GENETIC ANALYSIS OF PHOTOTACTIC BEHAVIOR IN *DROSOPHILA SIMULANS*[1]

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ABSTRACT

Phototaxis mazes have been employed to select photopositive and photonegative strains of *Drosophila simulans*. The results suggest that phototactic behavior in *D. simulans*, as in other Drosophila species, is a polygenic trait. Hybridization using divergent strains revealed that the genes controlling negative phototactic behavior in *D. simulans* are autosomal, as opposed to *D. melanogaster* in which negative phototactic behavior is known to be very strongly sex-linked.

THE use of Hirsch-Hadler classification mazes has generated a large amount of literature dealing with genetic aspects of the phototactic and geotactic behavior of *Drosophila melanogaster* (HIRSCH 1959; HADLER 1964b; WALTON 1970; MARKOW 1975a, b; WATANABE and ANDERSON 1976), *D. pseudoobscura* (DOBZHANSKY and SPASSKY 1967, 1969; WOOLF 1972) and *D. persimilis* (DOBZHANSKY and SPASSKY 1969; and POLIVANOV 1975). Most natural populations of Drosophila are geotactically and phototactically neutral when tested in the maze, although much individual variation exists. A genetic component to this variation has allowed for the creation of highly divergent geo- and photo-negative and positive strains of flies, using the mazes as selection devices.

Populations of *D. melanogaster* show a gradual response to selection for negative or positive phototactic behavior, consistent with a polygenic mode of inheritance (HADLER 1964a; MARKOW 1975a). Subsequent genetic analysis has shown that in this species, the genes giving photonegative behavior reside in the X chromosome and that genes for photopositive behavior are largely autosomal (MARKOW 1975b). Similar observations have been made for every population of *D. melanogaster* examined (HADLER 1964b, WALTON 1970), making a sex-linked mode of inheritance for negative phototactic behavior an apparent characteristic of this species.

D. simulans and *D. melanogaster* are sibling species having similar appearance and karyotype. Characterization of the mode of inheritance of phototaxis in *D. melanogaster* suggested that the genetics of this behavior be investigated in *D. simulans*. The results of genetic analysis of phototactic behavior in *D. simulans* is presented below and discussed with respect to the evolutionary relationship between *D. simulans* and *D. melanogaster*.

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MATERIALS AND METHODS

Photactic behavior was measured in a Hirsch-Hadler classification maze (HADLER 1964a). Flies entering the maze make 15 light/dark choices and finally emerge in 16 collecting tubes. The maze is constructed so that flies emerging in tube number 1 have made 15 consecutive dark choices and are, therefore, highly photonegative. Those flies appearing in tube number 16 have made 15 light choices (highly photopositive). Flies making an equal number of light/dark choices emerge in tube 8 or 9. About three hundred flies are tested at one time and the number appearing in each tube are counted and a mean phototactic score is computed. Photoneutrality is represented by a phototactic score of 8.5. Flies were four days old at the time of testing. Males and females were tested separately. The light source was provided by G.E. cool white lights which gave 200 foot candles of illumination at the surface of the mazes.

Cultures of *D. simulans*, originally collected from 10 different localities, were obtained from the University of Texas Drosophila stock center. The ten cultures were pooled in a population cage for 5 generations (1000 flies each generation) to establish a highly variable base population.

Selection was initiated by testing approximately 300 males and 300 virgin females from the base population in the phototaxis maze. Fifty of the most photopositive females were mated with fifty of the most photopositive males to found a photopositive population. A photonegative population was begun with fifty of the most photonegative pairs of flies. Every generation, selection was carried out in the photopositive and photonegative populations by using the fifty most extreme pairs out of 300 tested. Populations undergoing selection were always raised in tupperware population cages containing 12 food cups.

After 21 generations of selection, reciprocal hybridizations were carried out between the photonegative and photopositive lines. Fifteen males from one divergent population and fifteen virgin females from the other divergent population were placed together in each of five half-pint bottles. Flies were allowed to lay eggs for three days and were transferred to fresh bottles. This procedure was repeated to obtain three broods of F_1 flies. The F_2 generations were raised in a similar way.

RESULTS

The results of 21 generations of selection for positive and negative phototactic behavior in *D. simulans* are shown in Figure 1. Realized heritabilities of photonegative and photopositive behavior were calculated (FALCONER 1960) for the first 10 generations of selection and are presented in Table 1. The gradual divergence seen in Figure 1 and the low heritabilities are consistent with a polygenic mode of inheritance.

At the 21st generation of selection, reciprocal hybridizations were carried out between the photopositive and the photonegative populations. Results are shown in Table 2. When photonegative females are mated to photopositive males, both the F_1 males and F_1 females are photoneutral and do not differ significantly from

TABLE 1

Heritabilities of photopositive and photonegative behavior realized over the first ten generations of selection

Behavior	Sex	Realized $h^2 \pm SE$
Photonegative	Females	0.0976 ± 0.0026
Photonegative	Males	0.0921 ± 0.0059
Photopositive	Females	0.0648 ± 0.0063
Photopositive	Males	0.0409 ± 0.0054

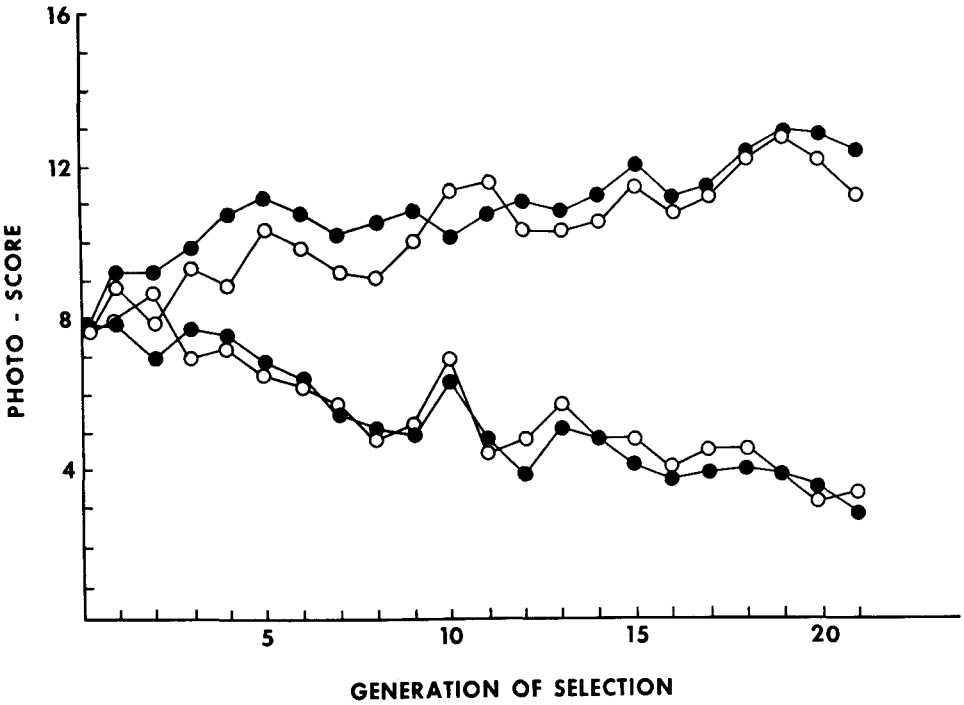

FIGURE 1.—The response to 21 generations of selection for photopositive and photonegative behavior in *D. simulans*.

each other. This finding indicates that negative phototaxis is under the control of autosomal loci. The phototactic scores of F_1 males from the reciprocal cross are significantly more photopositive than their sisters, suggesting some degree of sex linkage for genes influencing photopositive behavior. While photoscore variation exists between replications for a given sex, F_1 males are consistently more photopositive than the F_1 females. The F_2 variances tend to be slightly larger than the variances seen in the F_1 generations, an observation which lends support to a polygenic mode of inheritance.

DISCUSSION

The gradual response to selection and the low heritability of phototactic behavior in *D. simulans* is similar to that observed in other Drosophila species (DOBZHANSKY and SPASSKY 1969; MARKOW 1975a; and POLIVANOV 1975), and suggests that phototactic behavior in *D. simulans* has a polygenic basis. Curiously, in *D. simulans* photonegative behavior is autosomally inherited, but in *D. melanogaster* genes for negative phototaxis are strongly sex linked (MARKOW 1975b). Either chromosomal rearrangements have moved genes for phototaxis between the autosomes and *X* chromosomes during recent evolution, or phototactic behavior is controlled by nonhomologous loci in these two sibling species.

While the metaphase figures of *D. melanogaster* and *D. simulans* are indis-

TABLE 2

P_1, F_1, and F_2 photoscores from reciprocal hybridization of photopositive and photonegative populations of Drosophila simulans

		$\bar{X}$	s^2	n			$\bar{X}$	s^2	n	H^*	P†
		P_1: Photopositive ♀♀ ($\bar{X} = 11.111 \pm .15$) × Photonegative ♂♂ ($\bar{X} = 3.471 \pm .099$)									
F_1 ♀♀	1	8.24 ± .26	14.86	227	F_1 ♂♂	1	9.36 ± .29	14.22	175	8.67	.005>P>.001
	2	6.85 ± .29	7.80	229		2	7.71 ± .22	9.19	193	8.88	.005>P>.001
	3	8.02 ± .25	16.02	254		3	8.57 ± .26	18.88	272	1.84	0.2 >P>.1
	Pooled	7.71 ± .14	13.32	710		Pooled	8.52 ± .15	15.08	640	14.72	P<.0005
F_2 ♀♀	1	7.63 ± .17	12.84	455	F_2 ♂♂	1	8.22 ± .16	13.02	487	6.16	.025>P>.01
	2	8.26 ± .18	19.64	608		2	8.00 ± .22	23.20	480	1.23	0.3 >P>0.2
	Pooled	8.00 ± .13	16.84	1063		Pooled	8.13 ± .14	18.04	964	0.40	0.6 >P>0.5
		P_1: Photonegative ♀♀ ($\bar{X} = 3.458 \pm .11$) × Photopositive ♂♂ ($\bar{X} = 12.011 \pm .12$)									
F_1 ♀♀	1	8.49 ± .20	16.28	406	F_1 ♂♂	1	8.04 ± .28	19.68	257	2.06	0.2 >P>0.5
	2	9.11 ± .20	14.74	358		2	9.31 ± .29	15.85	190	0.33	0.6 >P>0.5
	3	8.86 ± .21	15.44	358		3	8.98 ± .21	17.37	378	0.08	0.8 >P>0.7
	Pooled	8.79 ± .12	15.52	1118		Pooled	8.76 ± .15	17.95	825	.079	0.8 >P>0.7
F_2 ♀♀	1	7.37 ± .19	18.19	496	F_2 ♂♂	1	8.69 ± .23	19.82	369	18.61	P<.0005
	2	8.12 ± .19	16.85	458		2	9.32 ± .20	16.14	386	18.93	P<.0005
	Pooled	7.72 ± .14	17.66	954		Pooled	9.01 ± .15	18.01	755	38.75	P<.0005

* The Kruskal-Wallis H is a nonparametric group comparison test which does not assume normal distributions or homogeneous variances.
† A χ^2 table is used to determine probability values (Woolf, 1968).

tinguishable under the light microscope, HORTON (1939), found evidence of 10 clear chromosomal rearrangements by looking at the salivary chromosomes of *D. melanogaster-D. simulans* hybrids. The tip of the *X* chromosome in *D. melanogaster* shows one band (1 E 3–4) which is absent from the corresponding position in the *D. simulans X* chromosome. Extra bands appear at the ends of the *D. simulans* second chromosome which do not appear in *D. melanogaster*. On these bases, HORTON (1939) suggested that a translocation occurred from the *X* chromosome to the second chromosome during the evolution of these two species. If this were true, there is no reason to assume that this small translocated region contained the photonegative factors. Additional differences between the chromosomes of *D. simulans* and *D. melanogaster* have been revealed more recently by quinacrine fluorescent staining techniques (ELLISON and BARR 1971) and, while the molecular basis of quinacrine differentiation of chromosomes is still not well understood (LATT, BRODIE and MUNROE 1974), the possibility must be mentioned that these differences might relate to phototactic loci. Finally, and perhaps more likely, is the possibility that phototaxis is genetically heterogeneous between species, that is, that similar phenotypes may be controlled by nonhomologous loci.

The locations of photonegative loci in the *D. melanogaster X* chromosome are currently being determined. Hybrids between photonegative lines of *D. melanogaster* and *D. simulans* and between their photopositive lines are now being created and should give insight into the question of homology of phototactic genes in these two sibling species.

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