

## Effect of Age and of Screening Pigment Mutations on the Phototactic Behavior of *Drosophila melanogaster*

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*Five different eye color mutations of *Drosophila melanogaster* have been tested for their effect on phototactic behavior. All five mutations seem to cause flies to be less photonegative than Canton-S control flies. The mutation sepia was found to produce this effect when heterozygous as well. It was also found that wild-type flies from highly photopositive and photonegative strains seem to be more photoneutral with age.*

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**KEY WORDS:** phototaxis maze; eye color mutants; *Drosophila*.

### INTRODUCTION

Phototactic behavior in *Drosophila* has been investigated by many different workers (Benzer, 1967; Brown and Hall, 1936; Markow, 1975*a,b*). As noted in the review by Rockwell and Sieger (1973), much variation exists in the experimental methods employed in the different investigations. Our own investigations (Woolf, 1972; Markow, 1975*a,b*) are carried out using Hirsch-Hadler classification mazes in which flies make a series of light/dark choices and are assigned phototactic scores from 1 (photonegative) to 16.0 (photopositive) (Hadler, 1954). Natural populations of *Drosophila melanogaster* and *Drosophila pseudoobscura* are, for the most part, photoneutral when tested in the maze. Much individual variation in phototactic score exists among members of a population. The variation observed has been attributed to both genetic and environmental factors (Hadler, 1964*b*;

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Dobzhansky and Spassky, 1969; Woolf, 1972; Markow, 1975b). In the present article, we report results of experiments designed to evaluate the effects of age and the effects of homozygosity and heterozygosity for recessive eye color variants on the behavior of *Drosophila melanogaster* in the phototaxis maze.

## MATERIALS AND METHODS

### Classification Mazes

The phototactic behavior of the *Drosophila* described in this report was measured using Hirsch-Hadler phototaxis mazes. Detailed accounts of the phototactic maze can be found elsewhere (Hadler, 1964*a,b*; Markow, 1975*a*). Flies entering a maze make 15 consecutive light/dark choices and emerge in 16 collecting tubes at the end of the maze. Flies making 15 dark choices emerge in tube number 1 (highly photonegative). Flies making 15 light choices appear in tube number 16 (highly photopositive). A population of flies which make an approximately equal number of light and dark choices will have a mean phototactic score of 8.5 (photoneutral). All testing was carried out under General Electric cool-white lights which provided 180 ft-candles of light at the surface of the maze.

### Eye Color Mutants

Five mutants, white (*w*), sepia (*se*), vermilion (*v*), brown (*bw*), and purple (*pr*), were obtained from the *Drosophila* stock center at the California Institute of Technology at Pasadena (see Lindsley and Grell, 1968, for further description of the mutants). The wild-type strain Canton-S, obtained from the same stock center, served as the control in experiments testing the effects of these five screening pigment mutations. The individual mutations were inserted into Canton-S genetic background using inversions and multiply marked chromosomes. Following "Cantonization," each screening pigment mutation was tested in the homozygous condition in the phototaxis maze. The effects of mutations when heterozygous were determined by mating virgin females from the Cantonized mutant stock to Canton-S males. F<sub>1</sub> virgin females were then backcrossed to males from the Canton-S strain. Virgin females from this backcross were tested in the maze. Females from each collecting tube were placed individually in vials with males homozygous for the mutant in question. The appearance of mutant progeny indicated a heterozygous female. Flies were always tested in the maze at 4 days of age.

### Age Effects

Flies from the Canton-S strain were tested in the maze at 4 days of age and again at 14 days of age. In addition, flies from a highly photopositive strain, strain O(+), and flies from a highly photonegative strain, strain O(-), which had both been created by artificial selection (Markow, 1975a), were tested in the same way.

## RESULTS

### Screening Pigments

Several replications for each mutation when homozygous are presented in Table I. Flies from the Canton-S strain tend to be photonegative. The mutations tested here all bring about qualitative or quantitative changes in the screening pigments of the compound eye. White-eyed flies are described as essentially lacking both red and brown pigments (Nolte, 1952) and are photoneutral in the maze. Flies homozygous for purple contain approximately 25% of the amount of red pigment and 137% the amount of

Table I. Phototactic Scores of Wild-Type Flies and Eye Color Mutants<sup>a</sup>

Experiment	Females	Males
Canton-S	5.60 ± 0.13 (485)	5.37 ± 0.13 (486)
	5.83 ± 0.21 (220)	5.44 ± 0.17 (280)
	6.21 ± 0.12 (562)	6.31 ± 0.12 (601)
White ( <i>w;cs</i> )	7.96 ± 0.21 (318)	8.80 ± 0.21 (337)
	8.12 ± 0.18 (358)	8.47 ± 0.19 (332)
	8.83 ± 0.17 (408)	8.63 ± 0.18 (386)
Sepia ( <i>se;cs</i> )	7.43 ± 0.22 (261)	7.50 ± 0.26 (210)
	7.37 ± 0.15 (534)	7.26 ± 0.13 (634)
Vermillion ( <i>v;cs</i> )	6.32 ± 0.17 (364)	6.44 ± 0.15 (405)
	7.57 ± 0.17 (525)	7.13 ± 0.11 (590)
	6.40 ± 0.17 (358)	6.32 ± 0.18 (288)
Brown ( <i>bw;cs</i> )	7.07 ± 0.17 (346)	7.33 ± 0.15 (334)
	7.44 ± 0.16 (347)	7.79 ± 0.12 (457)
Purple ( <i>pr;cs</i> )	6.06 ± 0.22 (317)	5.30 ± 0.20 (326)
	5.63 ± 0.17 (307)	5.78 ± 0.16 (324)
	6.06 ± 0.19 (256)	6.19 ± 0.17 (273)

<sup>a</sup> Number of flies is given in parentheses.

brown pigment found in the wild-type eye (Nolte, 1955). Purple-eyed flies do not appear to behave appreciably differently from the wild-type control flies. Vermillion-eyed flies cannot synthesize brown pigment (Nolte, 1952) and are not as photonegative as Canton-S flies. Flies homozygous for brown lack red pigment (Nolte, 1950) and are much less photonegative than wild type. Homozygosity for sepia causes very dark eye color because of the absence of red pigment and the presence of sepia pterin (Nolte, 1953*b*). Flies homozygous for sepia are the least photonegative of all the mutants tested except white. The variation observed between replications is probably due to unknown and therefore uncontrolled environmental factors.

A second series of experiments tested the effects on phototaxis of the same five mutations when heterozygous. Photoscores of heterozygous wild-type females and their homozygous wild-type sisters appear in Table II. Variation exists between replications, but the phototactic scores of the heterozygous wild-type females and the homozygous wild-type females are remarkably consistent within each experiment. In every case but one, the heterozygous flies have the same phototactic scores as do the homozygous flies. Females heterozygous for sepia (*se*<sup>+</sup>/*se*) are much less photonegative than are *se*<sup>+</sup>/*se*<sup>+</sup> females. Therefore, it appears that at least one screening pigment mutation is not entirely recessive in its effect on phototactic behavior.

### Age Effects

Flies tested in the phototaxis maze at 14 days of age behave differently than flies tested at 4 days (Table III). After 14 days, the phototactic scores

**Table II.** Effect on Phototaxis of Mutations in the Heterozygous Condition<sup>a</sup>

Experiment	Wild-type females homozygous	Wild-type females heterozygous
<i>w</i> /+ × +/ <i>Y</i> (256)	6.15 ± 0.31 (125)	6.15 ± 0.29 (131)
(220)	6.81 ± 0.38 (115)	6.61 ± 0.37 (105)
<i>se</i> /+ × +/+ (299)	6.39 ± 0.30 (160)	7.16 ± 0.35 (139)
(306)	4.89 ± 0.28 (141)	6.45 ± 0.29 (165)
<i>bw</i> /+ × +/+ (347)	4.90 ± 0.25 (168)	4.97 ± 0.24 (169)
(394)	5.86 ± 0.24 (191)	5.99 ± 0.21 (203)
<i>v</i> /+ × +/ <i>Y</i> (316)	6.16 ± 0.29 (149)	6.16 ± 0.28 (167)
(400)	5.98 ± 0.24 (186)	5.72 ± 0.22 (214)
<i>pr</i> /+ × +/+ (396)	4.83 ± 0.25 (191)	4.89 ± 0.25 (205)
(299)	6.07 ± 0.26 (158)	6.29 ± 0.27 (141)

<sup>a</sup> Number of flies is given in parentheses.

Table III. Effect of Age on Phototactic Behavior of *Drosophila* Strains<sup>a</sup>

Strain	4 days		14 days	
	Females	Males	Females	Males
Canton-S	5.95 ± 0.19 (255)	6.04 ± 0.19 (244)	7.84 ± 0.20 (321)	8.1 ± 0.18 (408)
	5.60 ± 0.13 (485)	5.37 ± 0.13 (486)	7.98 ± 0.17 (362)	7.83 ± 0.14 (531)
w(CS)	5.83 ± 0.23 (220)	5.43 ± 0.17 (280)	7.13 ± 0.17 (222)	6.64 ± 0.14 (302)
	8.11 ± 0.18 (358)	8.47 ± 0.19 (332)	6.68 ± 0.19 (172)	9.75 ± 0.21 (270)
	7.95 ± 0.21 (318)	8.79 ± 0.21 (337)	7.49 ± 0.21 (242)	10.91 ± 0.19 (291)
O(+)	8.83 ± 0.17 (408)	8.63 ± 0.18 (386)	7.39 ± 0.20 (237)	10.25 ± 0.14 (432)
	13.46 ± 0.14 (223)	13.34 ± 0.18 (186)	8.07 ± 0.25 (363)	10.96 ± 0.22 (305)
	12.74 ± 0.09 (425)	13.30 ± 0.12 (348)	9.76 ± 0.29 (238)	10.77 ± 0.28 (233)
O(-)	13.17 ± 0.11 (318)	13.34 ± 0.12 (256)	9.42 ± 0.19 (396)	10.01 ± 0.22 (327)
	2.55 ± 0.09 (441)	2.61 ± 0.08 (458)	4.63 ± 0.23 (235)	3.47 ± 0.27 (204)
	2.55 ± 0.12 (238)	2.62 ± 0.13 (256)	3.16 ± 0.10 (464)	3.38 ± 0.09 (337)
	2.56 ± 0.13 (203)	2.59 ± 0.12 (202)	4.87 ± 0.17 (356)	5.13 ± 0.18 (433)

<sup>a</sup> Number of flies is given in parentheses.

of flies having pigmented eyes move toward photoneutrality from their 4-day-old scores. This is especially true of Canton-S and strain O(+) and less so in strain O(-). To determine if an age effect is present in the absence of screening pigments, flies from the w(CS) strain were tested at 4 and 14 days. The white-eyed flies are photoneutral at 4 days, but at day 14 females tend to be slightly photonegative and males slightly photopositive.

## DISCUSSION

Screening pigments or eye color pigments in dipterans are distinguished from visual pigments by their location and their function. Eye color pigments are located in the primary and secondary pigment cells between ommatidia. These pigments prevent light from passing between adjacent ommatidia, thereby increasing visual acuity (Burkhardt, 1964). The eye color of wild-type *Drosophila* is due to the presence of a red pigment or drosopterin and a brown pigment or ommachrome.

The effect of screening pigment mutations on the phototactic behavior of *Drosophila melanogaster* has been investigated previously under substantially different experimental conditions. Brown and Hall (1936) used a Y-tube apparatus which had a light source illuminating one arm of the Y. Flies were shaken into the base of the Y and then allowed to run into the light or the dark arm. It was observed that a slightly lower percentage of white-eyed flies chose the light arm than did wild-type flies. Fingerman (1952) used a similar test situation to investigate the response of mutant flies to various wavelengths of light. While the response of the mutants to

white light was never measured, sepia was found to exhibit the strongest photopositive behavior at all individual wavelengths. White-eyed flies showed the least orientation to light, presumably due to loss of acuity.

The variety of conditions employed in testing *Drosophila* phototactic behavior has complicated the comparison of results from one test system to another (Rockwell and Sieger, 1973). An important factor was shown by Lewontin (1959) to be the kinetic state of the flies during testing. Agitated wild-type flies were found to move quickly to a light source while undisturbed flies seemed to be photonegative (see Rockwell *et al.*, 1975). The countercurrent apparatus measures phototaxis in flies which have been agitated (Benzer, 1967). Because of its strong photopositive behavior in the countercurrent device, Canton-S is the strain used as a base population by some of the laboratories which routinely induce visual mutations. When tested in the phototaxis maze where flies walk undisturbed through the apparatus, Canton-S is quite photonegative. Under the test conditions employed by Brown and Hall (1936) and by Fingerman (1952), flies were shaken prior to each test. This prevents direct comparison of our results with theirs. Further, neither of these previous investigations included any attempts to control the genetic backgrounds of the mutant flies being tested.

None of the eye color mutations examined here causes flies to behave more photonegatively than Canton-S. However, certain alterations of pigment content and composition are seen to result in less photonegative behavior. Whether these alterations are bringing about a tendency toward photoneutrality or actually influencing the flies to be more photopositive is difficult to know. Under either agitated or quiet conditions, a smaller proportion of white-eyed flies move into the light arm of Y-tubes. White-eyed flies are characterized by a reduced optomotor response (Kalmus, 1943), presumably due to the lowered visual acuity that results from absence of screening pigments. It would be expected that reduced visual acuity would make choices in the maze more random, resulting in a neutral phototactic score.

There is some evidence suggesting that darker eye color contributes to photopositive behavior. Visual inspection of flies from strain O(+) reveals many individuals with dark eye color variations (compared to wild type), while all flies from strain O(-) appear to have normal wild-type eyes. Evidently selection for photopositive behavior in the maze has favored alleles which darken eye color. The fact that the mutation sepia affects phototactic behavior even when heterozygous implies that eye-darkening alleles may not have to be homozygous to affect the phenotype and be utilized by selection.

While aging seems to make *D. melanogaster* more photoneutral, apparently there is no similar effect of age on *D. pseudoobscura* (Dobzhansky *et al.*, 1974). Factors contributing to the age effect in the *D.*

*melanogaster* strains having screening pigments are unknown, but may include a reduction in acuity or in the ability to discriminate the presence of light. The difference between the behavior of aged white-eyed females and aged white-eyed males is even more difficult to explain. The age phenomenon in white could reflect a neurophysiological process or change that does not express itself if screening pigments are present.

Polygenic traits are usually difficult to dissect genetically. Photomaze behavior is generally assumed to have a polygenic basis (Hadler, 1964*b*; Woolf, 1972; Markow, 1975*a,b*), but the function of the genes involved is unknown. The results of the present investigation suggest that some of the genetic factors influencing phototaxis control synthesis of screening pigments. The age effect observed for phototactic behavior in *D. melanogaster* suggests that this variable should be carefully controlled in future investigations.

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