

Light-Dependent Pupation Site Preferences in *Drosophila*. II. *Drosophila melanogaster* and *Drosophila simulans*

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Drosophila melanogaster prefers to pupate in the dark, while its sibling species, *D. simulans*, prefers the light when the species are tested in isolation and when cultured and tested together. Reciprocal interspecific hybridizations were carried out and the F_1 individuals were tested. Progeny from the cross of *D. melanogaster* females with *D. simulans* males chose pupation sites exactly intermediate between those of the two parental species, while the reciprocal-cross offspring preferred light pupation sites. The pupation site preferences (PSPs) of the hybrids are compatible with a sex-linked locus or loci influencing light-dependent PSP in this pair of species. Examination of light preferences of larvae prior to the late third instar demonstrates that these preferences are highly specific, being restricted to the time just before pupation. During the first two larval instars *D. melanogaster* is quite photopositive while *D. simulans* is comparatively photoneutral. These differences in light-dependent behavior could aid in reducing competition between the two species.

KEY WORDS: *Drosophila*; photobehavior; pupation site selection; sibling species.

INTRODUCTION

Attempts at understanding genetic and environmental determinants of habitat choice in *Drosophila* have generally focused on adult flies. However, a growing body of literature is beginning to reveal the existence of an interesting variety of preimaginal behavioral adaptations (Fogleman

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and Markow, 1980; Sokolowski, 1980; Markow, 1979; Godoy-Herrera, 1977). One particular stage of preadult life, the third-instar larva, appears to be subject to strong selective pressures on habitat preferences, for it is directly following this stage that the larvae pupate, remaining immobile for a number of days during which they are exposed to the possibility of desiccation, predation, and disease.

When moving to pupation sites, third-instar larvae of *Drosophila* show definite responses to a number of environmental variables including light (Markow, 1980; Rizki and Davis, 1953), moisture (Sameoto and Miller, 1968), gravity (Markow, 1979), larval density (Sokal *et al.*, 1960), and the presence of other species (Rizki and Davis, 1953). The interactions between these environmental factors and the genotype pose some interesting questions for the behavioral and ecological geneticists interested in adaptations.

In view of the large body of literature on the phototactic behavior of adult *Drosophila* (Markow, 1980; Markow and Smith, 1977, 1979; Kawanishi and Watanabe, 1978), we have chosen to examine the genetic aspects of light dependence in pupation site preferences (PSPs). Species differences in light-dependent PSPs have already been reported (Rizki and Davis, 1953; Markow, 1980; Fogleman and Markow, 1980). In the present paper we report upon the PSPs of two sibling species, *D. melanogaster* and *D. simulans*, their F₁ hybrids, and the results of a study of the developmental expression of light responses through the life cycle stages preceding pupation.

MATERIALS AND METHODS

The strains of *D. melanogaster* and *D. simulans* used in these experiments were descendants of flies caught in a deserted citrus grove approximately 5 mi from Arizona State University in Tempe. Experiments were initiated about five generations after their capture. All flies were reared in half-pint glass milk bottles containing a standard cornmeal-molasses-agar yeast medium at $24 \pm 1^\circ\text{C}$.

The first experiment testing for light dependence in pupation site preference (PSP) followed the procedure described by Markow (1980). Flies were placed in culture bottles seeded with yeast for 24 h before being transferred to egg collection containers. The egg collection containers consisted of empty half-pint milk bottles inverted over Stender dishes lined with a paper towel moistened with yeast suspension. Eggs were collected 18 to 20 h later from the surface of the disk and placed in Wheaton glass staining dishes containing 6 mm of medium. Three hundred eggs were arranged along the midline of each dish. Three hundred was

the sample size used throughout in order to avoid the effects of variable larval densities on the pupation site (Sokal *et al.*, 1960). With the lid in place, each dish was fitted with a jacket of double-layered black felt that covered half the dish. The dishes were placed in lighttight incubators at $24 \pm 1^\circ\text{C}$ and were constantly illuminated by G.E. circular cool white lights positioned in the incubator ceiling. The light intensity at the surface of the boxes was 160 lux. Eight to nine days later the boxes were removed from the incubators and the numbers of pupae in the light and dark halves were counted. Completely light boxes served as controls.

The second experiment was set up to see if either species showed a change in PSP when cultured together. Mixed-species boxes were set up with the same total number of eggs ($N = 300$), 150 eggs from each species. Morphological features that distinguish adult *D. simulans* from *D. melanogaster* are most reliably scored in males. Therefore, pupae were collected and only those containing male flies were scored. Previous studies indicate no sexual dimorphism for PSP.

The PSP of hybrids between *D. melanogaster* and *D. simulans* was measured in the third experiment. Virgin males and females from each species were separated under light ether anesthesia and stored until 3 or 4 days old. Flies were placed together in the reciprocal combinations: *D. melanogaster* females with *D. simulans* males and *D. simulans* females with *D. melanogaster* males. The flies were kept on yeasted medium for 2 days before being placed in egg collection containers. Since hybrid eggs have a decreased hatchability compared to eggs of each species, boxes for testing PSPs were set up with 500 individuals instead of 300.

In the fourth experiment, the responses of earlier stages (first-through third-instar larvae) to light were measured by setting up boxes in the same way as described above but recording the positions of individuals prior to pupation. The distributions of larvae were determined at 24, 48, 62, 86, 110, and 134 h, which corresponded to the first, second, early third, middle third, and late third instars and the prepupal stage. Larvae were harvested by first dividing the medium into eight sections and then using a hand strainer and some netting to separate them from the medium. Some boxes were allowed to remain in the incubators until pupation and served as further controls.

Each experiment was replicated at least three times and the data were pooled.

RESULTS AND DISCUSSION

The PSPs of *D. melanogaster* and *D. simulans* obtained in the first experiment are shown in Table I. *D. melanogaster* shows a clear pref-

Table I. Dark Pupal Distribution^a

	Percentage ($\bar{X} \pm \text{SE}$)	Arcsine $\sqrt{\text{percentage}}$ ($\bar{X} \pm \text{SE}$)	N	R
<i>D. melanogaster</i>	64.06 \pm 3.70	53.36 \pm 2.26	1912	9
<i>D. simulans</i>	40.18 \pm 3.30	39.26 \pm 1.94	1836	8
<i>D. melanogaster</i> with <i>D. simulans</i> ^b	63.12 \pm 6.38	52.81 \pm 3.94	139	4
<i>D. simulans</i> with <i>D. melanogaster</i> ^b	44.93 \pm 7.63	42.04 \pm 4.43	171	4
<i>D. melanogaster</i> ♀ \times <i>D. simulans</i> ♂	52.85 \pm 8.15	46.69 \pm 4.83	308	6
<i>D. simulans</i> ♀ \times <i>D. melanogaster</i> ♂	23.60 \pm 4.40	28.94 \pm 2.92	103	3
<i>D. melanogaster</i> controls	48.90 \pm 2.76		1125	6
<i>D. simulans</i> controls	54.87 \pm 1.50		911	5

^a Mean percentages of pupae in the dark halves of the boxes. *N* is the total number of pupae in the light and dark halves. *R* is the number of experiment replications. Arcsine transformed means utilized in statistical analyses are also given.

^b Males only.

erence for the dark, while *D. simulans* elects to pupate in the light ($P < 0.01$). It is interesting that any *Drosophila* species would pupate in the light, since in nature, sunny, exposed areas would allow more predation and desiccation than dark areas due to an increased visibility. Boxes with no covers served as controls for this experiment. These results, also given in Table I, show that with no choice of a light or dark pupation site, the pupae are evenly distributed in the dish.

In the second experiment, in which the two species were cultured together, no significant effect of the presence of one species on the behavior of the other was detected. The lack of character displacement for *D. melanogaster* PSPs is especially interesting in view of the observation by Rizki and Davis (1953) that, when cultured with *D. willistoni*, *D. melanogaster* does move its pupation site away from sites occupied by the other species. However, Budnik and Brncic (1974), who examined larval viability in *D. pavani*, *D. willistoni*, *D. simulans*, and *D. melanogaster* when cultured under mixed conditions, found that when two species that do not coexist in the same substrate in nature were placed together, the viability was significantly reduced. Possibly some biotic residue of one species is deleterious to the other. Budnik and Brncic (1974) found no effect on viability when the two species used were known to coexist in the wild. It would certainly be advantageous to larvae to be able to recognize and avoid the presence of individuals that may be harmful. That larvae are able to recognize individuals from their own strain has been demonstrated by Pruzan and Bush (1977). Larvae of the two species used here, *D. simulans* and *D. melanogaster*, are found together in the same substrate in nature. Culturing them together in the laboratory does not result in any reduction in the viability of either species (M.

Manning, unpublished). These two species have probably coexisted long enough that some sort of niche partitioning has already arisen. Observations on the light preferences of first- and second-instar larvae of both species (see below) support this contention.

The results of the third experiment are also given in Table I. A significant difference in PSP was found between the two types of interspecific F_1 , with the hybrids with *D. melanogaster* mothers preferring dark PSPs and the hybrids from the other cross pupating in the light. A Scheffé's analysis showed no significant difference between the means of *D. melanogaster* individuals and *D. melanogaster* \times *D. simulans* F_1 . The F_1 hybrids tend to be intermediate between the two parental species, showing little if any preference. On the other hand, comparing *D. simulans* to the F_1 of reciprocal cross, the F_1 hybrids are even more photopositive (76.4%) than their *D. simulans* mothers (59.8%, $P < 0.01$). These results are compatible with the hypothesis that genes controlling light-dependent PSPs are sex linked. Assuming more than one allele at PSP loci, some degree of heterozygosity would be expected in the hybrid females. Heterozygosity for positive and negative PSP alleles, in the absence of dominance, would give an intermediate PSP as seen in the cross that gives females (*D. melanogaster* females with *D. simulans* males). The reciprocal cross gave males that pupated in the light, as did larvae from the strain from which they received their X chromosomes.

An analysis of variance was performed with the first six groups listed in Table I which yielded an F value of 7.58 ($P < 0.05$). A posteriori comparisons among group means were also made using pairwise Scheffé's analysis. Significant differences were found between group 1 and group 2 and between group 5 and group 6.

An earlier study (Markow, 1980) failed to reveal any correlation between photopreferences of adults and light-dependent PSPs, suggesting that these two behaviors are under the influence of different sets of genes. The question arose whether the light or dark preferences expressed by late third-instar larvae are characteristic only of that stage or of larval behavior in general. The distributions of first-, second-, and third-instar larvae are reported in Table II. Numbers of the first-instar larvae were lower than those of the second and third instars owing to their small size, visibility, and vulnerability to hazards of the counting procedure. In *D. melanogaster*, larvae are slightly photopositive during the first and second instars, are photoneutral during the third instar, and have become photonegative as prepupae. In this species, the "decision" to pupate in the dark is highly stage specific. *D. simulans* larvae, on the other hand, are slightly photopositive during the first instar, photoneutral during the second and early third instars, and then slightly photopositive from the middle

TABLE II. A Comparison of Light-Dark Distributions for the Three Larval Instars of Each Species^a

Stage	% in dark		<i>t</i>	df	<i>P</i>
	<i>D. melanogaster</i>	<i>D. simulans</i>			
1st instar (χ^2)	25.70 ± 7.00 (320) 68.46 35.35 ± 4.50 (511) 24.98	43.20 ± 8.74 (94) 0.38 54.17 ± 9.90 (372) 4.74	2.512 0.50 < <i>P</i> < 0.70 2.683 <i>P</i> < 0.05	9 17	0.02 < <i>P</i> < 0.05 0.01 < <i>P</i> < 0.02
2nd instar (χ^2)	54.92 ± 3.88 (395) 0.02	57.16 ± 7.64 (399) 0.72	0.865 0.30 < <i>P</i> < 0.50	19	0.30 < <i>P</i> < 0.50
3rd instar (a) (χ^2)	50.31 ± 6.26 (1008) 9.52	44.99 ± 4.32 (347) 4.38	1.323 <i>P</i> < 0.05	25	0.10 < <i>P</i> < 0.20
3rd instar (b) (χ^2)	46.23 ± 2.50 (1769) 8.56	47.19 ± 3.40 (873) 0.72	0.312 0.30 < <i>P</i> < 0.50	21	0.70 < <i>P</i> < 0.80
3rd instar (c) (χ^2)	83.96 ± 22.68 (60) 11.26	41.99 ± 11.02 (171) 1.69	3.414 0.10 < <i>P</i> < 0.20	5	0.01 < <i>P</i> < 0.02
Prepupae (χ^2)					

^a A chi-square test tests the deviation of the distribution of larvae from each stage from a 50:50 light:dark distribution, and the two species are compared stage by stage by a *t* test. The *t* tests were performed on arcsine transformed data.

third instar onward. The preference for light apparently expresses itself at an earlier stage in *D. simulans*. At three of the six stages of development at which these two sibling species were compared, their distributions differed significantly. Since these two species may coexist in nature, these behavioral differences could serve as a means of reducing interspecific competition by partitioning preimaginal substrates.

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