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A Survey of Intra- and Interspecific Variation for Pupation Height in *Drosophila*

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Pupation heights of various natural and laboratory populations of Drosophila melanogaster, D. simulans, *and* D. pseudoobscura *were observed in the laboratory under conditions of continuous darkness or continuous light. Generally higher mean pupation heights were observed under conditions of darkness.* D. melanogaster *tended to pupate higher than the* D. pseudoobscura *populations, and* D. pseudoobscura *tended to pupate higher than* D. simulans. *The order of these species differences was similar whether pupation was measured in light or in darkness. Results of selection for pupation height in* D. melanogaster *suggest the presence of genetic variation for this character. The possibility that a relationship exists between adult and larval behaviors was explored by measuring the pupation heights of larvae from strains selected for geotactic behavior as adults, and also by measuring geotaxis of adults from strains selected for pupation height.*

KEY WORDS: *Drosophila melanogaster*; *D. simulans*; *D. pseudoobscura*; pupation height; geotaxis; species differences; selection for pupation height.

INTRODUCTION

Most of the *Drosophila* behavior genetics literature deals with adult fly behaviors such as courtship, phototaxis, and geotaxis, and with neurological aberrations. The behaviors of preimaginal stages, which constitute a sizable portion of the drosophilid life span, have received comparatively little attention, despite their probable importance to the total fitness of the organism.

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In the present study we looked at one preimaginal trait: how high third instar larvae pupate. We measured pupation height in freshly caught samples of *D. melanogaster*, *D. simulans*, and *D. pseudoobscura* from diverse geographic localities to get an idea of how much intra- and interspecific variation exists for this trait. The measurements were made in the light and in darkness in order to assess the effect of illumination on pupation height. We also attempted to select for high and low pupation in *D. melanogaster*. The effects of selection for pupation height on adult geotactic behavior and of selection for adult geotaxis on pupation heights were also examined.

MATERIALS AND METHODS

Measurement of Pupation Height

In all experiments, eggs were placed on the surface of a 2-inch-deep layer of cornmeal-molasses-agar medium contained in the bottom of a 20- by 4-cm plexiglas cylinder. The cylinder was lined with parafilm to enable removal of all pupae for counting and for retrieval of individuals for selection experiments. Pilot studies showed no effect of the parafilm linear on pupation height. Height of pupation was determined as the distance in centimeters of each pupa from the surface of the medium. Measurements were made when all the larvae had pupated but prior to the time of eclosion. Pupation occurred under conditions of continuous illumination of 90 ft-candles provided by G.E. cool white (FC16T10-CW-RS) lights or in continuous darkness in a light-tight incubator. Inside the cylinders, the temperature under both conditions was 24 ± 1°C and the humidity was 35%. Samples of each population were tested in light and in darkness simultaneously, and several replications were carried out at random for each population. Each sample consisted of 150 eggs collected within 6 hr of each other. In the various experiments, between 86% and 97% of the eggs reached the pupal stage. One hundred and fifty eggs were used consistently in all experiments to avoid effects of varying density on pupation site (Sokal *et al.*, 1960). Since pilot studies showed no sex differences in pupation heights, mean heights were calculated for the total number of pupae in each sample.

Selection for Pupation Height

Selection was begun by measuring the pupation height of the base population in the light and then, from a total of 150, taking the 35 highest and 35 lowest pupae, allowing adults to eclose from them, and using them as parents of the next generation. Selection, in light, was continued for 21 generations.

Measurement of Adult Geotaxis

Geotactic behavior of adult *Drosophila* was measured using Hirsch classification mazes (Hirsch, 1959). Flies entering a maze made 15 consecutive up-down choices and emerged in 16 numbered collecting tubes at the end. Flies which made 15 downward choices emerged in tube No. 16 (geopositive). Flies making 15 upward choices emerged in tube No. 1 (geonegative). About 200 flies were tested at once, and after 24 hr all flies completed the maze. The flies in each collecting tube were counted in order to calculate a mean geotactic score. A geoneutral population has an expected mean of 8.5. Two replications of each maze run were conducted and pooled if appropriate following homogeneity tests.

Strains of Flies

Wild-caught samples of *D. melanogaster*, *D. simulans*, and *D. pseudoobscura* were tested within one to three generations of their capture. The localities are shown in Table I.

For the selection experiments, a heterogeneous base population of *D. melanogaster* was employed. This population was founded by combining five different wild-type laboratory strains (Canton-S, Oregon-R, Urbana, Lausanne, and Stephenville) in a population cage for ten generations prior to the onset of selection.

Our laboratory has been maintaining strains of *D. melanogaster* and *D. pseudoobscura* that have been selected as adult flies for positive and negative geotactic behavior in Hirsch classification mazes for over 100 generations (Hirsch, 1959; Pyle, 1976; Woolf *et al.*, 1978). The pupation heights of larvae from each of these strains were measured.

RESULTS

In Table I, the mean pupation heights (in centimeters) for several populations of *D. melanogaster*, *D. simulans*, and *D. pseudoobscura* are shown. Pupation heights in the light and dark were compared by T-tests. While some variation existed between replications, in every case comparatively higher pupation sites were characteristic of the flies tested in darkness. Statistically significant light-dark differences did not occur for all *D. simulans* populations, but the trend toward higher pupation in darkness was consistently present. There was no apparent relationship between how high larvae from a given population pupated in the light and how high they pupated in the dark. For example, *D. melanogaster* from Orlando, Florida, showed the lowest pupation sites in light but tended to pupate rather high in darkness.

Table I. Pupation Heights of *Drosophila* Populations (in cm)

Origin	Replication	Light $\bar{X} \pm$ SE (n)	Dark $\bar{X} \pm$ SE (n)	t
Amherst, Mass. (1976)	1	3.14 ± 0.60 (141)	4.59 ± 0.62 (143)	4.39[a]
D. melanogaster	2	4.14 ± 0.60 (142)	5.26 ± 0.68 (146)	3.39[a]
Cornville, Ariz. (1976)	1	1.82 ± 0.60 (142)	4.52 ± 0.58 (142)	8.18[a]
D. melanogaster	2	2.66 ± 0.60 (142)	5.41 ± 0.79 (143)	8.59[a]
	3	2.63 ± 0.61 (141)	4.87 ± 0.81 (145)	5.74[a]
Orlando, Fla. (1976)	1	1.42 ± 0.52 (145)	6.03 ± 0.51 (145)	12.80[a]
D. melanogaster	2	1.89 ± 0.57 (142)	4.00 ± 0.54 (141)	6.03[a]
	3	1.45 ± 0.59 (146)	4.20 ± 0.59 (142)	8.33[a]
Omaha, Nebr. (1976)	1	1.77 ± 0.58 (144)	3.59 ± 0.61 (141)	5.69[a]
D. melanogaster	2	1.73 ± 0.59 (146)	3.41 ± 0.66 (142)	4.67[a]
	3	1.78 ± 0.60 (145)	3.58 ± 0.67 (143)	2.15[a]
Provo, Utah (1976)	1	2.16 ± 0.56 (143)	3.83 ± 0.59 (146)	4.91[a]
D. melanogaster	2	2.72 ± 0.58 (143)	3.67 ± 0.59 (141)	3.06[a]
	3	2.86 ± 0.59 (146)	3.97 ± 0.68 (141)	3.36[a]
Tempe, Ariz. (1976)	1	3.18 ± 0.62 (141)	4.96 ± 0.67 (141)	4.94[a]
D. melanogaster	2	3.08 ± 0.55 (145)	4.64 ± 0.68 (144)	4.00[a]
Riverside, Calif. (1976)	1	2.24 ± 0.57 (143)	3.43 ± 0.55 (142)	3.61[a]
D. melanogaster	2	2.22 ± 0.62 (145)	3.83 ± 0.59 (146)	4.24[a]
Oregon-R	1	3.52 ± 0.42 (141)	4.27 ± 0.42 (144)	2.58[a]
D. melanogaster	2	3.02 ± 0.46 (144)	5.99 ± 0.49 (141)	9.28[a]
	3	2.71 ± 0.53 (144)	6.27 ± 0.56 (145)	10.47[a]
Riverside, Calif. (1976)	1	0.68 ± 0.55 (140)	1.56 ± 0.56 (141)	2.93[a]
D. simulans	2	0.67 ± 0.56 (138)	1.74 ± 0.56 (144)	3.57[a]
Honolulu, Hawaii (1977)	1	0.10 ± 0.39 (140)	0.56 ± 0.54 (139)	2.19
D. simulans	2	0.07 ± 0.42 (140)	0.68 ± 0.53 (140)	2.18
	3	0.22 ± 0.54 (144)	0.53 ± 0.54 (138)	1.24
	4	0.06 ± 0.16 (142)	0.40 ± 0.54 (143)	1.41
Amecameca, Mex. (1976)	1	0.75 ± 0.72 (134)	3.89 ± 0.61 (135)	8.97[a]
D. pseudoobscura	2	0.86 ± 0.62 (136)	3.74 ± 0.67 (130)	9.00[a]
Riverside, Calif. (1976)	1	1.14 ± 0.47 (133)	2.12 ± 0.48 (130)	3.27[a]
D. pseudoobscura	2	1.11 ± 0.43 (133)	2.75 ± 0.38 (129)	5.12[a]
Mather, Calif. (1976)	1	1.12 ± 0.49 (130)	3.94 ± 0.57 (128)	8.81[a]
D. pseudoobscura	2	1.06 ± 0.66 (136)	3.84 ± 0.64 (135)	7.94[a]

[a] $p < 0.01$.

The pupation heights of all samples of the three species were subjected to Duncan's multiple-range test. In the light, there were three significantly different subgroups ($p < 0.05$), which corresponded to the three different species. In the dark, two significantly different subgroups ($p < 0.05$) occurred. The first subgroup contained all populations of *D. melanogaster* and the Amecameca, Mexico, and Mather, California, samples of *D. pseudoob-*

Table II. Pupation Heights of *Drosophila* (in cm)

Origin	Replication	Light $\bar{X}$ ± SE (n)	Dark $\bar{X}$ ± SE (n)
D. melanogaster	1	2.16 ± 0.54 (139)	7.09 ± 0.51 (140)
geonegative	2	1.72 ± 0.59 (139)	8.36 ± 0.63 (141)
D. melanogaster	1	2.63 ± 0.50 (142)	4.84 ± 0.43 (144)
geopositive	2	2.75 ± 0.58 (144)	4.50 ± 0.58 (143)
D. pseudoobscura	1	0.68 ± 0.69 (138)	6.33 ± 0.31 (140)
geonegative	2	0.69 ± 0.65 (139)	5.78 ± 0.73 (138)
D. pseudoobscura	1	0.40 ± 0.68 (139)	2.56 ± 0.68 (143)
geopositive	2	0.40 ± 0.71 (138)	2.31 ± 0.72 (140)

scura. All *D. simulans* samples and the *D. pseudoobscura* from Riverside were included in the second subgroup.

To explore whether selection for adult geotactic behavior had any effect on height of pupation, we looked for pupation height differences between the geopositive and geonegative strains of both *D. melanogaster* and *D. pseudoobscura* (Table II). Again, larvae showed higher pupation in the dark than in the light, especially when from geonegative strains. The pupation heights of each species were subjected to a 2 × 2 factorial analysis of variance. For *D. melanogaster*, between-strains df = 1, MS = 2.656, F = 10.97, between-conditions df = 1, MS = 30.149, F = 124.52, and interaction df = 1, MS = 7.239, F = 29.90. For *D. pseudoobscura*, between-strains df = 1 MS = 7.62, F = 167.2, between-conditions df = 1, MS = 27.41, F = 601.25, and interaction df = 1, MS = 5.56, F = 121.96.

The response to selection for high and low pupation site for 21 generations is shown in Fig. 1. Realized heritabilities were calculated over the first

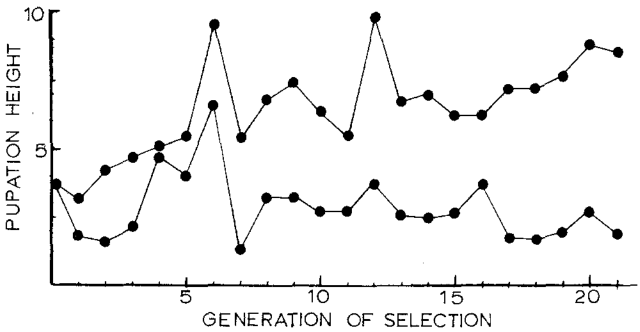

Fig. 1. Response to selection for pupation height, measured in centimeters, in *D. melanogaster*.

Table III. Geotactic Scores of Flies from Selected Larval Strains

	Light	Dark
High	12.14 ± 0.18 (400) 12.29 ± 0.21 (361)	12.92 ± 0.23 (380) 12.70 ± 0.20 (362)
Low	10.44 ± 0.25 (401) 10.40 ± 0.19 (448)	10.59 ± 0.19 (345) 10.39 ± 0.18 (301)

ten generations:

	Realized h^2	t	p
High pupation	0.31 ± 0.08	6.73	<0.01
Low pupation	0.10 ± 0.09	3.83	<0.01

Selection for low pupation has a limit imposed by the surface of the medium. This lower limit is undoubtedly reflected by the lower heritability in this direction. It was noticed that in the low line developmental time had changed by generation 10 to 8 days but that it stayed at about 10 days in the high line. It was also observed at that time that while egg-laying rates remained high in the low strain, noticeably fewer eggs were laid per female in the high strain.

After 20 generations of selection, adult flies from the high and low strains were tested in geotaxis mazes (Table III). Since no differences were found between males and females, the sexes were pooled and the geotactic scores of flies from the low and high strains in light and in dark were compared by a 2 × 2 factorial analysis of variance. For conditions, df = 1, MS = 0.22, and F = 0.019. For strains, df = 1, MS = 10.49, and F = 0.916. For interactions, df = 1, MS = 0.43, and F = 0.038. Selection has apparently not resulted in individuals from the high-pupation strain walking higher as adults than flies from the low-pupation strain. The relatively greater geopositivity of adults from the high-pupation strain may be due to weakness or stumbling. There was no effect of light on adult geotaxis.

DISCUSSION

Differences in pupation site selection have previously been reported between laboratory strains of *D. melanogaster* and *D. simulans* with respect to preference for the center or sides of culture bottles (Sameoto and Miller, 1968; Barker, 1971) and between laboratory *D. melanogaster* and *D. willistoni* for dark and light areas of a culture dish (Rizki and Davis, 1953). The influence of environmental factors such as larval density and temperatures on pupation site selection by laboratory strains of *D. melanogaster* (Sokal *et al.*, 1960) and humidity on pupation sites of *D. willistoni* (De Souza *et al.*, 1970) has been known for some time.

Selection of the optimal pupation site is an important step in fly development. Variables such as predation, desiccation, and competition could potentially interfere with successful emergence of adult individuals. The influence of environmental factors on prepupal orientation is supported by the results of the present study. The "light" experiments were all conducted under overhead lights. Since pupation sites were always lower in light than in darkness, it is probably safe to assume that light is not attracting third instar larvae inward. In fact, presence of light overhead appears to be preventing third instar larvae from crawling very high. This assumption is supported by a separate study (Markow, manuscript in preparation) in which we have found that most third instar *Drosophila* prefer dark over light pupation sites when given a choice. The advantages of avoiding bright places where desiccation or exposure to predation might occur can be easily imagined. The effect of light on pupation height is illustrated by this interesting aside: after 20 generations of selection for high and low pupation site, we attempted to measure the pupation height of the divergent strains in the dark. As described above, the testing cylinders were 20 cm high. In the dark, larvae of the high strain crawled so high that they reached the top of the cylinder, pushed their way through the foam plug and out of the container, and continued up the incubator shelf!

In wild collections of adult flies, several species of *Drosophila* are usually retrieved at any single locality. For example, certain samples of the *D. melanogaster*, *D. simulans*, and *D. pseudoobscura* in the present study were all found in the same collections at Riverside, California. The species differences reported here for pupation site measured in the laboratory probably have some significance for habitat use and niche partitioning. We report pupation heights for populations from a wide variety of geographic locations, and among the populations examined it can be generalized that *D. melanogaster* pupates higher than does *D. pseudoobscura* and that *D. simulans* shows the lowest pupation sites of all. This order was found to be basically the same in experiments conducted in the light and in the dark. Futhermore, the results of the present study using freshly caught samples support the species differences reported among laboratory populations of *D. melanogaster* and *D. simulans* (Sameoto and Miller, 1968; Barker, 1971). More species should be examined in order to assess the amount of interspecific variation for this trait and to determine if any relationship exists between the pupation heights and the preimaginal ecology of particular species.

Changes in pupation height appear to be a correlated effect of selection for adult geotactic behavior which manifests itself only when pupation is measured in the dark. Populations of *D. melanogaster* and *D. pseudoobscura* previously selected for geopositive or geonegative behavior as adults show small differences in pupation height when measured in the light. In darkness, however, larvae from strains selected to walk upward as adults crawl dramatically higher to pupate than larvae from geopositive strains. While selection for pupation site has had little effect on adult geotaxis, selection for adult geotaxis on the other hand may have utilized genetic variation affecting behavioral processes common to both larvae and adults, processes most likely rooted in the brain.

While the response of larvae to crawl upward can certainly be referred to as "negative geotaxis," we hesitate to employ this term at this time. The nature of the testing cylinders allows larvae to go up only. In addition to moving against gravity, the larvae may be responding to the presence of the medium, moisture, other larvae, or even some unknown factors. That genetic variation is present for the larval behavior being looked at is apparent from the selection responses reported here. The importance of this intraspecific variation is still unclear, but such genetic varitation would be helpful to natural populations in the event that environmental changes alter availability of particular pupation niches.

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