*ARTIFICIAL AND NATURAL SELECTION FOR TWO BEHAVIORAL TRAITS IN DROSOPHILA PSEUDOOBSCURA**

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Communicated October 23, 1968

Abstract and Summary.—In an attempt to elucidate the genetic architecture of two behavioral traits, populations of *Drosophila pseudoobscura* were selected for positive and for negative phototaxis and geotaxis.

The selected populations diverged rapidly in their behavior (Figs. 1 and 2). The selection was relaxed after 20 generations in the phototactic, and after 30 generations in the geotactic populations. The relaxation resulted in convergence almost as rapid as was the divergence under selection. The average phototactic and geotactic neutrality of natural populations is an adaptive trait protected by genetic homeostasis. This does not preclude rapid responses to artificial, and presumably to natural, selection.

Hirsch and his students[1-4] have constructed an ingenious apparatus—classification mazes—in which the reactions of *Drosophila* flies to gravity and light may be studied quantitatively. Using these mazes, they have selected populations of *Drosophila melanogaster* genetically geopositive or geonegative, and photopositive or photonegative. Our experiments were made on *D. pseudoobscura* with mazes modeled on those of Hirsch and of Hadler.[4] We also have obtained geopositive, geonegative, photopositive, and photonegative populations.[5-8] Benzer,[9] using a countercurrent distribution technique, selected mutants in *D. melanogaster* which reacted differently to light. If we understand his technique correctly, he may be dealing with kinds of behavior not identical with those studied in Hadler's and in our experiments—an escape reaction rather than a choice by the flies of light or of dark passages. However that may be, the behavioral variations involved clearly have genetic components.

When tested in the Hirsch-Hadler classification mazes, most strains of *D. pseudoobscura* are, on the average, neutral to light and to gravity.[10] Strains of a related species, *D. persimilis*, are on the average photopositive, but neutral to gravity.[10] The behavior of all populations with which experiments were made proved to be easily modifiable, in the positive as well as in the negative directions, by artificial selection. The average neutrality of wild flies is evidently the result of a balance between the genetically positive variants and the genetically negative ones. Though we do not know why this should be so, populations of *D. pseudoobscura* in nature must be maintained by natural selection in a state of average photo- and geoneutrality. The experiments reported in this article were designed to throw some light on the genetic architecture of the populations responsible for this situation.

Materials and Methods.—The experiments were started in April 1965, with F_2 hybrid flies obtained by crossing ten strains of *D. pseudoobscura* homokaryotypic for the *AR* gene arrangement with ten strains homokaryotypic for the *CH* gene arrangement in the third chromosomes. All strains were derived from flies collected at Piñon Flats, Mount

San Jacinto, California. Photographs of the mazes used have been published.[6, 8] In every generation approximately 300 virgin females and 300 males from each population were run separately through either the phototaxis or the geotaxis maze; 25 females and 25 males which showed the most pronounced positive or negative behavior were selected to serve as progenitors of the next generation.

The mazes are so constructed that the flies assort themselves into 16 terminal tubes. Tube no. 1 is reached by flies making 15 upward choices (on the geotaxis maze) or 15 dark choices (on the phototaxis maze). Tube no. 16 is reached by 15 downward or 15 light choices. Equal numbers of upward and downward or dark and light choices lead to the tubes nos. 8 and 9. The mean phototactic and geotactic scores are computed from the number of flies which have entered the different terminal tubes. A mean score of 8.5 corresponds to photo- or to geotactic neutrality.

Results.—The experiments were carried for 42 generations. The mean phototactic and geotactic scores of the females are reported in Table 1, while those of the males are shown graphically in Figures 1 and 2. The founder populations were, within the limits of sampling errors, on the average neutral to light and to gravity, the means for the females being 8.7 and 8.2, and for the males 8.6 and 8.7. The selection resulted in clear-cut divergence. In the populations selected for positive and for negative phototaxes the divergence became so great that by about the 15th generation very few flies entered the tubes in the middle of the mazes (nos. 7–10). In other words, the variation curves became scarcely overlapping. The populations selected for positive and negative geotaxes are characterized by appreciably greater variances than those selected for the phototaxes; the distributions of the geopositive and geonegative flies continued to overlap in the neutrality zone until the end of the experiments.

Realized heritability was computed for the first 15 generations of the selection as explained in reference 6. The results are summarized in Table 2 (we are obligated to Mr. Rollin Richmond for making the necessary calculation on a Data Processing computer). The heritability of the reaction to light is clearly greater than that of the reaction to gravity, but in both cases it is quite low

TABLE 1. *Mean scores of females selected for positive and negative phototaxis and geotaxis.*

	Phototaxis		Geotaxis			Phototaxis		Geotaxis	
Generation	Positive	Negative	Positive	Negative	Generation	Positive	Negative	Positive	Negative
P	8.7	8.7	8.2	8.2	18	13.4	2.7	13.2	4.5
1	7.8	6.8	10.7	7.9	19	14.4	2.2	12.9	4.5
2	9.1	6.9	10.0	7.8	20	13.6	2.6	12.4	4.7
3	8.6	5.4	10.4	7.7		Relaxation			
4	10.3	5.5	11.1	7.1	21	—	—	14.6	4.3
5	10.8	5.1	10.3	5.9	22	—	—	13.5	4.7
6	10.4	5.8	10.3	6.1	23	—	—	13.6	3.8
7	11.2	5.0	11.9	8.4	24	—	—	13.8	3.6
8	10.7	5.0	11.2	6.0	25	13.3	3.1	14.1	4.0
9	12.3	5.4	12.0	5.2	26	—	—	13.7	2.9
10	11.4	4.4	11.3	8.2	27	—	—	13.4	3.7
11	13.1	4.8	12.2	7.9	28	—	—	13.6	4.0
12	13.2	3.2	11.6	5.9	29	—	—	13.8	4.8
13	13.2	3.7	11.4	5.9	30	12.3	6.1	14.2	5.2
14	12.4	3.3	12.3	6.5				Relaxation	
15	13.4	2.4	12.1	4.7	31	11.5	4.3	—	4.2
16	14.4	2.8	12.4	5.4	35	9.5	6.7	13.1	7.3
17	14.2	2.9	12.6	5.1	40	11.1	9.0	12.8	8.6

compared to most traits that are used in selection experiments. The reason we were nevertheless able to obtain a striking divergence is the intensity of the selection, made possible by the high efficiency of the Hirsch mazes.

After approximately 15 generations, the selections were making little or no progress. The populations had reached selection "ceilings" or "plateaus." In the populations selected for geotaxis, an attempt was made to break through these ceilings by further increasing the intensity of the selection. The mazes are so constructed that no fly going through a maze once can make more than 15 upward or downward choices. From the 20th generation on, we accordingly resorted to the following procedure. As before, 300 females and 300 males were run through the geotaxis mazes. After the flies had assorted themselves in the terminal tubes (which took about 24 hr), the 100 females and 100 males that had entered the two uppermost or the two lowermost tubes (nos. 1 and 2, or 15 and 16) were taken and run through the mazes again, the two sexes of course separately. As before, 25 "best" females and 25 "best" males were selected to be the parents of the next generation. With this procedure, a fly that had entered tube no. 1 twice had made not 15 but 30 upward choices, and a fly found twice in tube no. 16 had made 30 downward choices. This was continued for ten generations, from the 20th to the 30th, in the populations selected for geotaxis.

TABLE 2. *Estimates of heritability realized during the first 15 generations of selection.*

Selection	Phototaxis	Geotaxis
Positive, ♀♀	0.0996 ± 0.0092	0.0280 ± 0.0057
" ♂♂	0.1005 ± 0.0083	0.0206 ± 0.0067
Negative, ♀♀	0.0900 ± 0.0112	0.0244 ± 0.0111
" ♂♂	0.0630 ± 0.0098	0.0336 ± 0.0089

In Figure 2, the triangles connected by solid lines represent the mean scores in the geotaxis mazes obtained after single runs. The circles in the same figure represent the mean scores of the 100 "best" flies after the second consecutive runs. It can be seen that, with a single exception (23rd generation in the negative selection population), the second runs "improved" the scores. This clearly means that whatever causes, genetic or environmental, make a fly choose upward or downward passages in the maze on the first run continue to operate on the second run as well. Some, though rather slight, further progress of the selection was observed in the negative population.

In the 20th generation the selection was relaxed in the populations selected for phototaxis, and in the 30th generation in those selected for geotaxis. In the following 20 generations in the former, and in 10 generations in the latter, the populations were perpetuated by transferring about 500 randomly chosen flies, females and males together, to a fresh population cage. The generations were thus discrete as before; however, since the amount of food remained constant, the populations were more crowded. From time to time, groups of virgin females and males were selected and run through the mazes to test their reactions to light or to gravity. These tests were made in the 25th, 30th, 31st, 35th, and 40th generations with flies selected formerly for phototaxis, and in the 31st, 35th, and 40th generations with those selected for geotaxis.

As can be seen in Figures 1 and 2 (points connected by dashed lines) and in Table 1, the relaxation resulted in rapid loss of the divergence achieved by the artificial selection. The selection gains were lost most rapidly in the populations selected in the negative direction; the point of convergence will presumably be on the positive side of neutrality. The rapidity with which the relaxation of selection led to the convergence is impressive. In the population previously selected for 20 generations for negative phototaxis, the rate of loss on relaxation was greater than the rate of gain under artificial selection (Fig. 1). A similar situation seems to be observed in the population selected for negative geotaxis (Fig. 2). Natural selection opposes the changes in the negative direction more strongly than those in the positive one.

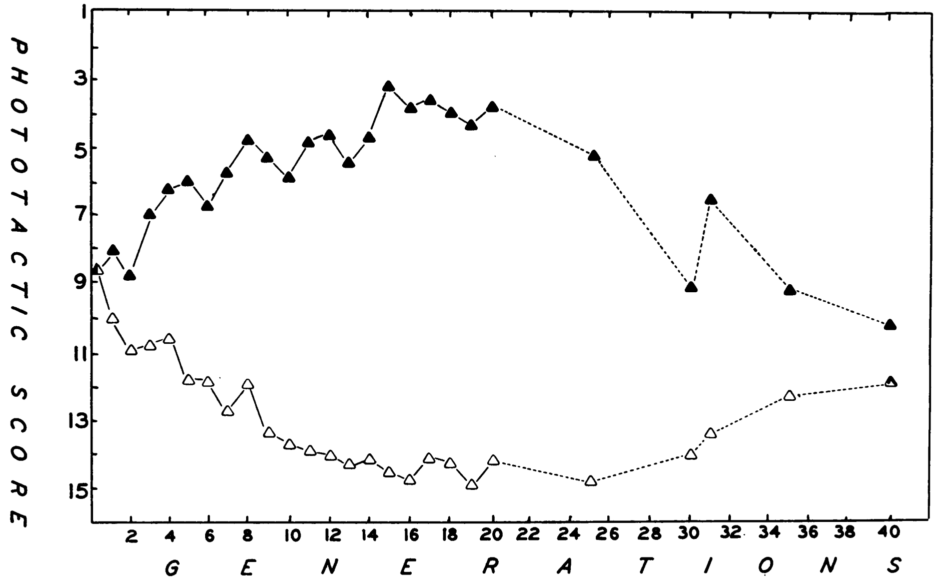

FIG. 1.—Selection for phototaxis. Positive, open triangles; negative, solid triangles. Dashed line indicates that selection was relaxed.

The flies in the populations whose behavior was altered by selection did not become easily distinguishable morphologically. However, a careful biometric study by Pasteur[11] has detected several slight but significant changes. Thus, the body size in the populations selected for positive geotaxis was greater than in the negative ones. However, this difference proved to be unstable after selection was relaxed. Other correlated effects of selection for behavior included slight changes in eye size, numbers of branches in the aristae, testis color, and wing venation. These changes also showed some indications of instability after relaxation of selection. A further correlated effect concerns the chromosomes. As stated above, in the *Materials and Methods* section, all populations were polymorphic for the *AR* and *CH* gene arrangements in the third chromosomes. The initial frequencies were 50 per cent of each. Tests made in the 15th and the 41st generations, using samples of 300 chromosomes per population, gave the following percentages of *AR* chromosomes:

	Generation 1	*Generation 15*	*Generation 41*
Phototaxis, positive	50.0	86.3	94.7
“ negative	50.0	73.7	79.0
Geotaxis, positive	50.0	85.3	82.7
“ negative	50.0	32.0	68.3

The chromosomal polymorphism in populations not selected for the behavioral traits has been extensively studied; in experimental populations kept at 25°C on standard laboratory food media, equilibria are reached at the level of 70–80 per cent *AR* chromosomes. The value of 32 per cent in the 15th generation of the population selected for negative geotaxis is thus strikingly low. However, after the relaxation of selection for geotaxis, this population rapidly lost its exceptional chromosomal composition, and in the 41st generation was close to what is expected without selection for geotaxis. In contrast, the population selected for positive phototaxis showed a prevalence of *AR* chromosomes, and this prevalence was conserved or increased after the relaxation of selection for phototaxis.

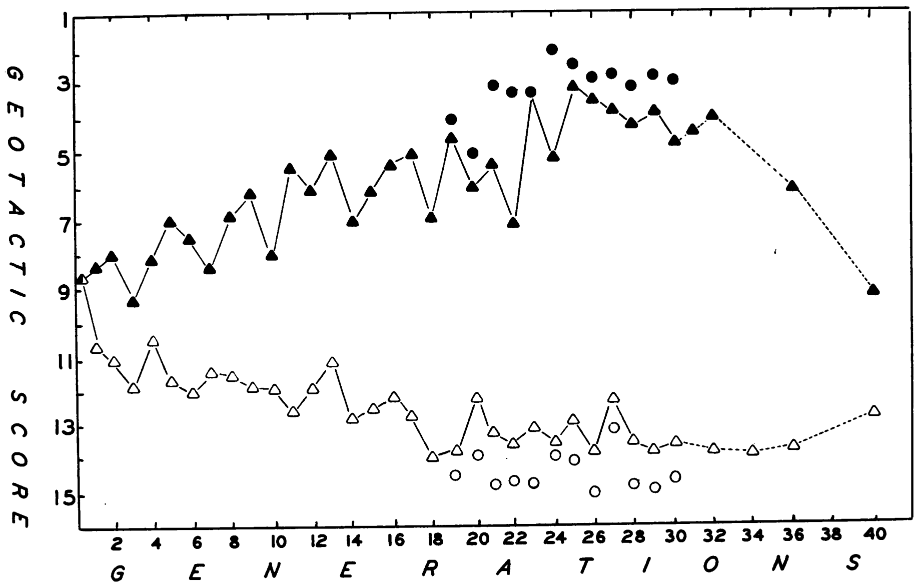

Fig. 2.—Selection for geotaxis. Positive, open triangles; negative, solid triangles. Mean scores on retests of 100 “best” flies, circles. Dashed line indicates that the selection was relaxed.

Conclusions.—Lerner[12] has defined genetic homeostasis as “the property of the population to equilibrate its genetic composition and to resist sudden changes.” Genetic homeostasis is manifested in that “attempts to shift populations too rapidly and too far from adapted mean values for specific traits, either by artificial selection or by changes in the breeding system, are counteracted by natural selection which is directed toward the maintenance of a phenotypic balance between fitness-determining characters." In our experiments, changes in either direction from the average photo- and geotactic neutrality were induced by artificial selection, and rapidly counteracted by natural selection after the artificial selection was relaxed.

While it is probable that average photo- and geotactic neutrality is adaptive in nature, it seems quite unlikely that it is directly selected for in the flies living in laboratory bottles and population cages. The striking genetic homeostasis displayed in our experiments is due to correlated effects of the selection. One of these can be specified with confidence—the abnormal equilibrium of the chromosomal polymorphs. We may then infer that the neutrality is not genetically fixed by making the populations homozygous for some "optimal genotype" that guarantees such neutrality. Far from this, the populations contain ample genetic variance affecting behavior. If the environment happens to favor phototactic or geotactic positivity or negativity, the population can respond rapidly to the natural selection that this environment brings into action. It can equally easily change back to the original state if the environment changes. Genetic plasticity is thus combined with high adaptedness.

Professor J. Hirsch has kindly informed us that according to his unpublished data, populations of *Drosophila melanogaster* selected for positive and for negative geotaxis tend to relapse toward neutrality following relaxation of the artificial selection.

* Work supported under contract no. AT-(30-1)-3096, U.S. Atomic Energy Commission.

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