

Light-Dependent Pupation Site Preferences in *Drosophila*: Behavior of Adult Visual Mutants

THERESE ANN MARKOW

Department of Zoology, Arizona State University, Tempe, Arizona 85281

A method for measuring the influence of light on pupation site preferences, PSP, of late-third-instar *Drosophila* is described. Wild-type strains of *D. melanogaster* all are characterized by dark PSPs. Strains of flies having mutations that render adults blind due to structural abnormalities of the compound eye show no alterations in PSP. Flies carrying the mutant *trp^{CM}* also show normal PSPs. However, severe mutations at the *norpA* locus appear to leave larvae blind as well as cause blindness in adults. The *norpA* locus is believed to control a protein intermediate in the phototransduction process. It is hypothesized that the step controlled by the *norpA* locus is common to the photoreceptors of both larvae and adult flies. Genes controlling structural components of the compound eye of adults are evidently not important for light-dependent behavior in preimaginal stages.

A tremendous amount of literature exists describing phototactic responses of adult *Drosophila* at the neurological level (Pak & Grabowski, 1978) and populational level (Markow, 1975; Markow & Smith, 1977, 1979). In adult flies the major photoreceptor is the compound eye and almost all light-dependent adult behaviors appear to be mediated by this organ. Extensive mutagenesis studies have generated numerous mutants producing aberrations in visually dependent behaviors that have been traced to lesions in the structure and/or function of the compound eye. These mutants have been indispensable to the enormous progress made in the field of insect vision.

On the other hand, a little-studied area of *Drosophila* behavioral and neural biology is phototactic responses in preadult stages. In *D. melanogaster* the first three larval instars last 4 to 5 days and the pupal stage makes up the other half of preimaginal life. Responses to environmental variables such as light, density, temperature, and gravity during these stages are not random (Rizki & Davis, 1950; Sokal, Ehrlich, Hunter,

¹ Supported by NIH Grant GM 25424. The technical assistance of Ms. Melanie Manning, Ms. Patricia Merha, and Ms. Michino Oishi is greatly appreciated. Drs. William Pak and John Merriam kindly provided mutant stocks used in this study.

& Schlager, 1960; Markow, 1979) and are quite critical to the successful emergence of the adult fly.

In my laboratory we have become interested in whether or not any relationship exists between adult and preadult phototactic behavior. We have decided to focus initially on one preimaginal behavior, pupation site preference, PSP, since this step is followed by a long and vulnerable period during which the larva is transformed to a fly. This paper is the first of a series dealing with the effect of light on PSP. A system for measuring the influence of light on PSP is described and experiments testing the effect of adult visual-system mutants on PSP are reported. Two types of visual mutants were tested, those which affect structure of the adult compound eye and those which affect the phototransduction process. The expectation that only the latter group effects preimaginal behavior is verified.

MATERIALS AND METHODS

Two wild-type strains, Canton-S and Oregon-R, and six mutant strains were tested for PSP. The mutants had all initially been isolated on the basis of abnormal adult phototactic behavior and were subsequently found to have aberrations of the electroretinogram of the compound eye. Four of the mutants tested reside at the *norpA* ("no receptor potential") locus ($I-6.5 \pm 0.1$, Pak, Ostroy, Deland, & Win, 1976). Another mutant, *trp^{CM}* ("transient receptor potential"), is autosomally located (Chromosome III, Cosens & Manning 1969). The other strain, *w t sev ora*, is a multiply mutant stock ("white," $I-1.5$; "tan," $I-27.5$; "sevenless," $I-33.2 \pm 0.2$; "outer rhabdomeres absent," $III-65.3 \pm 0.4$, Harris, Stark & Walker, 1976; Koenig & Merriam 1977).

The above strains were obtained from Dr. William Pak at Purdue University and Dr. John Merriam at UCLA. Two other strains were tested, photomaze negative and photomaze positive. These strains were created by over 300 generations of continuous selection for adult photopreferences in Hirsch-Hadler multiple-unit phototaxis mazes (Markow 1975). On a photopreference scale of 1 (photonegative) through 16 (photopositive) the photonegative strain had a photoscore of 2.8 ± 0.12 and the photopositive strain had a score of 14.96 ± 0.15 at the time of this study. Both strains have normal ERGs in spite of their extreme preferences for light or dark.

All strains were raised on standard cornmeal-molasses-agar medium at $24 \pm 1^\circ\text{C}$. To collect eggs for PSP determinations, groups of about 30 pairs of flies were placed in empty half-pint bottles inverted over stendor dishes containing paper towels moistened with a yeast suspension. Eighteen hours later eggs were collected from the surfaces of the paper towels and transferred to Wheaton glass staining dishes which contained $\frac{1}{2}$ in. of medium in the bottom. Each glass dish was set up with 300 eggs arranged on a line in the central axis of the dish. The lids were placed on the dishes

and each dish was then half covered with a double-layered jacket of black felt as shown in Fig. 1. Dishes were placed in incubators having cool white lights in their ceilings and set at a temperature of $24 \pm 1^\circ\text{C}$. The light intensity at the surfaces of the medium in the dishes was 15 fc. Eight days after the boxes were set up, the distribution of pupae in the light and dark halves of the boxes was computed. Two kinds of control experiments were conducted, either in completely light or in completely dark boxes.

RESULTS AND DISCUSSION

All of the visual mutants used in this study were originally isolated on the basis of their failure as adults to respond to light in a countercurrent device or a similar apparatus (Benzer 1967, Pak, Grossfield, & White, 1969). Such devices test reactions of agitated flies to light. When phototactic behavior of adult flies was measured in the countercurrent apparatus, the distributions of adult flies seen in Fig. 2 were obtained. Flies from the wild-type strains and the two photomaze strains run quickly to light. It is not surprising that selection for maze behavior has had no apparent effect upon the countercurrent distributions of the photostains since the maze and the countercurrent device measure different behaviors (Lewontin 1959). Individuals from all mutant strains show marked deviations from the wild-type pattern: they are not attracted to light. Flies from mutant strains cannot respond normally to light because of defects in the compound eye.

Pupation site preferences of these same strains of flies are shown in Table 1. On the average 87% of the eggs reached the pupal stage. Each mean represents at least three replications. The four nonmutant strains, Canton-S, Oregon-R, "photomaze negative," and "photomaze positive," all show a similar degree of dark PSP. Larvae from the *trp*^{CM}, *w tan sev ora*, and *norpA*^{p16} strains also show strong PSPs for darkness. The other three alleles at the *norpA* locus all show striking deviations from wild type for PSP. One, *norpA*^{p64}, still prefers the dark, but less so than wild type. The distributions of pupae in *norpA*^{p24} and *norpA*^{H64} are identical to those of controls run in complete darkness.

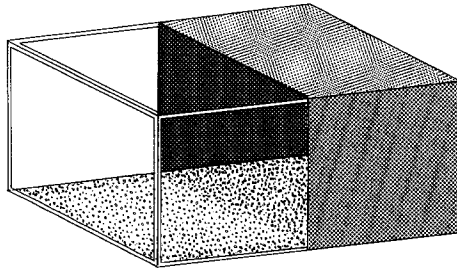


FIG. 1. Chamber employed in testing pupation site preferences of third-instar *Drosophila* larvae.

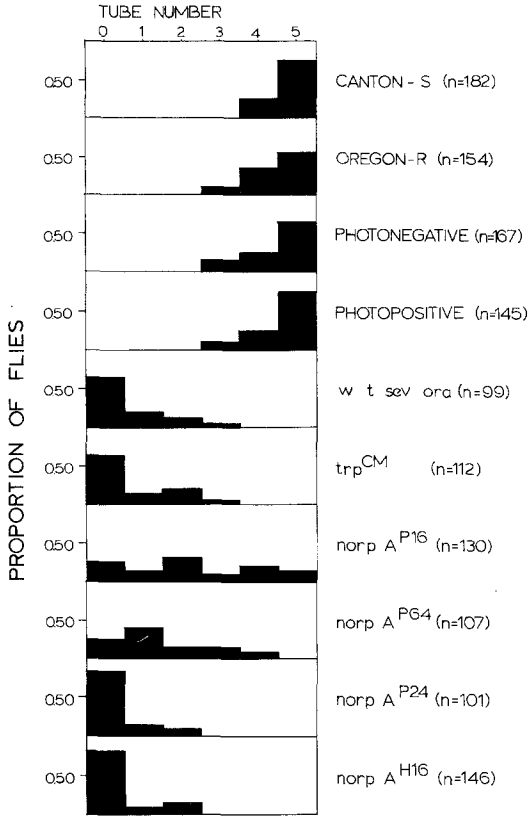


FIG. 2. Behavior of adult flies in the countercurrent apparatus. Each tube has a number which corresponds to the number of times flies ran toward light.

The mutant strains of flies used in this study fall into two categories with respect to the nature of neuroanatomical and/or neurophysiological abnormalities they produce in the compound eye. The first type affects the structural features of the compound eye and is represented by the *w t sev ora* strain. Adult flies from this strain lack function in both the outer retinula cells R_{1-6} , and in R_7 , a situation characterized by an abnormal ERG (Harris et al., 1976). By behavioral criteria, adult flies from this strain are blind. The compound eyes of flies from the second category, while structurally intact, appear to render flies blind due to some defect in intermediate steps in the phototransduction process. The remainder of the mutants tested here are of this type. Flies homozygous or hemizygous for strong *norpA* alleles, such as *norpA^{P24}* or *norpA^{H44}* are behaviorally blind and show a flat ERG. Less extreme alleles such as *norpA^{P16}* and *norpA^{P64}* give reduced visual abilities and less by behavioral criteria and an altered ERG form.

TABLE I
Proportion of Pupae Found in the Light and in the Dark from Wild-Type and Mutant Strains of *D. melanogaster*

Strain	Percentage in light	Percentage in dark	<i>n</i>
Canton-S	21.8	78.2	1845
Oregon-R	22.9	77.1	802
Photomaze +	23.0	77.0	835
Photomaze -	29.9	70.1	861
w tan sev ora	22.5	77.5	888
trp ^{CM}	17.8	82.2	868
norpA ^{p16}	20.3	79.7	893
norpA ^{p64}	37.5	62.5	1015
norpA ^{H44}	51.4	48.6	718
norpA ^{p24}	49.2	50.8	1056
Light control (Canton-S)	48.9	51.1	1270
Dark control (Canton-S)	48.1	51.9	1192

Note. a-c: The percentage data were transformed to angles prior to being subjected to a Duncan multiple range test, $\alpha = .05$. Three non-overlapping subsets, a, b, and c, were found.

While *Drosophila* larvae lack compound eyes, they do possess non-image-forming photoreceptors at their anterior ends (Demerec, 1950). However, these photoreceptors have not yet been described in detail for any species in the genus. One purpose of this study was to use genetic methods to see if any commonalities exist between structural and functional components of the larval and adult visual systems. Since the adult compound eye develops during the pupae stage it is not surprising to find that genes controlling its structure are not functioning in the larval photoreceptor system. The multiple mutant w t sev ora which affects compound eye structure has no effect on PSP. Neither does the weakest norpA allele, norpA^{p16}. However, strong norpA alleles produce PSPs suggestive of an inability either to perceive light or to process its reception, just as in adults. Curiously trp^{CM} has no effect on PSP. Possibly the trp locus controls a phototransduction step which exists only in the compound eye (Minke 1977). Taken together, these results suggest that while genes controlling characteristic compound eye structures do not contribute to the larval compound eye, the phototransduction process, or at least part of it, is the same.

The PSPs of larvae from photomaze strains are interesting. Preferences of adults flies for light or darkness can be modified in the laboratory by artificial selection (Markow 1975). But judging from the PSPs of these strains, it is evident that genes which influence flies to go to light or to dark as adults are either not active earlier or act on CNS processes different from those used by larvae in selecting a pupation site.

In a different test device, Rizki and Davis (1953) also found that *D. melanogaster* larvae tend to seek out dark pupation sites. In addition, other studies with our own system have shown that fresh samples from wild populations of *D. melanogaster* prefer to pupate in the dark (Manning, 1980). These findings, taken with the observation that all wild-type strains in the present study also exhibit dark PSPs, suggest that this trait has been under strong selection in this species. Selection of dark pupation sites may act as an adaptation against the perils of predation and desiccation.

REFERENCES

- Benzer, S. (1967). Behavioral mutants of *Drosophila* isolated by countercurrent distribution. *Proceedings of the National Academy of Sciences*, **58**, 1112–1119.
- Cosens, D. J., & Manning, A. (1969). Abnormal electroretinogram from a *Drosophila* mutant. *Nature (London)* **224**, 285–287.
- Demerec, M. (Ed.). (1950). *The Biology of Drosophila*. New York: Hafner.
- Harris, W., Stark, W., & Walker, J. (1976). Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. *Journal of Physiology*, **256**, 415–439.
- Koenig, J. & Merriam, J. (1977). Autosomal ERG mutants. *Drosophila Information Service*, **42**, 50–51.
- Lewontin, R. C. (1959). On the anomalous response of *Drosophila pseudoobscura* to light. *American Naturalist*, **93**, 327–328.
- Manning, M. (1980). Pupation Site selection in *Drosophila*. *Journal of Arizona–Nevada Academy of Sciences*, **15**, 33.
- Markow (1975). A genetic analysis of phototactic behavior in *Drosophila* I. Selection in the presence of inversions. *Genetics*, **79**, 527–534.
- Markow (1979). A survey of intra- and interspecific variation for pupation height in *Drosophila*. *Behavior Genetics*, **9**, 209–217.
- Markow, T. & Smith, W. L. (1977). Genetic analysis of phototactic behavior in *Drosophila simulans*. *Genetics*, **85**, 273–278.
- Markow, T. & Smith, L. D. (1979). Genetics of phototactic behavior in *Drosophila ananassae*, a member of the melanogaster species group. *Behavior Genetics*, **9**, 61–67.
- Minke, B. (1977). *Drosophila* mutant with a transducer defect. *Biophysical and Structural Mechanisms*, **3**, 59–64.
- Pak, W. L., & Grabowski, S. (1978). Physiology of the visual and flight systems. In M. Ashburner & T. Wright (Eds.). *The Genetics and Biology of Drosophila*, Vol. 2a. New York: Academic Press.
- Pak, W. L., Grossfield, J., & White, W. V. (1969). Non-phototactic mutants in a study of vision of *Drosophila*. *Nature (London)*, **222**, 351–354.
- Pak, W. L., Ostroy, S. E., Deland, M. C., & Win, C. F. (1976). Photoreceptor mutant of *Drosophila*: Is protein involved in intermediate steps of phototransduction? *Science*, **194**, 956–959.
- Rizki, R. M. & Davis, C. (1953). Light as an ecological determinant of interspecific competition between *D. willistoni* and *D. melanogaster*. *American Naturalist*, **87**, 389–392.
- Sokal, R. R., Ehrlich, P., Hunter, P., & Schlager, G. (1960). Some factor affecting pupation site of *Drosophila*. *Annals of the Entomological Society of America*, **53**, 174–182.