Behavior in Different Environments of Populations of *Drosophila pseudoobscura* Selected for Phototaxis and Geotaxis

(genetic selection/light color and intensity)

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ABSTRACT When tested in Hirsch-Hadler mazes, *Drosophila pseudoobscura* descended from natural populations is phototactically and geotactically neutral on the average. Decidedly positive and negative populations have been obtained by artificial selection. The behavior is independent of temperature (15-27°) and age (2-17 days). The flies perceive light as weak as 0.2 lux; from 7 to 3000 lux the behavior difference between positive and negative strains increases little, and perhaps decreases at 8000 lux. A remarkable transformation occurs with red light; the population that is negatively phototactic with shorter wave lengths or with white light becomes photopositive.

The responses of *Drosophila* flies to gravity and to light have been measured in Hirsch-Hadler classification mazes (1, 2). By selecting in a series of generations flies that respond in a given way, populations are obtained with phototactic or geotactic behavior quite different from the original source population (1-7). Populations of *Drosophila pseudoobscura* descended from wild-collected progenitors are photo- and geoneutral on the average. After 10 or more generations of selection, populations have average phototactic and geotactic scores between 14 and 15 (for positive flies, maximum possible score being 16), or between 2 and 3 (for negative flies, lowest possible score being 1). The neutrality score in our mazes is, then, 8.5. Natural populations of *D. persimilis*, a sibling species of *D. pseudoobscura*, is geoneutral but significantly photopositive on the average (8). Photoneutral and photonegative populations can be obtained by selection also in *D. persimilis* (9). All our past experiments were made under standard conditions of temperature, age of the flies, and light intensity. The purpose of the work reported below is an exploratory study of the effects of modified age, temperature, light intensity, and color on the behavior of populations that had been selected for photo- or geopositivity or negativity. More precise studies can be based on the preliminary results here described.

MATERIALS AND METHODS

Experimental population cages nos. 25, 26, 31, and 32 were started in November 1966 with *D. pseudoobscura*, the ancestors of which were collected at Pinon Flats, Mount San Jacinto, Calif. For 20 generations, the selection was made as described (5). From July 1968 on, populations nos. 25 and 26 were subjected to directional selection for positive and negative phototaxis, respectively, and nos. 31 and 32 for negative and positive geotaxis. Approximately 300 females and 300 males in every generation were run, separately, through the phototactic or geotactic mazes. The next generations were started with 25 females and 25 males that showed the most positive or most negative behavior in the maze tests. The experiments described below were started in December 1971 after 60 generations of selection (20 + 40 genera-

tions); 23 to 25 additional generations of selection accumulated by the time the experiments were completed. As will be seen below, the positive and negative populations were quite different in their behavior in mazes.

Unless otherwise specified, the experiments were made under "standard" conditions: in a room at a constant temperature of 25°, virgin females and males 3-6 days old, phototactic mazes with circular fluorescent white lights 32 cm above the maze, providing about 1250 lux at the maze level, and geotactic mazes with vertical fluorescent light near the terminal vials. Photographs of the mazes have been published (10). Changes in light characteristics were made in several ways. In experiments with white light (other than the 1250-lux standard) a horizontal battery of fluorescent lamps (Sylvania F20T12-D, Daylight), containing various numbers of lamps at various heights above the maze, were used, yielding intensities from 2 to 8000 lux. Low intensities of 0.2 and 0.4 lux, and the green to red series of colors, were obtained with a Zeiss photo-enlarger head mounted above the maze. G.E. incandescent lamps of 75-150 W, in combination with neutral density, narrow pass or sharp-cut Wratten or glass-mounted acetate filters were used to obtain the intensities or spectral regions desired. Ultra-violet light was obtained with a G.E. R-15-9-8 lamp suspended above the maze. Light intensities were determined with either a Kahl foot candle meter (model 268-WA620), or a Photovolt photometer (model 501-M with type C phototube). No effort was made to obtain equal radiant energy levels at the various wavelengths, although the intensity of the deepest red light used was equal to the lowest intensity of white light tested (0.2 lux).

RESULTS

Independence of Temperature and Age. The constant-temperature room was set at 15°, 20°, or 27°. The mean photo- or geotactic scores with the standard errors, and the differences between the scores of the populations selected for positivity or for negativity, are shown in Table 1. No consistent difference in the response to light at different temperatures was observed. The abnormally high score of the photonegative males at 27° is probably accidental. The geotactic scores tend to be slightly higher at cooler than warmer temperatures, but the differences are neither large nor consistent within the temperature range examined.

Flies grown either at 25° or at 16° were aged 2-17 days at 25°, and then run through the mazes at 25°. The phototactic scores did not change within this age interval. Old flies were very slightly more geopositive than the young ones.

Light Intensity. Populations selected for many generations for positive or for negative phototaxis under standard light intensity (1250 lux) were tested with weaker or stronger light.

TABLE 1. *Phototactic and geotactic scores at different temperatures*

Temperature and sex	Phototaxis			Geotaxis		
	Positive	Negative	Difference	Positive	Negative	Difference
27°						
♀ ♀	14.57 ± 0.09	2.86 ± 0.12	11.71	12.83 ± 0.18	2.85 ± 0.15	9.98
♂ ♂	14.09 ± 0.11	5.25 ± 0.20	8.84	14.17 ± 0.11	5.00 ± 0.23	9.17
25°						
♀ ♀	14.60 ± 0.09	3.22 ± 0.13	11.38	12.50 ± 0.19	2.97 ± 0.13	9.53
♂ ♂	13.86 ± 0.12	3.07 ± 0.12	10.79	13.04 ± 0.13	4.93 ± 0.17	8.11
20°						
♀ ♀	13.10 ± 0.14	3.17 ± 0.15	9.93	13.53 ± 0.19	3.27 ± 0.15	10.26
♂ ♂	13.30 ± 0.12	3.71 ± 0.13	9.59	14.50 ± 0.11	7.21 ± 0.17	7.39
15°						
♀ ♀	14.53 ± 0.09	3.60 ± 0.18	10.93	14.12 ± 0.16	3.54 ± 0.16	10.58
♂ ♂	12.90 ± 0.13	3.62 ± 0.15	9.28	15.42 ± 0.07	8.02 ± 0.20	7.40

As was to be expected (Table 2), photopositive and photonegative populations in the dark give similar mean scores in the vicinity of the neutrality point. In two of the four tests, the photopositive population had an ostensibly lower, and in the other two ostensibly higher, scores than the photonegative population when tested without light. In three instances the differences are below, and in one at the conventional border of, statistical significance.

The lowest light intensity tried was 0.2 lux. This gave a small but statistically reliable difference, with the mean score of the photopositive population being above that of the photonegative one. The flies are clearly influenced in their choices of the light or dark passages even by so weak an illumination. Twice and 10 times stronger illumination (0.4–2 lux) increases the mean scores of the positive and decreases those of the negative populations. From 7 to 3000 lux there is no further

discernible increase in the differences between the photopositive and photonegative populations. At the highest intensity, 8000 lux, there seems to be a decrease in the difference. The score of the photopositive population is unchanged, but that of the photonegative one, quite unexpectedly, becomes less, rather than more, averse to light. The behavior of geopositive and geonegative populations tested in the dark was the same as with light.

Color Discrimination. The transmission characteristics of the color filters used were obtained with a Beckman Acta II spectrophotometer. They were as follows (W = Wratten, and A = acetate filters, Edmund Scientific Co.):

Color	nm	% Transmission
Green, W4 + 58	500–572	>10
Yellow, W23A + 58	570–600	>1
Red I, W25	>590	10
	>600	50
Red II, W25 + A857	>670	10
	>700	50
Red III, W25 + A866	>710	10
	>750	50

TABLE 2. *Phototactic scores at different light intensities*

Light (lux)	Sex	Positive	Negative	Difference
Dark	♀	9.66 ± 0.19	9.78 ± 0.21	−0.12
Dark	♂	8.03 ± 0.20	7.36 ± 0.21	+0.67
Dark	♀	9.55 ± 0.20	9.13 ± 0.17	+0.42
Dark	♂	8.68 ± 0.21	8.89 ± 0.18	−0.21
0.2	♀	9.62 ± 0.19	8.23 ± 0.23	+1.39
0.2	♂	10.56 ± 0.18	6.63 ± 0.18	+3.93
0.4	♀	9.00 ± 0.22	4.82 ± 0.18	+4.18
0.4	♂	10.47 ± 0.19	4.10 ± 0.14	+6.37
2	♀	10.12 ± 0.12	4.74 ± 0.16	+5.38
2	♂	11.23 ± 0.14	4.42 ± 0.15	+6.81
7	♀	13.13 ± 0.15	2.68 ± 0.11	+10.45
7	♂	13.19 ± 0.12	2.43 ± 0.10	+10.76
300	♀	14.26 ± 0.09	2.08 ± 0.09	+12.18
300	♂	13.85 ± 0.11	1.51 ± 0.07	+12.34
300	♀	14.71 ± 0.08	2.48 ± 0.10	+12.23
1250	♀	14.79 ± 0.08	3.73 ± 0.15	+11.06
1250	♂	13.80 ± 0.11	2.66 ± 0.11	+11.14
1250	♀	14.98 ± 0.07	2.95 ± 0.10	+12.03
1250	♂	14.39 ± 0.09	2.76 ± 0.13	+11.63
1250	♀	14.30 ± 0.08	3.56 ± 0.17	+10.74
1250	♂	13.72 ± 0.10	3.57 ± 0.13	+10.15
3000	♀	14.83 ± 0.07	3.86 ± 0.17	+10.97
3000	♂	14.73 ± 0.08	3.07 ± 0.10	+11.66
8000	♀	14.20 ± 0.12	5.05 ± 0.21	+9.15
8000	♀	14.55 ± 0.11	5.62 ± 0.20	+8.93
8000	♂	14.99 ± 0.14	6.41 ± 0.20	+8.58

Table 3 shows that with the standard white light the average scores of the photopositive population varied from 13.52 to 14.62, and of the photonegative one, from 2.31 to 3.15 in different generations. The differences between the positive and the negative populations ranged from 10.64 to 11.94. Their behaviors were obviously quite different. With ultraviolet and with green light the differences are about as great as with white light, although under green light the average scores of both the positive and the negative populations seem to be significantly lower. With yellow light, the mean scores of the photopositive population decline, and those of the photonegative one increase. The difference between the two populations is reduced to between one-half and one-third of what it is with shorter wave lengths.

With red light an interesting and unexpected situation develops. The photopositive population remains photopositive, even under Red III, which has, as stated above, an intensity of only 0.2 lux. The behavior of the photonegative population changes dramatically—with Red II and Red III it becomes distinctly photopositive in 6 of 8 trials, and is photoneutral in 2 of 8 trials. The mean scores obtained in the six tests are higher than the scores of the same population in the dark (compare Tables 2 and 3). The differences between

TABLE 3. Phototactic scores with light of different colors

Color	Sex	Positive	Negative	Difference
White	♀	14.62 ± 0.06	2.95 ± 0.15	+11.67
	♀	14.55 ± 0.07	2.26 ± 0.14	+12.29
	♀	13.52 ± 0.11	2.62 ± 0.10	+10.90
	♂	13.79 ± 0.09	3.15 ± 0.14	+10.64
	♂	13.72 ± 0.10	2.95 ± 0.15	+10.77
	♂	14.25 ± 0.10	2.31 ± 0.09	+11.94
Ultraviolet	♀	14.66 ± 0.09	3.16 ± 0.13	+11.50
	♂	13.72 ± 0.12	2.43 ± 0.11	+11.29
Green	♀	11.52 ± 0.18	1.81 ± 0.08	+9.71
	♂	12.46 ± 0.15	1.51 ± 0.05	+10.95
Yellow	♀	9.67 ± 0.19	4.96 ± 0.21	+4.71
	♂	10.00 ± 0.19	3.82 ± 0.14	+6.18
Red I	♀	9.20 ± 0.21	4.40 ± 0.17	+4.80
	♂	9.44 ± 0.19	7.72 ± 0.24	+1.72
Red II	♀	12.24 ± 0.13	12.39 ± 0.15	-0.15
	♀	12.51 ± 0.17	8.39 ± 0.25	+4.12
	♀	12.80 ± 0.15	11.60 ± 0.19	+1.20
	♂	11.99 ± 0.15	12.00 ± 0.16	-0.01
	♂	11.98 ± 0.17	9.95 ± 0.27	+2.93
	♂	11.63 ± 0.16	10.00 ± 0.20	+1.63
Red III	♀	11.20 ± 0.21	10.58 ± 0.20	+0.62
	♂	9.90 ± 0.21	10.50 ± 0.18	-0.60

the positive and the negative populations with Red II and III range from -0.60 to +4.12; some of the differences fall below the border of statistical significance. Not only do both photopositive and photonegative populations behave differently in the red light from their behavior in the dark, but their choices become almost equally biased in favor of the passages illuminated by red light.

DISCUSSION

Diverse kinds of behavior are lumped under the names phototaxis and geotaxis. This makes attempts to invent precise definitions of these terms fall short of success (11). Every species of *Drosophila* has, as far as we know, an escape reaction. The flies fly upwards and towards light when disturbed. In the countercurrent distribution apparatus of Benzer (12) and his collaborators, *D. melanogaster* is recorded as phototactically positive, except for some mutants that are photo-neutral. *D. pseudoobscura* behaves similarly. In the Hirsch-Hadler mazes, undisturbed flies are offered repeated choices of moving into or away from light or dark passages. They move through the maze because the passages are funnel-shaped, and going backwards is difficult. Both *D. melanogaster* and *D. pseudoobscura* are then geo- and photoneutral on the average, although pronounced positivity and negativity can be obtained by selection over a series of generations (1-10). Genetic differences between positive and negative strains are usually polygenic, although Woolf (13) found a positively and a negatively geotactic population that differed mainly in sex-linked genes. Medioni (14), Rockwell and Seiger (15), and Kekic and Marinkovic (16) have designed still different instruments, the common feature of which is that the flies can move into areas with light intensity, and also temperature and humidity, of their preferences. Strains of *D. melanogaster*, *D. pseudoobscura*, *D. persimilis*, and *D. subobscura* evinced a diversity of preferences, which in the last-named species have been modified by artificial selection (16).

Responses to light and to gravity are almost certainly adaptively functional, although their ecological roles are still little known. In their natural habitats the flies are most active for a few hours after sunrise and before sunset, their diurnal periodicity being more sharply pronounced in the dry American Southwest than in the humid tropics (17, 18). Grossfield (19, 20) found that while some species mate equally successfully with and without light (*D. pseudoobscura* is one of these), other species are stimulated by light or fail altogether without light. *D. subobscura* requires light for mating, but the intensity threshold required can be modified by selection (16).

Under laboratory conditions, the photo- and geopositive and negative populations of *D. pseudoobscura* seem to be equal in adaptedness. Marinkovic (21) compared the mating success of flies from these populations with and without light; they are equally successful in light, but the photopositive population is less so in the dark. In our geotactic mazes the flies face alternative choices of moving up or down, and in phototactic mazes in light or dark passages. They cannot choose a preferred degree of illumination or inclination to the vertical. It is noteworthy that the behavior greatly modified by genetic selection is within wide limits insensitive to such factors as temperature and age, and changes only in degree with light intensities varying as much as 0.2-8000 lux. The only remarkable qualitative change is the transformation with red light of the photonegative into photopositive behavior in the population selected for photonegativity. With the longest wave length tested (above 710 nm) the flies did not behave as they do without light. They clearly perceived the red light, and directed their movement towards it, while with shorter wave lengths the flies from photonegative populations moved away from light. The physiological basis of this transformation would be most interesting to discover.

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