Obs	187	43	1	9.7	231
	Exp	187.4	41.3	2.2		
Near Kropotovo, July	Obs	70	55	4	24.4	129
	Exp	73.8	47.6	7.6		
Near Kropotovo, August	Obs	80	63	7	25.7	150
	Exp	82.8	57.3	9.9		
Near Kropotovo, September	Obs	10	5	0	16.7	15
	Exp	10.8	3.9	0.3		
Far Kropotovo, July	Obs	52	16	2	14.3	70
	Exp	51.8	16.8	1.4		
Far Kropotovo, August	Obs	88	26	1	12.2	115
	Exp	89.0	24.2	1.7		
Besovo, August	Obs	37	9	0	9.8	46
	Exp	37.3	8.2	0.5		
Besovo, September	Obs	67	4	0	2.8	71
	Exp	68.2	2.8	0.05		
Maloe Kropotovo, August	Obs	12	5	0	14.7	17
	Exp	12.5	4.2	0.4		
Maloe Kropotovo, September	Obs	107	8	0	3.5	115
	Exp	109.1	5.8	0.1		

and Standard homozygotes and of heterozygotes, and the expected numbers computed according to Hardy's formula. The column marked "q" gives the gametic frequencies of the II-1 inversion in percentages. The data in table 1 indicate the diffusion rates of the inversion in the micropopulations studied, as well as the dynamics of the concentrations of this inversion at different times.

The release of the experimental flies caused the frequency of the inversion at

the Biological Station to rise from its normal value of 0.35 percent to 49.5 percent in July. The frequency in the near part of Kropotovo in July was lower than at the Biological Station—namely, 24.4 percent. In the more remote part of Kropotovo it was still lower—namely, 14.3 percent. Thus we have a clear picture of the diffusion of the inversion proceeding from the point of the release, at the Biological Station, and permeating the surrounding regions.

In August the concentration of the inversion at the Biological Station fell to 23.8 percent, and in near Kropotovo it stayed at about the same level—25.7 percent. In Far Kropotovo it was 12.2 percent, in Besovo 9.8 percent, and in Maloe Kropotovo 14.7 percent. The rate of diffusion of the inverted chromosomes through our system of micropopulations is noteworthy. In only two months after the release of the experimental flies, all the populations showed the inverted and the Standard gene arrangements to be not only present but to occur both as homozygotes and heterozygotes in relative frequencies conforming to Hardy's equilibrium. Unfortunately, we have not followed the rates of diffusion, because the high rate of dispersal which has been observed was unexpected by us. However, the rates of diffusion can be estimated indirectly. The distribution of homozygotes and heterozygotes in accordance with Hardy's equilibrium can become realized only in two generations. About 40 days are required for the development of two generations in nature. Since a random distribution has been found 60 days after the release of the flies, the released flies must have become dispersed in all populations in at most 20 days. Since the Biological Station is located in the center of the system of populations studied, the migration rate must amount to 50–100 meters per day on the average. Our evidence, consequently, contradicts that of the TIMOFEEFF-RESSOVSKYS, according to whom *Drosophila funebris* migrates about four meters per day. Nor can we agree with the inference of DOBZHANSKY and WRIGHT (1943) that *Drosophila funebris* is a much more slowly dispersing form than *Drosophila pseudoobscura*. Both species seem to migrate about equally fast.

The fact that on all months the Hardy's equilibrium proportions were realized in all localities suggests that the populations of these localities experience no appreciable migration pressure. It seems that in July, August, September, and October our system of micropopulations is broken up into separate foci without much interchange of individuals among them. This, in turn, suggests the existence of seasonal phases in the migration of the flies. Intense movements of the flies took place early in June after their liberation. It is possible that these intense movements were due to a scarcity of food and of breeding places at that time. In two or three weeks the released flies became distributed, though not uniformly, in the whole system of populations. This period of migration was then followed by a period of more sedentary existence, possibly caused by abundance of food in the environment.

Assuming that the above hypothesis of a sedentary period is correct, it follows that the changes in the incidence of the inversion which took place in subsequent months were due to the action of natural selection. Indeed, the

concentrations of the inversion fell with time (table 1). At the Biological Station, the inversion frequency was 49.5 percent in July, 23.8 percent in August, 17.2 in September, and 9.7 in October. Similar trends toward decrease of the concentration of the inversion were observed also in other localities.

Table 1 shows the observed numbers of the three possible karyotypes (inversion and Standard homozygotes and heterozygotes) in each population sample examined, as well as the expected frequencies of these karyotypes calculated with the aid of Hardy's equilibrium formula. A comparison of the observed and the expected figures indicates a systematic excess of heterozygotes and a deficiency of both classes of homozygotes. This suggests that natural selection has a differential influence on these karyotypic groups, favoring the heterozygotes at the expense of the homozygotes. Table 2 shows the

TABLE 2

Expected and observed numbers of homozygotes and heterozygotes in the combined data.

	STANDARD HOMOZYGOTES	HETERO- ZYGOTES	INVERSION HOMOZYGOTES
Observed	924	359	20
Expected	949.1	317.5	36.4
Deviation	-25.1	41.5	-16.4
Deviation in percentage of the expected number	2.8%	13%	45%

total numbers of homozygotes and heterozygotes found in all population samples except that taken in July at the Biological Station. Numbers of the homozygotes and heterozygotes expected on the basis of Hardy's equilibrium are also given. It can be seen that the deficiency of homozygotes for the Standard gene arrangement amounts to only 2.8 percent of the expected number, while that of the inversion homozygotes is 45 percent. In other words, the negative selection directed against the inversion homozygotes is sixteen times stronger than that against the Standard homozygotes.

The heterozygotes are favored by the natural selection at work in the populations studied. The excess of the heterozygotes amounts to 13 percent of their expected number. It is evident that we are dealing here with a case of an intrapopulation heterosis. The increased survival of heterozygotes retains the newly introduced inversion in the population. The selection directed against the inversion homozygotes, however, is so intense that all the populations undergo changes toward decreases of the frequencies of the inversion.

DISCUSSION

If individuals of *Drosophila funebris* homozygous and heterozygous for the inversion II-1 and for the corresponding Standard gene arrangement are induced to hibernate, inversion homozygotes and heterozygotes are discriminated against by natural selection to almost the same degree (DUBININ and TINIAKOV 1946a). Here, then, the genetic contents of the inversion act as a dominant to those of the Standard chromosomes. But the action of selection is

quite different in summer, particularly during the period when the populations of the species expand in numbers. For at this time the Inversion/Standard heterozygotes possess the highest adaptive value, the inversion homozygotes the lowest, and the Standard homozygotes an intermediate one. This difference in the action of natural selection during hibernation on one hand and during the time of the expansion of the populations in summer on the other shows how complex may be the influence of natural selection on the genetic structure of populations in nature. It may be noted here that while in the city populations the frequency of the inversion heterozygotes waxes from month to month during the summer, this is not the case in rural populations. In the latter the concentration of the inversion is lower than in the city, and it does not change appreciably during the summer. A somewhat analogous situation has been discovered by DOBZHANSKY (1943) and WRIGHT and DOBZHANSKY (1946) in *Drosophila pseudoobscura*; here certain inversion heterozygotes also possess an adaptive value much higher than the corresponding homozygotes during a part of the annual seasonal cycle (spring and summer), while during other seasons (autumn and winter) they seem to be equivalent. Furthermore, the least favored homozygote during the spring season becomes the relatively more favored one during the summer.

The data presented in this paper establish, independently from the similar findings of WRIGHT and DOBZHANSKY (1946), the fact of intrapopulational heterosis involving an inferiority of inversion homozygotes to inversion heterozygotes. This confirms the theoretical conclusions reached by STURTEVANT and MATHER (1938), MALINOVSKY (1940), WRIGHT and DOBZHANSKY (1946), and others. S. J. BESSMERTNAYA (unpublished), working in our laboratory, obtained in 1939-1940 experimental results indicating that in *Drosophila melanogaster* there is a preferential survival of heterozygotes for the inversions CIIL-CIIR. In these experiments she used artificial populations kept in population cages of the type proposed by L'HERITIER and TEISSIER. Experiments of this type have been more recently developed by WRIGHT and DOBZHANSKY (1946).

The data on the intrapopulational heterosis prove that the persistence of low concentrations of inversions in rural populations of *Drosophila funebris* is caused by natural selection favoring the respective inversion heterozygotes, with selection against the homozygotes inhibiting the growth of the concentrations of these inversions. Although it is possible that the interrelations between the environment and the inversions are more complex than indicated above, the importance of heterosis is evident.

SUMMARY

In early June, 1945, a total of about 100,000 *Drosophila funebris* flies homozygous for a certain inversion were liberated at the Biological Station near Kropotovo, 115 kilometers southwest of Moscow. In July, the descendants of the released flies were present in the populations of localities about one kilometer distant from the point of release. The relative frequencies of the

inversion homozygotes and heterozygotes was found to be approximately that expected on the basis of the Hardy's equilibrium formula, with the heterozygotes being somewhat more frequent. It is estimated that the rate of diffusion of *Drosophila funebris* during the early part of the summer amounts to about 50-100 meters per day on the average.

Natural selection favors the inversion heterozygotes more than it does the Standard homozygotes, and the latter more than the inversion homozygotes. We are dealing here with a case of intrapopulation heterosis. This phenomenon is very important in the dynamics of natural populations. The action of natural selection, however is, far more complex than the data on the intrapopulation heterosis would by themselves suggest. Thus, during hibernation both inversion homozygotes and heterozygotes are discriminated against in favor of the Standard homozygotes. The action of natural selection is also different in rural and in urban populations.

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