Journal of Insect Behavior, Vol. 7, No. 3, 1994

Development Time and Pupation Behavior in the *Drosophila melanogaster* Subgroup (Diptera: Drosophilidae)

Philip Welbergen[1] and Marla B. Sokolowski[1,2]

Accepted September 10, 1993; revised December 20, 1993

This study is an in-depth analysis of intersexual, intraspecific, and interspecific variability in larvopupal developmental time, pupation site preference, and larval and pupal survival of a number of isofemale lines of the species Drosophila mauritiana, D. melanogaster, D. sechellia, D. simulans, D. teissieri, *and* D. yakuba. *There was no significant sex differences in pupation height, but females eclosed significantly earlier than males in all species. In addition, the suggestion of a strong negative correlation between larval developmental time and pupation height could not be confirmed in this study. The hypothesis that differences in pupation height provide a basis for niche partitioning between closely related species with overlapping distributions was tested by three planned orthogonal contrast analyses of variance. First, the two species* D. teissieri *and* D. yakuba, *with largely overlapping distribution, were significantly different in pupation height. Second, the two allopatric, nonoverlapping island species* D. mauritiana *and* D. sechellia *did not significantly differ in pupation height. However, the absence of a significant difference in the final contrast between the two cosmopolitan species* D. melanogaster *and* D. simulans, *which are often found together, makes us cautious to accept the hypothesis.*

KEY WORDS: *Drosophila melanogaster* subgroup; development time; pupation behavior.

INTRODUCTION

In *Drosophila*, the choice of a suitable pupation site directly influences the successful emergence of the adult (Sokolowski, 1985; Rodriguez *et al.*, 1992).

[1]Department of Biology, York University, North York, Ontario, Canada M3J 1P3.
[2]To whom correspondence should be addressed.

Previous studies show that differences in pupation height, a continuous measure of pupation site preferences, are influenced by the abiotic factors moisture, lighting conditions, and temperature and by the biotic factors density, sex, developmental time, and species measured (for reviews, see Sokolowski, 1985; Sokolowski *et al.*, 1986).

Pupation site choice by *Drosophila* larvae could provide a basis for larval habitat choice and niche separation between species. Schnebel and Grossfield (1986) have done the only systematic study of interspecific variability in pupation behavior in *Drosophila*. They found significant differences between the two most closely related species in four species triads, each triad coming from a different ecosystem ranging from desert to tropical rain forest. Earlier, Markow (1979) observed that *D. melanogaster* pupated higher than its sibling, *D. simulans*, and that *D. pseudoobscura*, a more distantly related species, pupated higher than *D. simulans* but lower than *D. melanogaster*. This, and Schnebel and Grossfield's (1986) findings, strongly suggests the presence of niche separation among closely related *Drosophila* larvae.

The present study is a systematic study of intersexual, intraspecific, and interspecific variability in pupation behavior and developmental times in the *D. melanogaster* subgroup. The phylogenetic relationship among the members of the *D. melanogaster* subgroup has recently been considered in detail using genetic information from all available sources (Lachaise *et al.*, 1988; Singh, 1989). We first addressed the question of effects of sex on pupation height and tested the hypothesis that if pupation height is related to development time, fast developers should pupate in different locations than slow developers. Subsequently, we tested the hypothesis that among species that are sympatric, if larval behavior is important ecologically, pupation heights should differ. Two species pairs from the *D. melanogaster* subgroup are distributed sympatrically: first, the well-known sibling species pair *D. melanogaster* and *D. simulans*, which are cosmopolitan, are generalists, and occur together in many places; and second, the closely related species *D. teissieri* and *D. yakuba*, which are generalists and overlap with regard to their distributions on the African mainland and the host plants exploited by their larvae (Lachaise *et al.*, 1988). The alternative hypothesis, that among species that are allopatric, pupation heights should not differ, was tested with the closely related species pair, *D. mauritiana* and *D. sechellia*. They live allopatrically on the island of Mauritius and the islands of Seychelles, respectively. *Drosophila sechellia* breeds on the fruits of the rubiaceous shrub *Morinda citrifolia* (Lachaise *et al.*, 1988; Legal *et al.*, 1992). *Drosophila mauritiana* is widespread all over Mauritius and is an abundant, broad-niched, opportunistic, and domestic species (Lachaise *et al.*, 1988). The two species are allopatric to their most closely related relatives *D. simulans* and *D. melanogaster*.

MATERIALS AND METHODS

Flies and Rearing Conditions

We examined isofemale lines of *D. mauritiana*, *D. melanogaster*, *D. sechellia*, *D. simulans*, *D. teissieri*, and *D. yakuba* (Table I). All lines were reared in sterilized plastic bottles in an incubator kept at 24 ± 1°C, with a photocycle of 12 h of light followed by 12 h of darkness, with lights on at 0800 h. Except for the two lines of *D. sechellia*, flies of each line were reared on 45 ml of dead yeast, sucrose, and agar (culture) medium with added minerals and propionic acid as the antifungal agent. *Drosophila sechellia* was reared on 45 ml of dead yeast, bananas, sucrose, and agar (culture) medium with tegosept as the antifungal agent.

Experimental Conditions and Procedures

The handling and test procedures for measuring pupation height are described in detail by Bauer and Sokolowski (1985). For each isofemale line, 10 newly hatched larvae (±2 h in age) were placed into vials (2 cm in diameter

Table I. Isofemale Lines Used in this Study

Line	Origin	Received from
D. mauritiana		
G102	Mauritius, 1979	Mid-American Stock Centre
G29	Mauritius, 1979	Mid-American Stock Centre
No. 75 David	Mauritius, 1985	J. A. Coyne
Cambridge	—	Mid-American Stock Centre
D. melanogaster		
WC8	Windsor, 1988	R. S. Singh
NA11	Nashville, 1988	R. S. Singh
LA10	Louisville, 1988	R. S. Singh
CF3	Carterville, 1988	R. S. Singh
D. sechellia		
Robertson	Stock No. 3591	Mid-American Stock Centre
Cambridge	—	Mid-American Stock Centre
D. simulans		
Isiolo	Kenya, 1988	Mid-American Stock Centre
Florida City	—	J. A. Coyne
BRW9	Australia, 1991	Mid-American Stock Centre
No. 135.2	Stock No. 135.20	Mid-American Stock Centre
D. teissieri		
No. 128.2	Stock No. 128.2	Mid-American Stock Centre
Umea	—	Umea Stock Centre
D. yakuba		
No. 115	Stock No. 115	Mid-American Stock Centre
Umea	—	Umea Stock Centre

and 11 cm in height) using a dissecting probe. Each vial contained 5 ml of a 2-day-old dead yeast–sucrose–agar medium. This medium was also used for *D. sechellia*. Vials were stoppered with standard-size cotton balls, placed in the outside longitudinal rows of test tube racks, and incubated under the same conditions as used for rearing. The positions of the vials containing 10 larvae for each isofemale line (1610 larvae in total) were completely randomized within and between test tube racks and the racks were positioned randomly in the incubator under evenly distributed overhead fluorescent illumination.

The 18 isofemale lines were tested simultaneously for six measures: time to pupation (hours), pupation site, number of pupae, pupation height (millimeters), time to eclosion (hours), and number of adults. In addition, sex of pupae and adults was recorded. Pupae were sexed as described by Bauer and Sokolowski (1985). Pupation site was classified into three classes, i.e., in the center of the food plug, at the periphery of the food plug (a pupa was attached to the glass wall but still partially embedded in food), and on the glass wall, not touching food. The height of peripherally located pupae and off-food pupae located on the glass wall was measured as the distance from the surface of the medium to a point between the two anterior spiracles of the pupa. The measures were taken every 12 h starting at 0800 and at 2000 h. We recorded data up to 400 h after the start of the experiment. Newly emerged adults were removed from the vials within 12 h of eclosion.

Analysis of Data

We used general linear models procedure to analyze the results. The first analyses of variances used a hierarchal model with isofemale lines as a random effect nested within species, which was the fixed-effect factor (Winer, 1971, p. 360). This was done for the measure, time to pupation and the derived measures survival to the pupal stage and survival to the adult stage. For each vial, we used the median time to pupation in the analysis of variance, because it is difficult to obtain time measurements on all larvae (i.e., some larvae may respond very slowly). Furthermore, by using the median it is not necessary to measure the right-hand tail of the distribution, because the sample sizes are known (Sokal and Rohlf, 1981). The two derived measures expressed as percentages were first $\arcsin\sqrt{}$-transformed.

The second analyses used a partially hierarchal model (Winer, 1971, p. 464) to analyze the pupation height and time to eclosion measures. The model had three factors, species (fixed effect), isofemale lines (random effect; nested within species), and sex (fixed effect). The average pupation height and the median time to eclosion were used from each vial. The reasons for using median time are given above. These data were subsequently used in the analyses of variance. Before we employed the analyses of variance, we tested for homo-

geneity of variances and normal distributions. In cases in which the assumptions of homogeneity of variance were not met, a transformation on the scale of measurement was utilized.

Since the sample sizes of vials of the isofemale lines were unequal (Table II), and therefore the ANOVAs were not balanced, we calculated type III sums of squares and estimable functions using the SAS Institute (1990) general linear model (GLM) procedure.

RESULTS AND DISCUSSION

Table IIA shows the median times to pupation and 90% confidence limits of all isofemale lines. The results of the nested ANOVA are given in Table IIB. The differences between the isofemale lines within a species are significant. The variation among the six species for larval developmental time was not significant.

The nested analysis of variance for the survival to the pupal stage data indicates that there are no significant differences among the isofemale lines within the six species [$F_{(12,143)} = 1.55$, $P = 0.11$]. In addition, the species do not differ significantly in larval survivorship [$F_{(5,12)} = 0.68$, $P = 0.65$]. So the density of larvae throughout larval development remained the same.

The average median times to eclosion and 95% confidence limits of females and males of all the lines are given in Table IIIA. Contrary to the analysis of larval developmental time, the analysis of median times to eclosion shows a highly significant among species effect (Table IIIB). *Drosophila sechellia* has the longest developmental time and *D. teissieri* the shortest. The direction of differences between the species is, however, similar for both the time to pupation and the time to eclosion. This means that the largest differences in development between the species occur during the pupal stage, Lachaise (1983) investigated preadult development of 23 species of Drosophilidae. Our results are consistent with the differences he found among the three species *D. melanogaster*, *D. teissieri*, and *D. yakuba*, although he included embryonic development and measured developmental time at a temperature of 25°C.

The analysis of variation also detects a significant intraspecific effect. The isofemale lines within a species significantly differ in the larvopupal developmental time. In addition, females eclosed significantly earlier than males in all species (Table IIIB). This is consistent with the general findings for *D. melanogaster* (Bakker and Nelissen, 1963; David *et al.*, 1976).

Significant differences between the species are also detected in the analysis of variance in the survival from first instar to adulthood [$F_{(5,12)} = 3.42$, $P = 0.04$]. Inspection of means and corresponding 95% confidence limits (Fig. 1) reveals that the species had an average survival to the adult stage at about 75%, except for the *D. teissieri* isofemales lines. Their average is between 57 and

Table II. (A) Mean and 95% Lower (L_1) and Upper (L_2) Confidence Limits of Median Time to Pupation (h); (B) Results of the Nested ANOVA with Unequal Sample Sizes of Vials (N) on Natural Log Time to Pupation

A

Line	N	L_1	Mean	L_2
D. mauritiana				
G102	10	133.6	140.3	147.3
G29	4	125.4	135.5	146.3
No. 75 David	10	122.1	128.2	134.6
Cambridge	10	135.0	141.7	148.8
D. melanogaster				
WC8	10	141.4	148.5	156.0
NA11	9	139.5	146.9	154.6
LA10	3	114.9	125.6	137.3
CF3	7	139.3	147.7	156.5
D. sechellia				
Robertson	9	159.4	167.8	176.7
Cambridge	10	157.8	165.7	174.0
D. simulans				
Isiolo	10	136.9	143.8	151.0
Florida City	10	152.3	159.9	167.9
BRW9	10	143.9	151.1	158.6
No. 135.2	10	125.5	131.8	138.4
D. teissieri				
No. 128.2	9	139.8	147.2	155.0
Umea	10	119.8	125.8	132.1
D. yakuba				
No. 115	10	137.7	144.6	151.9
Umea	10	121.6	127.6	134.0

B

Source	df	MS	F	P
Species	5	0.1271	2.870	0.063
Lines w. species	12	0.0443	7.145	0.0001
Error	143	0.0062		

66%. The ANOVA further indicates that the differences between the isofemale lines within the species are not significant [$F_{12,143)} = 1.05$, $P = 0.41$].

The distributions of the pupae in the vials are given in Table IV. The comparisons among the species by means of log-linear models is not possible, because the number of isofemale lines per species is unequal. However, close inspection of Table IV reveals species differences. *Drosophila sechellia* and *D. teissieri* clearly pupated in the food, whereas in *D. melanogaster* and *D. simulans* the majority of pupae was found on the glass wall. *Drosophila mauritiana* pupae were located mainly in the food or at the periphery of the food plug.

Table III. (A) Mean and 95% Lower (L_1) and Upper (L_2) Confidence Limits of Median Time to Eclosion (h); (B) Results of the Partially Hierarchical ANOVA with Unequal Sample Sizes of Vials on Untransformed Time to Eclosion

A

Line	Females				Males			
	N^a	L_1	Mean	L_2	N^a	L_1	Mean	L_2
D. mauritiana								
G102	10	249.0	258.0	267.0	10	252.6	261.6	270.6
G29	4	247.3	261.5	275.7	4	239.8	254.0	268.2
No. 75 David	9	234.2	243.7	253.2	10	242.8	251.8	260.8
Cambridge	10	249.0	258.0	267.0	10	262.8	271.8	280.8
D. melanogaster								
WC8	10	254.2	263.2	272.2	10	257.8	266.8	275.8
NA11	9	249.7	259.2	268.7	9	255.7	265.2	274.7
LA10	2	229.9	250.0	270.1	3	233.3	249.7	266.1
CF3	7	262.5	273.7	283.9	7	262.5	273.7	283.9
D. sechellia								
Robertson	9	279.7	289.2	298.7	9	287.1	296.6	306.0
Cambridge	10	279.4	288.4	297.4	9	290.9	300.3	309.8
D. simulans								
Isiolo	10	240.0	249.0	258.0	9	249.9	259.3	268.8
Florida City	10	258.6	267.6	276.6	10	268.2	277.2	286.2
BRW9	10	242.6	251.6	260.6	10	257.0	266.0	275.0
No. 135.2	10	235.4	244.4	253.4	10	243.2	252.2	261.2
D. teissieri								
No. 128.2	8	229.9	240.0	250.1	9	239.9	249.3	258.8
Umea	9	211.2	220.7	230.2	10	220.2	229.2	238.2
D. yakuba								
No. 115	10	236.0	245.0	254.0	10	241.4	250.4	259.4
Umea	10	220.1	229.1	238.1	10	221.9	230.9	239.9

B

Source	df	MS	F	P
Species	5	16,342.31	10.74	0.0004
Lines w. species	12	1,520.97	7.22	0.0001
Sex	1	2,925.86	35.14	0.0001
Species * sex	5	155.14	1.86	0.1749
Lines w. species * sex	12	83.26	0.40	0.9647
Error	280	210.65		

[a] Number of medians used in the calculations.

More than 50% of the *D. yakuba* pupae were found at the periphery of the food plug. Table IV also reveals that the distribution of pupae over the three classes differs considerably between the isofemale lines within a species.

To analyze the effect of sex on pupation height, centrally and peripherally

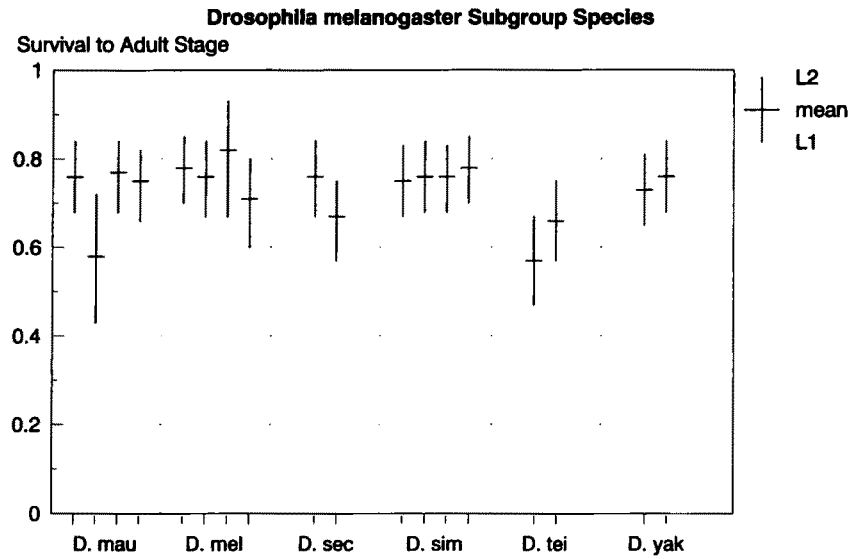

Fig. 1. Mean proportion of adult survival ± 95% confidence limits of the 18 isofemale lines. The order of lines per species corresponds to the order listed in Table I.

located pupae in food were considered to have zero height. Pupae could not be removed from the food plugs for sex determination, because otherwise time to eclosion and survival to the adult phase (see below) could not be measured. The definition of zero height, however, enabled us to determine the sex of all pupae in most vials, first, by recording the sex of pupae on the glass wall and, second, by recording the sex of adult flies. The difference in the number of females and males could then be attributed to the pupae embedded in food. Since we computed mean heights of female and male pupae per vials, we discarded those vials from the analyses that did not give complete certainty about the sex of all pupae. The average pupation height and standard errors of females and males are given in Table V.

A partially hierarchical three-factor ANOVA, as described under Materials and Methods, could not be done, because the mean pupation height of one or both sexes of the isofemale lines G102 of *D. mauritiana*, Cambridge of *D. sechellia*, and No. 128.2 and Umea of *D. teissieri* was zero. Isofemale line LA10 of *D. melanogaster* had to be discarded, because the sample size was too small. Instead, two-factor analyses of variance done separately for each species revealed that the interaction effects between isofemale line and sex of pupae were not significant. We decided to pool the interaction mean square with the error mean square as by Sokal and Rohlf (1981, box 10.2).

The analyses show that females and males do not significantly differ in

Table IV. Distribution of Pupae in Three Classes, i.e., on the Wall (G), at the Border Between Food and Glass Wall (B), and in the Food (I)

Line	N^a	G	B	I
D. mauritiana				
G102	10	2.5	22.8	74.7
G29	4	36.0	48.0	16.0
No. 75 David	10	10.5	15.1	74.4
Cambridge	10	22.0	31.7	46.3
D. melanogaster				
WC8	10	94.6	4.3	1.1
NA11	9	35.5	53.9	10.5
LA10	3	61.5	38.5	0.0
CF3	7	80.4	16.1	3.6
D. sechellia				
Robertson	9	30.7	10.7	58.7
Cambridge	10	5.0	23.8	71.2
D. simulans				
Isiolo	10	72.8	8.7	18.5
Florida City	10	49.4	28.7	21.8
BRW9	10	18.9	31.1	50.0
No. 135.2	10	76.8	18.9	4.2
D. teissieri				
No. 128.2	9	7.8	27.3	64.9
Umea	10	6.0	22.6	71.4
D. yakuba				
No. 115	10	25.0	52.1	22.9
Umea	10	25.3	54.0	20.7

[a]Number of vials observed.

pupation height in *D. mauritiana* [$F_{(1,35)} = 0.310$, $P > 0.50$], *D. melanogaster* [$F_{(1,48)} = 0.02$, $P > 0.75$], *D. simulans* [$F_{(1,47)} = 1.65$, $P > 0.10$], and *D. yakuba*[$F_{(1,1)} = 2.34$, $P > 0.35$]. The *t*-test comparison of the means of female and male pupae in *D. sechellia* Robertson also reveals no significant difference in pupation height [$t_{(8)} = 0.681$, $P > 0.50$].

The absence of a sex effect on pupation height in *D. melanogaster* and *D. simulans* is consistent with the results of Markow (1979) and Bauer and Sokolowski (1985). In contrast, Bauer (1984), Bauer and Sokolowski (1988), and Casares and Carrecedo (1987) reported higher pupation height of male larvae. Identified variables exerting a differential influence on female and male pupation height are humidity (Casares and Carrecedo, 1987), density (Bauer and Sokolowski, unpublished), and number of strains used in a study (Sokolowski and Bauer, 1989). We did not control for humidity, but the number of first-instar larvae was the same in every vial and the analysis of survival to the pupal stage shows that larval density remained the same in every vial (see above). Hence,

Table V. Mean and 95% Lower (L_1) and Upper (L_2) Confidence Limits of Mean Pupation Height (mm) of Female and Male Pupae of the Six Species

Lines	Females				Males			
	N^a	L_1	Mean	L_2	N^a	L_1	Mean	L_2
D. mauritiana								
G102	8	0.0	0.3	0.6	8	0.0	0.0	0.0
G29	3	0.0	1.6	7.6	3	0.0	1.1	6.0
No. 75 David	8	0.0	0.8	2.7	9	0.0	0.7	2.4
Cambridge	8	0.5	2.1	6.4	8	0.0	1.2	3.5
D. melanogaster								
WC8	10	10.4	16.9	27.0	10	13.4	20.0	31.9
NA11	9	0.6	1.6	3.2	9	0.6	1.6	3.2
CF3	7	3.7	7.0	12.7	7	3.2	6.3	11.4
D. sechellia								
Robertson	5	0.0	0.7	4.4	5	0.0	0.4	1.8
Cambridge	8	0.0	0.0	0.0	7	0.0	0.0	0.0
D. simulans								
Isiolo	4	3.9	12.4	36.6	4	6.8	20.2	56.8
Florida City	6	2.5	7.0	17.1	6	3.3	8.8	21.2
BRW9	8	0.0	0.8	2.7	8	0.1	1.3	3.7
No. 135.2	8	5.8	12.8	27.1	8	10.4	22.2	46.2
D. teissieri								
No. 128.2	7	0.0	0.0	0.0	7	0.0	0.0	0.0
Umea	8	0.0	0.0	0.0	9	0.0	0.3	0.9
D. yakuba								
No. 115	4	0.0	1.1	6.0	4	1.1	6.2	23.4
Umea	6	0.0	1.1	6.8	6	0.0	1.8	6.6

[a] Number of means used in the calculations.

the differences in pupation height cannot be attributed to differential larval survivorship.

In addition, morphological (Bauer, 1984) and developmental differences between female and male larvae (Casares and Carrecedo, 1987), and differences in geotactic response (Casares and Carrecedo, 1987) are other possible explanations for the differences in pupation height between female and male larvae. However, we did not detect significant differences between the sexes in the present study.

Thus male and female data were pooled and mean pupation heights of the isofemale lines of the six species were subsequently analyzed and compared. In the following analyses of pupation height, we were able to include the heights of the peripherally located pupae. By taking account of these heights, we abandoned the problem of too many zeros in the analyses. In addition, those vials that were discarded in the previous analyses could be used here, because there was no need to know the sex of every pupa. Table VIA gives the average pupation height (±95% confidence limits) of all isofemale lines and species.

Table VI. (A) Mean and 95% Lower (L_1) and Upper (L_2) Confidence Limits of Mean Pupation Height (mm); (B) Results of the Nested ANOVA with Unequal Sample Sizes of Vials (N) on $\ln(x + 1)$-Transformed Mean Pupation Heights

A

Line	N	L_1	Mean	L_2
D. mauritiana				
G102	10	0.0	0.5	2.3
G29	4	0.6	2.1	5.1
No. 75 David	10	0.3	1.0	2.1
Cambridge	10	1.2	2.4	4.2
D. melanogaster				
WC8	10	11.1	17.7	27.7
NA11	9	1.8	3.3	5.8
LA10	3	2.1	5.7	13.6
CF3	7	4.3	7.8	13.7
D. sechellia				
Robertson	9	0.5	1.4	3.7
Cambridge	10	0.0	0.6	1.4
D. simulans				
Isiolo	10	8.7	13.9	21.9
Florida City	10	4.7	7.7	12.4
BRW9	10	1.1	2.2	4.0
No. 135.2	10	10.0	16.0	25.1
D. teissieri				
No. 128.2	9	0.1	0.7	1.7
Umea	10	0.0	0.6	1.4
D. yakuba				
No. 115	10	4.2	7.1	11.4
Umea	10	2.7	4.7	7.8

B

Species	5	15.587	5.74	0.0062
Lines w. species	12	2.717	5.66	0.0001
Error	142	0.480		

A strong negative correlation between developmental time and pupation height in *D. melanogaster* and *D. simulans* is suggested by Casares and Carrecedo (1987). They observed that the first larvae leaving medium to start pupation (i.e., larvae with a shorter developmental time) crawled up higher than later larvae (i.e., those with a longer developmental time). Sokal *et al.* (1960) observed that earlier-pupating larvae pupated more on the glass wall of the vial than on the medium at the bottom of the vial.

Table VII gives Spearman correlation coefficients of the relationship between median time to pupation (Table IIA) and mean pupation height (Table VIA) for the 18 isofemale lines. The analyses reveal that only 3 of the 18 correlation coefficients are significant. In *D. teissieri* Umea and *D. yakuba* No.

Table VII. Spearman's Coefficients of Rank Correlation Between Median Time to Pupation and Mean Pupation Height of the 18 Isofemale Lines

Lines	N^a	r_s	P
D. mauritiana			
G102	10	−0.254	0.479
G29	4	0.316	0.684
No. 75 David	10	0.058	0.873
Cambridge	10	0.592	0.072
D. melanogaster			
WC8	10	0.087	0.811
NA11	9	0.034	0.931
LA10	3	−0.500	0.667
CF3	7	0.270	0.558
D. sechellia			
Robertson	9	−0.243	0.529
Cambridge	10	0.814	0.004
D. simulans			
Isiolo	10	−0.624	0.054
Florida City	10	−0.500	0.142
BRW9	10	0.138	0.704
No. 135.2	10	−0.432	0.213
D. teissieri			
No. 128.2	9	0.513	0.158
Umea	10	−0.690	0.027
D. yakuba			
No. 115	10	−0.776	0.008
Umea	10	−0.177	0.624

[a] Number of vials observed.

115 time to pupation and pupation height are significantly negatively correlated, but in *D. sechellia* Cambridge the correlation coefficient is significantly positive. We conclude that there is no systematic relationship between the height of pupae and their larval developmental time, and therefore, we cannot confirm the suggestion of Casares and Carrecedo (1987) of a strong negative correlation between the two measures in the lines and species we studied. Also, Bauer and Sokolowski (1988) observed no correlation between larval developmental time (including embryonic development) and pupation height for *D. melanogaster* male and female larvae.

The nested analysis of variance with unequal sample sizes of vials on $\ln(x + 1)$-transformed mean pupation height (Table VIB) reveals highly significant differences between the isofemales lines within a species and between the species. To test the hypothesis that differences in pupation height provide a basis for niche partitioning between closely related species (Schnebel and Grossfield, 1986), we performed three planned orthogonal contrast analyses of variances. First, we compared the two cosmopolitan species *D. melanogaster* and *D. simu-*

lans. Second, *D. teissieri* and *D. yakuba* were compared as species with largely overlapping distributions. Third, we contrasted the two allopatric, nonoverlapping island species *D. mauritiana* and *D. sechellia*. With regard to the first two contrasts, the underlying hypothesis is that natural selection in the past by means of competition among the closely related species could have resulted in strikingly different pupation site preferences. The alternative hypothesis that among species who are allopatric, pupation heights should not differ, is tested with the third contrast.

The orthogonal contrast analyses reveal no significant difference between *D. melanogaster* and *D. simulans* [$F_{(1,12)} = 0.05$, $P = 0.819$], a significant difference between *D. teissieri* and *D. yakuba* [$F_{(1,12)} = 7.39$, $P = 0.019$], and no significant difference between *D. mauritiana* and *D. sechellia* [$F_{(1,12)} = 0.19$, $P = 0.673$]. The results of the last two contrasts analyses fit the Schnebel and Grossfield hypothesis of habitat use and niche partition. However, the absence of a significant difference between the two cosmopolitan species, in contrast to the findings of Markow (1979) and Schnebel and Grossfield (1986), makes us cautious about accepting the hypothesis. Although it is noted that the isofemale lines *D. melanogaster* and *D. simulans* are from different geographic regions, Markow (1979) used sympatric and allopatric populations of the two species and Schnebel and Grossfield (1986) did not indicate where the two species were sampled. Furthermore, conditions of continuous light or continuous darkness were used in both studies. There was no significant difference between *D. melanogaster* and *D. simulans* in the dark experiment by Markow (1979). Their results, therefore, cannot be used to explain pupation height choice in natural populations of *Drosophila* under a normal light regime.

The present study showed significant variations for larvopupal development and pupation behavior measures within and between species of the *melanogaster* subgroup tested under identical conditions. The observations indicate the possible relevance of genetic factors on larvopupal development and pupation behavior in common environments. In *D. melanogaster*, differences in pupal height are influenced additively by both the second and the third pair of autosomes, with the second pair having three times the effect of the third (Bauer and Sokolowski, 1985).

Our previous work with larval pupation behavior has shown a positive correlation between the locomotor aspect of larval foraging behavior and pupation height in laboratory and orchard-derived stocks of *D. melanogaster* (Sokolowski, 1980, 1985; Sokolowski and Hansell, 1983; Sokolowski *et al.*, 1986). Larvae with long path lengths (*rovers*) had higher pupal heights than larvae with shorter path lengths (*sitter*). At present we are investigating the locomotor aspect of the larval foraging behavior in the other species in the *melanogaster* subgroup.

ACKNOWLEDGMENTS

We thank Saeid Babaei, Gus Lagos, and Max Licht for technical assistance. Peggy Ng is kindly acknowledged for statistical advice. This work was supported by an International Fellowship from the Natural Sciences and Engineering Research Council of Canada (NSERC) awarded to P. W. and an Operating Grant from the NSERC to M.B.S.

REFERENCES

Bakker, K., and Nelissen, F. (1963). On the relations between duration of the larval and pupal period, weight and diurnal rhythm in emergence in *Drosophila melanogaster*. *Entomol. Exp. Appl.* **6:** 37–52.

Bauer,S. J. (1984). Sex difference in pupation site choice in *Drosophila melanogaster*. *Dros. Info. Serv.* **60:** 58.

Bauer, S. J. , and Sokolowski, M. B. (1985). A genetic analysis of path length and pupation height in a natural population of *Drosophila melanogaster*. *Can. J. Genet. Cytol.* **27:** 334–340.

Bauer, S. J., and Sokolowski, M. B. (1988). Autosomal and maternal effects on pupation behavior in *Drosophila melanogaster*. *Behav. Genet.* **18:** 81–97.

Casares, P., and Carrecedo, M. C. (1987). Pupation height in *Drosophila*: Sex differences and influence of larval developmental time. *Behav. Genet.* **17:** 523–535.

David, J., Bocquet, C., and Pla, E. (1976). New results on the genetic characteristics of the Far East race of *Drosophila melanogaster*. *Genet. Res. Cambr.* **28:** 253–260.

Lachaise, D. (1983). Reproductive allocation in tropical Drosophilidae: Further evidence on the role of breeding-site choice. *Am. Nat.* **122:** 133–146.

Lachaise, D., Cariou, M.-L., David, J. R., Lemeunier, F., Tsacas, L., and Ashburner, M. (1988). Historical biogeography of the *Drosophila melanogaster* species subgroup. In Hecht, M. K., Wallace, B., and Prance, G. T. (eds.), *Evolutionary Biology, Vol. 22*, Plenum Press, New York.

Legal, L., David, J. R., and Jallon, M.-M. (1992). Toxicity and attraction effects produced by *Morinda citrifolia* fruits on the *Drosophila melanogaster* complex of species. *Chemoecology* **3:** 125–129.

Markow, T. A. (1979). A survey of intra- and interspecific variation for pupation height in *Drosophila*. *Behav. Genet.* **9:** 209–217.

Rodriguez, L., Sokolowski, M. B., and Shore, J. S. (1992). Habitat selection by *Drosophila melanogaster* larvae. *J. Evol. Biol.* **5:** 61–70.

SAS Institute (1990). SAS/STAT User's Guide: Vol. 2, GLM-VARCOMP, SAS Institute, Cary, NC.

Schnebel, E. M., and Grossfield, J. (1986). The influence of light on pupation height in *Drosophila*. *Behav. Genet.* **16:** 407–415.

Singh, R. S. (1989). Population genetics and evolution of species related to *Drosophila melanogaster*. *Annu. Rev. Genet.* **23:** 425–453.

Sokal, R. R., and Rohlf, F. J. (1981). *Biometry*, Freeman, New York.

Sokal, R., Ehrlich, P., Hunter, P., and Schlager, G. (1960). Some factors affecting pupation site of *Drosophila*. *Ann. Entomol. Soc. Am.* **53:** 174–182.

Sokolowski, M. B. (1980). Foraging strategies of *Drosophila melanogaster*: A chromosomal analysis. *Behav. Genet.* **10:** 291–302.

Sokolowski, M. B. (1985). Genetics and ecology of *Drosophila melanogaster* larval foraging and pupation behaviour. *J. Insect Physiol.* **31:** 857–864.

Sokolowski, M. B., and Bauer, S. J. (1989). Genetic analysis of pupation distance in *Drosophila melanogaster*. *Heredity* **62:** 177–183.

Sokolowski, M. B., and Hansell, R. (1983). Elucidating the behavioral phenotype of *Drosophila*

melanogaster larvae: Correlations between larval foraging strategies and pupation height. *Behav. Genet.* **13:** 267–280.

Sokolowski, M. B., Bauer, S. J., Wai-Ping, V., Rodriguez, L., Wong, J. L., and Kent, C. (1986). Ecological genetics and behavior of *Drosophila melanogaster* larvae in nature. *Anim. Behav.* **32:** 403–408.

Winer, B. J. (1971). *Statistical Principles in Experimental Design*, McGraw-Hill, New York.