A GENETIC ANALYSIS OF PHOTOTACTIC BEHAVIOR IN *DROSOPHILA MELANOGASTER* I. SELECTION IN THE PRESENCE OF INVERSIONS[1]

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ABSTRACT

The effectiveness of selection for positive and negative phototactic behavior in populations of *Drosophila melanogaster* heterozygous for various multiple inversions was compared using the method of realized heritability. Selection in the presence of *FM6*, *SM1* or *TM3* alone was as effective as in populations carrying no inversions. However, the presence of *FM6* and *TM3* together reduced the effectiveness of selection for photopositive behavior and *FM6* and *SM1* and *TM3* restricted the response to selection for negative phototactic behavior. The results are discussed in terms of the organization of genes influencing phototactic behavior in this species.

HADLER (1964a) described a multiple unit maze for the quantitative classification of the phototactic behavior of *Drosophila melanogaster*. Wild-type *Drosophila melanogaster* tend to be slightly photonegative when tested in the maze, but much individual variation exists. This individual variation in phototactic behavior has permitted the creation by artificial selection of highly divergent photopositive and photonegative strains of flies (HADLER 1964b; DOBZHANSKY and SPASSKY 1967, 1969). The rate of divergence during selection is gradual. Heritability of phototaxis in Drosophila is low (RICHMOND 1969; DOBZHANSKY and SPASSKY 1967). These observations, in addition to the results of hybridizations of divergent phototactic strains of flies (HADLER 1964b; WOOLF 1972) suggest that phototactic behavior in Drosophila is polygenic.

Without genetic variation, selection cannot successfully operate. In short-term selection experiments where the mutation rate is low and migration is prevented, recombination and segregation are the two chief sources of new genetic variation. An enormous amount of variation may be concealed in the heterozygous state and by the linkage relationships of the loci influencing a particular trait. In *Drosophila melanogaster* recombination can be restricted in particular chromosomes by making them heterozygous for marked, multiple inversions known as balancers (LINDSLEY and GRELL 1968). Examining the effectiveness of selection when recombination in specific chromosomes is restricted provides an opportunity

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to investigate the organization of genes controlling the trait under selection. The objective of the investigation reported here was to determine the effectiveness of selection for negative and positive phototactic behavior in *Drosophila melanogaster* in the presence of marked, multiple inversions, as measured by the method of realized heritability.

MATERIALS AND METHODS

The sixteen different populations of flies used in this investigation were raised in population cages on standard cornmeal-molasses-agar-brewer's yeast medium at 24±1°. Flies were tested at least 24 hours after etherization.

Stocks were synthesized (Figure 1) with the intention of reducing genetic recombination as a source of variation in particular chromosomes and combinations of chromosomes. This was accomplished by using marked, multiple inversions. Recombination was restricted in chromosome *1* by *FM6* which was marked with Bar eye, in chromosome *2* by *SM1* having Curly wing, and in chromosome *3* by *TM3* marked with Stubble bristle and Serrate wing. The wild-type flies used were derived by combining 200 males and 200 females from each of twenty wild stocks present in the lab in a large population cage for several generations. The eight resultant strains of flies were designated by the members of their chromosome sets heterozygous for inversions. For example in Strain 1, *FM6* restricted recombination in the *X* chromosome; in Strain 12,

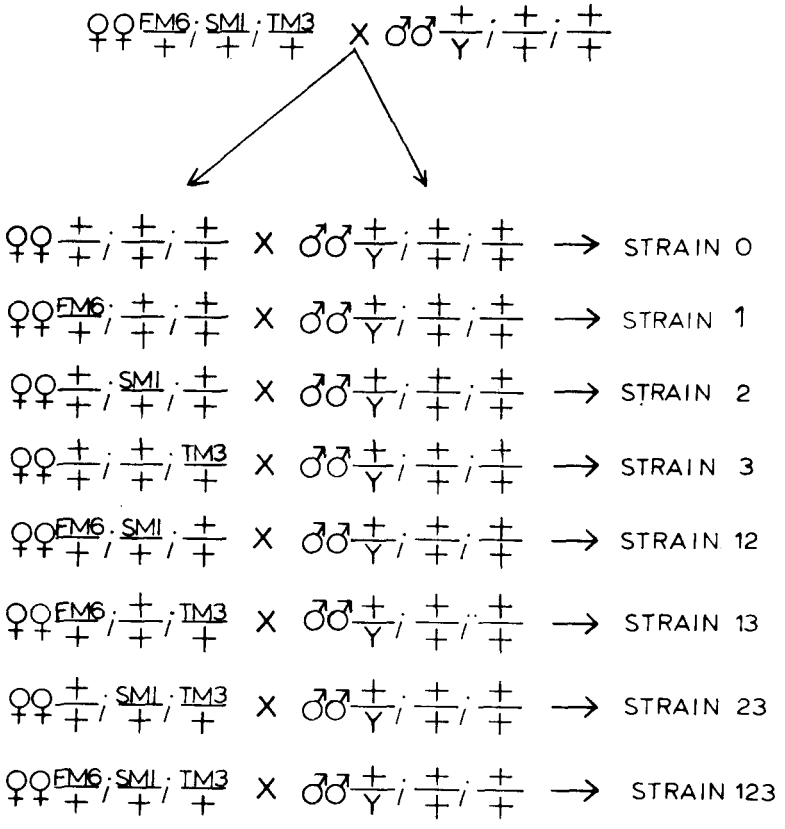

FIGURE 1.—Synthesis and coding of stocks.

recombination was reduced in chromosomes *1* and *2*. Each generation, inversion-bearing females and wild-type males were tested in the maze and used as the parents of the next generation. Since there is no crossing over in Drosophila or in the tiny *fourth* chromosome in females, recombination could be selectively reduced in each different strain.

The classification mazes used in this investigation were constructed according to the design of HADLER (1964a). Each maze consists of 15 consecutive Y-units. Flies are introduced into the stem of the first Y and each fly makes 15 light/dark choices while passing through the maze. A fly making 15 light choices will appear in tube number 16 while a fly making 15 dark choices will emerge in tube number 1. Mean phototactic scores for a population of flies are based on the total number of flies in the collecting tubes at the end of the maze. Neutrality is represented by a mean phototactic score of 8.5. The time required for all flies to pass through the maze is about 24 hours. The 60 most photopositive or most photonegative males and females were chosen to be the parents of the next generation. Because the females tested were not virgins, those females selected to be parents were deseminated by treatment at —10° for ten minutes (NOVITSKI and RUSH 1948).

RESULTS

Phototactic scores of males of each strain prior to selection are seen in Table 1. Selection for positive and negative behavior in the phototaxis maze was carried out for twenty generations in order to examine the response to selection under different conditions of recombination. After 20 generations of selection there was no appreciable change in the variances or the standard errors of the phototactic scores of any of the strains of flies. The results are presented in Figures 2, 3, and 4. Females of several strains were occasionally not tested due to the small number available. Strain 123 had very low viability and could not be maintained. While the results are shown for inversion-bearing females as well as for wild-type males, it should be remembered that only the photoscores of the wild-type males are without the possible influence of the marker chromosomes.

It can be seen from Figures 2 through 4 that each strain responded somewhat differently to selection for photopositive and photonegative behavior. Strain 0 diverged the most in both directions after 20 generations. Strains 1, 2 and 3 also diverged strongly in both directions. Structural heterozygosity in two chromosomes, especially in certain combinations, seems to have reduced the response to selection either in the positive or negative direction. The mean photoscores of males are usually more extreme than those of females, especially in strains with two inversions. This may be due partly to heterozygosity for genes in chromo-

TABLE 1

Photoscores of wild-type males at generation 0

Strain	$\bar{x} \pm$ SE	s^2
0	5.97 ± 0.17	7.82
1	6.59 ± 0.22	10.28
2	6.75 ± 0.19	10.44
3	6.60 ± 0.18	9.38
12	6.54 ± 0.22	12.26
13	7.09 ± 0.17	9.60
23	6.70 ± 0.18	8.89

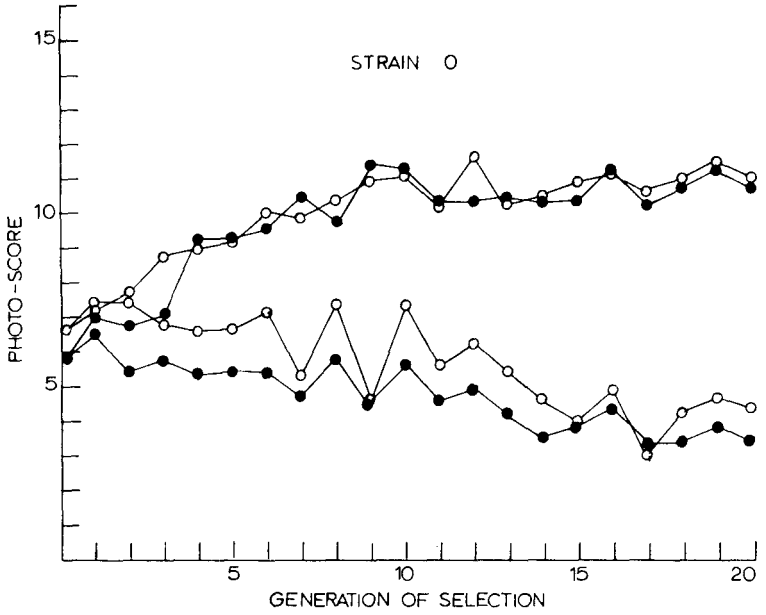

FIGURE 2.—The response of Strain 0 over twenty generations of selection for positive and negative phototactic behavior (○ = females, ● = males).

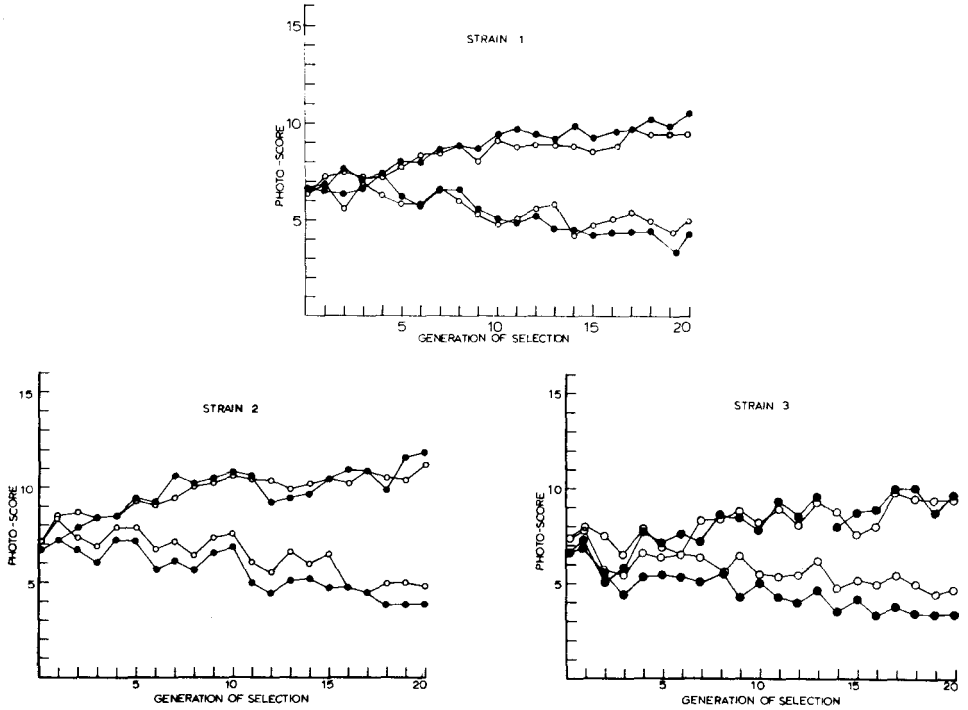

FIGURE 3.—The response of strains heterozygous for one inversion in one chromosome over twenty generations of selection for positive and negative phototactic behavior. (○ = females, ● = males).

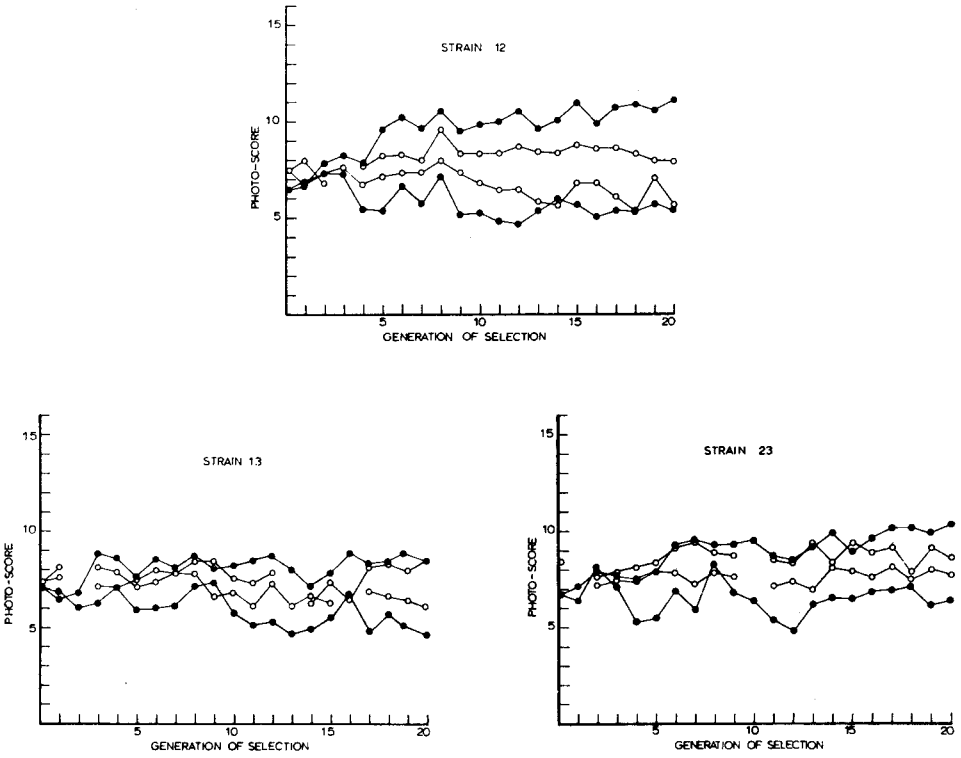

FIGURE 4.—The response of strains heterozygous for inversions in two chromosomes over twenty generations of selection for positive and negative phototactic behavior. (○ = females, ● = males).

somes balanced by inversions and partly to hemizygosity in males for genes in the *X* chromosome. In most strains divergence in the positive direction is somewhat greater than in the negative. Similar asymmetry in divergence is observed in many two-way selection experiments and FALCONER (1960) discusses several possible causes.

Heritability of phototaxis

Heritability is defined as the ratio of the additive genetic variance to the phenotypic variance, symbolized $h^2 = V_A/V_P$. The additive genetic variance, or breeding value, is the major determinant of the resemblance between relatives. Therefore, the heritability can be expressed by the regression of the breeding value on the phenotypic value, $h^2 = b_{AP}$. FALCONER (1960) has shown that the most accurate means of calculating the heritability of a trait is by regression of offspring on parent. The heritability of a trait has predictive value in that it can be used as a guide to the breeding value of an individual.

Because the deviation of the mean of the offspring from the population mean is by definition the breeding value of the parents, the response to selection can be expressed as $R = h^2S$, where S is the selection differential. Heritability can also be estimated from the response to selection, $h^2 = R/S$. The heritability of a particular

trait is not, however, constant, either between populations or within the same population under different conditions. Heritability can change during selection. The method of estimating the heritability realized over several generations will reflect this change and provides an excellent means of comparing the effectiveness of selection in different populations regardless of differences in the intensity of selection (FALCONER 1960).

Realized heritability was estimated for the wild-type flies of each strain, according to the method of FALCONER (1960). The generation means are plotted against the cumulated selection differentials and a regression line is fitted to the points. The slope of the line estimates the realized heritability. The heritability realized in Strain 0 during twenty generations of selection is seen to be low (Table 2). In the negative, realized heritabilities are 4.8% for females and 3.8% for males. Heritabilities realized in the positive line of Strain 0 are 4.5% and 4.2% for females and males respectively. A t-test shows that each of these regression lines deviates significantly from zero.

If the presence of inversions reduced the effectiveness of selection, the heritabilities realized for the wild-type males from the inversion-bearing strains should be lower than the heritabilities realized for Strain 0 males. The realized heritabilities for positive and negative phototaxis in the wild-type males of all strains are seen in Table 2. All regression lines except three are significantly different from zero. The nonsignificant heritabilities in Strain 13(+), Strain 12(−) and Strain 23(−) correspond to their reduced response to selection. To determine whether selection was as effective in the presence of inversions as in the wild-type strains,

TABLE 2

Realized heritabilities of phototaxis in wild-type flies over 20 generations of selection

Strain	Sex	$h^2 \pm$ SE	t
0(—)	♀ ♀	0.0484 ± 0.0077	6.23**
0(—)	♂ ♂	0.0381 ± 0.0046	8.27**
0(+)	♀ ♀	0.0449 ± 0.0078	4.75**
0(+)	♂ ♂	0.0425 ± 0.0097	5.01**
1(—)	♂ ♂	0.0417 ± 0.0041	10.29**
1(+)	♂ ♂	0.0399 ± 0.0041	9.87**
2(—)	♂ ♂	0.0362 ± 0.0045	7.89**
2(+)	♂ ♂	0.0446 ± 0.0078	5.74**
3(—)	♂ ♂	0.0357 ± 0.0057	6.21**
3(+)	♂ ♂	0.0432 ± 0.0073	5.97**
12(—)	♂ ♂	0.0108 ± 0.0073	1.49
12(+)	♂ ♂	0.0397 ± 0.0066	5.98**
13(—)	♂ ♂	0.0274 ± 0.0065	4.26**
13(+)	♂ ♂	0.0094 ± 0.0056	1.71
23(—)	♂ ♂	0.0033 ± 0.0082	0.39
23(+)	♂ ♂	0.0298 ± 0.0043	6.98**

Probability values are read at 18 degrees of freedom.
* Significant at 0.05 level.
** Significant at 0.001 level.

the realized heritabilities of phototaxis in each strain were compared to the realized heritabilities in Strain 0. Only in the three strains showing nonsignificant heritabilities was selection significantly less effective than it was in the absence of inversions: $t = 4.07$, 3.15, and 4.01 for strains 13(+), 12(−), and 23(−) respectively; $P < 0.001$ in all three cases.

DISCUSSION

ERLENMEYER-KIMLING and HIRSCH (1961) and HIRSCH and ERLENMEYER-KIMLING (1962), following the procedure of MATHER and HARRISON (1949), assessed the contributions of particular chromosomes to the negative and positive geotactic behavior of *Drosophila melanogaster*. This was done by determining deviations from expected geotactic behavior when marked chromosomal inversions from an unselected strain were substituted for particular chromosomes in a selected geonegative or geopositive strain of flies. While conclusions were made as to the roles of various chromosomes in positive and negative geotactic behavior, observations were made on flies carrying genetically marked inversions which themselves may have influenced the behavioral trait being examined. Further, analysis of particular chromosomes after selection may yield information about the genes which were involved in that particular selection response but tell little about the importance of recombination to the effectiveness of selection.

CARSON (1958) found that selection for high and low mobility in *Drosophila robusta* is not as effective in populations having a high degree of inversion heterozygosity as in more structurally homozygous populations. His results suggest the importance of recombination during selection but the degree of the suppression of crossing over and the particular chromosomes involved were not determined.

Polygenes influencing phototactic behavior in *Drosophila melanogaster* probably reside in all chromosomes. The presence of inversion heterozygosity in any one chromosome seems to make little difference in the effectiveness of selection. Recombination in the structurally homozygous chromosomes and segregation for the one balanced chromosome appear to have provided enough variation for selection to the effective.

The situation is different when two inversions are present. In certain combinations, suppression of crossing over in two chromosomes greatly reduced the response to selection. Restriction of recombination in chromosomes *1* and *3* interfered with selection for positive phototactic behavior. Restricting recombination in chromosomes *1* and *2* and *3* reduced the effectiveness of selection for negative phototaxis. Evidently the organization of genes controlling phototactic behavior in this species is such that in the absence of recombination in these particular chromosomes, selection is severely restricted.

Since more new combinations of genes are generated by recombination than by segregation, the restriction of recombination in two particular chromosomes may simply have eliminated an important source of variation without which selection could not make any progress. It might be expected that if particular chromosomes

contained an abundance of genes influencing photonegative of photopositive behavior, restricting the creation of new combinations of these genes could reduce the effectiveness of selection. Segregation of already existing combinations of genes simply may not have been able to provide enough variation. In addition to restricting recombination in structurally heterozygous chromosomes, inversions may increase the amount of crossing-over in the structurally homozygous members of the genome (LUCCHESI and SUZUKI 1968). This is especially true when two chromosomes are heterozygous for inversions. In the experiments described here, heterozygosity for two inversions may have increased recombination between structurally homozygous chromosomes, but the total amount of recombination for the whole genome was still probably less than in Strain 0. The loss of Strain 123 was very unfortunate, because the degree of its response to selection would have revealed the effectiveness of selection using variation generated almost totally by segregation.

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LITERATURE CITED

CARSON, H., 1958 Response to selection under different conditions of recombination in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **23**: 291–306.

DOBZHANSKY, TH. and B. SPASSKY, 1967 Effects of selection and migration on geotactic and phototactic behavior of Drosophila. I. Proc. Roy. Soc. London B **168**: 27–47. ——, 1969 Artificial and natural selection for two behavioral traits in *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci. U.S. **62**: 75–80.

ERLENMEYER-KIMLING, L. and J. HIRSCH, 1961 Measurements of the relationships between chromosomes and behavior. Science **134**: 1068–1069.

FALCONER, D. C., 1960 *Introduction to Quantitative Genetics*. Oliver and Boyd. Edinburgh, London.

HADLER, N., 1964a Genetic influence on phototaxis in *Drosophila melanogaster*. Biol. Bull. **126**: 264–273. ——, 1964b Heritability and phototaxis in *Drosophila melanogaster*. Genetics **50**: 1269–1277.

HIRSCH, J. and L. ERLENMEYER-KIMLING, 1962 Studies in experimental behavior genetics. IV. Chromosome analysis for geotaxis. J. Comp. Physiol. Psychol. **55**: 732–739.

LINDSLEY, D. and E. H. GRELL, 1968 Genetic Varations of *Drosophila Melanogaster*. Carnegie Inst. of Wash. Publ. No. 627.

LUCCHESI, J. C. and D. SUZUKI, 1968 The interchromosomal control of recombination. Ann. Rev. Genetics **2**: 53–86.

MATHER, K. and B. J. HARRISON, 1949 The manifold effect of selection. Heredity **36**: 1–52.

NOVITSKI, E. and G. RUSH, 1948 Tolerance of *Drosophila melanogaster* to subzero temperatures. Drosophila Inform. Serv. **22**: 55.

RICHMOND, R. C., 1969 Heritability of phototactic and geotactic responses in *Drosophila pseudoobscura*. Am. Naturalist **103**: 315–316.

WOOLF, C. M., 1972 Genetic analysis of geotactic and phototactic behavior in selected strains of *Drosophila pseudoobscura*. Behav. Genet. **2**: 93–106.

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