Habitat selection by *Drosophila melanogaster* larvae

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Abstract

Drosophila melanogaster larvae are used to examine habitat choice behavior and its effect on a component of preadult fitness (pupal survivorship). We established strains of flies by collecting pupae from two microhabitats from an orchard. Strain differences in pupation site choice (on versus off fruit) persisted in a field-like laboratory assay without artificial selection. To produce heterogeneous environments, air temperature and soil water content were varied in these assays. A habitat suitability difference measure was used to determine for each environment, which microhabitat (on or off fruit) resulted in greater pupal survivorship. We found 1) that habitat choice behavior had both plastic and heritable components, 2) that strain-by-environment interactions influenced habitat choice behavior and pupal survivorship and, 3) a significant positive correlation between habitat suitability and larval habitat choice behavior.

Introduction

Drosophila larvae are suitable organisms for studies of habitat selection (e.g. Cavener, 1979; Taylor and Condra, 1983; Sokolowski et al., 1986). *D. melanogaster* larval behavior is simple; foraging occurs until midway through the third larval instar after which larvae cease feeding and wander in search of a pupation site. In the laboratory, successful pupation and emergence depends on food availability during foraging, waste concentration and choice of pupation site during wandering behavior (Bakker, 1961; Botella et al., 1985; Sokolowski, 1980; Sokolowski et al., 1984). When larval density is low and food availability prior to pupation is not limited, the probability of emergence from the pupal case can be directly related to

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pupation site choice. Other advantages of using *D. melanogaster* larvae in studies of habitat selection are: 1) the well characterized genetic basis for variation in pupation site choice (Bauer and Sokolowski, 1985; 1988; Sokolowski and Bauer, 1989), 2) the occurrence of variation for pupation site choice in nature and in the laboratory (Sokolowski et al., 1986) and, 3) the ease with which pupae and emerging adults can be located and counted. The proportion of flies emerging from pupal cases in a particular habitat is a good measure of the suitability of a particular habitat choice. Hence it is possible to determine if there is a correlation between habitat choice and pupal survivorship.

The rover/sitter polymorphism found in natural populations of *D. melanogaster* is influenced by a single major gene at the *foraging* locus (de Belle et al., 1989). Rover larvae have significantly longer foraging paths than sitters (Sokolowski, 1980; de Belle and Sokolowski, 1987; de Belle et al., 1989). The trait shows a bimodal distribution in orchards in the Toronto area; rovers comprise approximately 70% and sitters, 30% of the population. The *foraging* locus appears to have pleiotropic effects on larval pupation behavior. A gene by environment association occurs as progeny of flies that emerged from pupae collected on the fruit behave as sitter larval foragers whereas those collected from soil behave as rovers (Sokolowski et al., 1986).

The ultimate goal of these studies is to determine the forces maintaining the rover/sitter polymorphism in nature. Spatial heterogeneity of the environment is one possible mechanism. Temporal variation may also be important as the available habitats change over time. In this paper we investigate the influence of spatial heterogeneity of the environments on 1) habitat choice behaviour, 2) pupal survivorship, a preadult component of fitness, and, 3) we provide experimental evidence for habitat selection by *D. melanogaster* larvae.

Materials and methods

Two strains of *D. melanogaster* were established in the fall of 1983 from a pear orchard in Southern Ontario by collecting pupae from each of two distinct pupal microhabitats: 1) the M1 strain was collected from the upper surface of fruit and behaves as a sitter larval forager and 2) the M4 strain was collected in soil at a distance approximately 12 cm away from the nearest fruit and behaves as a rover larval forager (Sokolowski et al., 1986). The present experiments were performed 3 years after establishing M1 and M4 strains in the laboratory. Rover/sitter phenotypes and differences in pupation heights of these strains were verified prior to the initiation of the present study. Differences in larval behavior have been maintained in a number of rover and sitter strains in our laboratory without artificial selection. Mixed populations of rovers and sitters maintain their polymorphic behavioral phenotypes using standard mass transfer rearing techniques (described below).

Flies were maintained using mass transfer techniques in 180 ml glass culture bottles on 45 ml of a standard dead yeast-sucrose-agar medium (culture medium); food was not limited under these conditions. Bottles were incubated under standard

conditions (24 °C ± 1 °C, 60% RH with a 12:12 L:D photoperiod; lights on at 0800 h) as described in Sokolowski et al. (1984).

The experimental assay was designed to resemble field conditions and allowed larvae to choose between two pupation sites, on versus off the fruit. Individual experimental assays consisted of glass dishes (8.5 cm in diameter and 4.8 cm in height) containing sterile sifted topsoil to a depth of 5 mm, grass (strips of oat seedlings), and half a grape covered with 1 ml of a yeast paste (1.5:1 ratio by weight of distilled water to Fleischmann's bakers' yeast). The soil in the dishes contained a measured amount of water, designated either 0%, 25%, 50%, 75% or 100% water content. The percent water content was calculated and defined using the formula:

$$\frac{\text{no. of grams of water}}{\text{no. grams of oven-dry soil}} \times 100 = \% \text{ water content in soil}$$

following Birkeland (1974). Third instar larvae (4 day post-hatching at 25 °C ± 1 °C) were carefully removed with a moist pain brush from plastic petri dishes (8.5 cm in diameter and 1.4 cm high) which contained 35 ml of culture medium and 100 larvae (following Sokolowski et al., 1984). Food was not limited under these conditions. Larvae were lightly rinsed with distilled water. Ten third instar larvae were randomly selected and placed onto a yeast paste located in the center of the half grape which was located on the soil in the center of the dish. Each dish was covered with a petri dish lid (8.8 cm in diameter and 0.9 cm in height) and was randomly assigned to an incubator at either 16 °C, 25 °C or 32 °C. Five replicate dishes were used for a total of 50 larvae per strain per soil water content per temperature giving a 3-way factorial design.

After the larvae had pupated, each half-grape was carefully placed into a box (3 cm in length × 3 cm in width × 2 cm in height) constructed of 0.2 mm brass wire mesh. Each box was then replaced in the center of its dish. The box separated the two pupal microhabitats (on versus off the fruit) in the experimental assay so that adult emergence from each microhabitat could be assessed. Pupal cases were individually located in each of the two pupal microhabitats and pupal survivorship was measured as the percentage of adults emerging from each microhabitat.

All percentage data were transformed using the arcsine square root transformation (Sokal and Rohlf, 1969). Statistical analyses were done using SAS procedures (SAS Institute Inc., 1985).

Results

Pupation behavior

Larvae had the choice to pupate on or off of the fruit. This choice was then nested within each environment (fifteen soil water content by temperature conditions). When each strain was analyzed separately in a 2-way analysis of variance (ANOVA), all factors (temperature, soil water content and their interaction) were
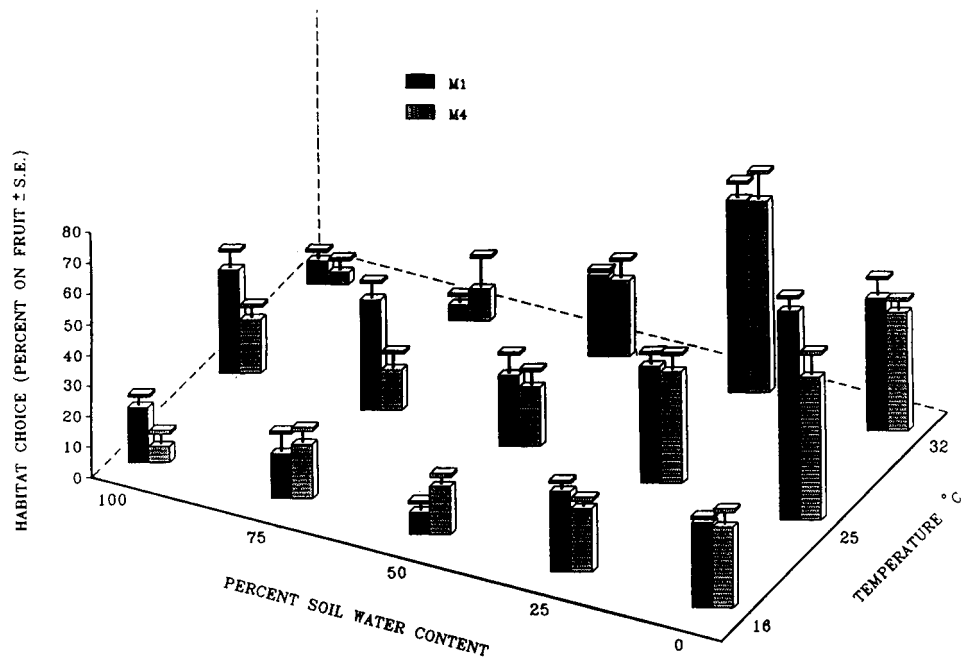
Fig. 1. A three dimensional representation of the transformed mean (± S.E.) percent of M1 and M4 *D. melanogaster* pupae found on the fruit when exposed to five soil water content and three air temperature conditions.

significant indicating significant plasticity for habitat choice behavior within each strain (for all combinations of environments, M1, $F_{(14,59)} = 12.06$, $p < 0.0001$ and M4, $F_{(14,60)} = 6.56$, $p < 0.0001$). As anticipated from the collection sites of the strains, on average, M1 larvae pupated on the fruit more frequently than M4 larvae, especially at 25° C. In general, larvae pupated more often on the fruit at lower soil water contents. Fewer larvae pupated on the fruit at 16° C and 32° C compared to at 25° C (Figure 1).

The results of a 3-way ANOVA on habitat choice behavior showed significant effects of strain, temperature, soil water content, and significant interactions between strain and temperature, and between temperature and soil water content (Table 1). The 3-way interaction was not significant.

Adult emergence

Strain-by-environment interactions for fitness-related traits are relevant to studies of habitat selection and the maintenance of genetic variation. A 3-way ANOVA of the proportion of adults emerging from each assay showed a significant strain-by-temperature interaction on survival as well as significant effects of temperature, soil

Table 1. ANOVA of the proportion of *D. melanogaster* pupae found on the fruit in different environments

Source	df	SS	F	P
Strain	1	1344.84	7.36	**
Temperature	2	3419.14	18.71	***
Soil Water Content	4	6143.43	33.62	**
Strain × Temperature	2	1378.87	3.77	*
Strain × Soil Water Content	4	808.57	1.11	NS
Temperature × Soil Water Content	8	10996.09	7.52	***
Strain × Temperature × Soil Water Content	8	1242.51	0.85	NS
Error	119	6892.88		

All data were arcsine square root transformed.
Significant differences are asterisked.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$,
NS = not significant.

Table 2. ANOVA of the proportion of *D. melanogaster* flies emerging from pupae in different environments.

Source	df	SS	F	P
Strain	1	427.07	2.20	NS
Temperature	2	7005.02	18.00	***
Soil Water Content	4	2258.32	2.91	***
Strain × Temperature	2	1789.55	4.61	**
Strain × Soil Water Content	4	134.45	0.17	NS
Temperature × Soil Water Content	8	5804.94	3.74	**
Strain × Temperature × Soil Water Content	8	849.69	0.55	NS
Error	119	23079.30		

All data were arcsine square root transformed.
Significant differences are asterisked.
**$p < 0.01$, ***$p < 0.0001$,
NS = not significant.

water content and significant interactions between temperature and soil water content (Table 2). Again, the 3-way interaction was not significant.

The mean number of adults emerging from each temperature condition (Figure 2) showed a change in the relative fitness rank of the strains (response curves intersect). At 16° C and 32° C, M4 had higher survival rates whereas at 25 °C, M1 had higher survival rates (Student Neuman Keul's Test, $p < 0.05$).

Habitat suitability

A strong positive correlation between behavior and habitat suitability is expected if habitat selection occurs. We define a habitat suitability difference measure as the proportion of adults emerging from larvae that pupated on the fruit
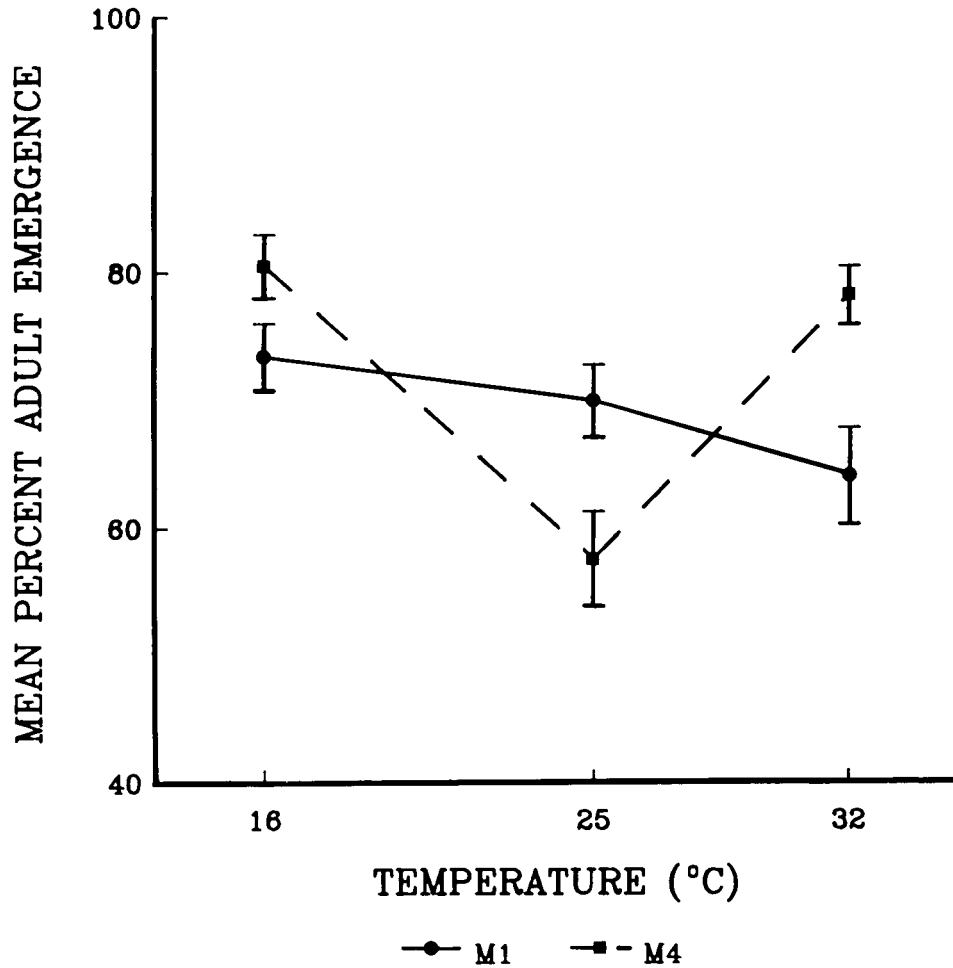
Fig. 2. The transformed mean ($\pm$ S.E.) number of flies emerging from each assay is shown for each temperature. The strain-by-temperature interaction found in Table 2 is reflected in the alternating ranks of the strains (line crosses).

minus the proportion of adults emerging from larvae that pupated off of the fruit (i.e. did a larva make the proper choice under the environmental conditions it faced).

$$\text{habitat suitability difference measure} = \left[\frac{\text{no. of flies emerging from the on-fruit location}}{\text{no. of larvae pupating on-fruit}} - \frac{\text{no. of flies emerging from the off-fruit location}}{\text{no. of larvae pupating off-fruit}} \right] \times 100$$
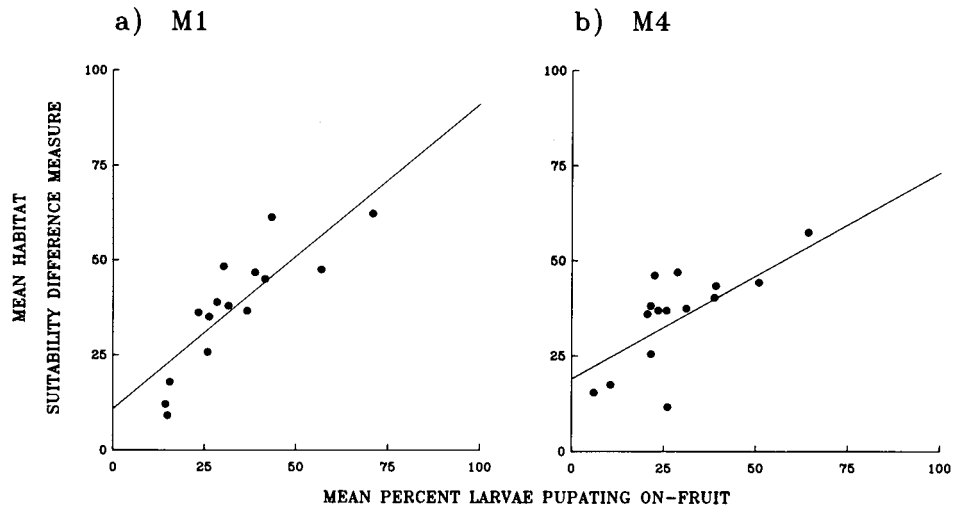
Fig. 3. The relation between the mean percent of larvae pupating on the fruit and the mean habitat suitability measure is shown for a) strain M1 and b) strain M4. An arcsine transformation was applied to all data.

After arcsine square root transformation [(habitat suitability + 100)/200] the measure ranges from 0 to 90 (untransformed it ranges from −100 to +100). A computer simulation (of 500 larvae) was carried out to ensure that there was no correlation between the habitat suitability difference measure and pupation site choice (behavior). When larvae choose habitats at random and emergence is random with respect to habitat, there is no correlation provided that behavioral scores are not 0 or 100%. Therefore, for replicates where all larvae pupated either on or off of the fruit, we used the average behavior (number of larvae pupating either on or off the fruit) for that treatment.

Adult emergence from the pupal case is used as a measure of fitness. The transformed habitat suitability difference measure for each strain is plotted against the proportion of larvae pupating on the fruit in Figure 3. With habitat selection, we expect more larvae to pupate on the fruit when the on-fruit site confers higher fitness (a transformed habitat suitability difference measure greater than 45). Similarly, when the off-fruit site is better (a value below 45) more larvae should pupate off the fruit. Therefore, we expect a positive correlation between pupation site choice and the habitat suitability difference measure. Indeed, Figure 3 shows a positive relation between habitat choice behavior and habitat suitability for both the M1 and M4 strains (M1, $r = 0.60$, $n = 74$, $p < 0.0001$; M4, $r = 0.54$, $n = 74$, $p < 0.001$, Pearson's correlation).

Discussion

Little experimental evidence is available to address the role of habitat selection on the maintenance of genetic polymorphism. Jones and Probert (1980) showed habitat selection of *Drosophila simulans* in heterogeneous environments. An eye color polymorphism (white and red eyes) was maintained in heterogeneous population cages containing sectors of red and white light. The polymorphism was not maintained in homogeneous red or white light control cages. de Souza et al. (1970) demonstrated that spatial heterogeneity maintained genetic variation for pupation behavior. Pupation behavior was measured as the tendency to pupate inside food cups as opposed to outside on the floor of the population cage. de Souza et al. (1970) suggest that a single gene can explain this difference in pupation behavior in *Drosophila willistoni*. Interestingly, Sokolowski et al. (1986) demonstrated that rover and sitter larvae show differences in pupation behavior (on versus off the food). Similarly, the rover/sitter polymorphism in *D. melanogaster*, is influenced by a single major gene (de Belle et al., 1989).

In the present study, we have presented evidence for habitat selection in fruit fly larvae. Habitat choice, survival and habitat suitability were influenced by larval strain, soil water content and air temperature. Habitat choice was correlated with habitat suitability. In other words, larvae tend to choose pupation sites in which they have a higher probability of surviving.

The evidence for a genetic basis to larval pupation behavior is, 1) the M1 and M4 strains have maintained their differences in pupation site choice since 1983 even though they were mass cultured and no artificial selection for pupation behavior was applied, 2) a chromosomal analysis of pupation behavior in the experimental assay indicated a significant effect of the second and third pair of autosomes (Sokolowski, unpublished), 3) there is significant (0.32) heritability for pupation distance (the distance larvae pupate from food) using a biometrical genetic analysis (Sokolowski and Bauer, 1989), and 4) the M1 and M4 strains show phenotypic differences in rover/sitter, a genetically based behavioral polymorphism in the locomotory component of larval foraging behavior (Sokolowski, 1980; de Belle and Sokolowski, 1987; de Belle et al., 1989).

Why do different pupal microhabitats confer differences in survival? Larvae and pupae desiccate on dry soil during the wandering stage and early hours of pupation. Rapid water loss occurs even at high humidities during periods away from moist substrates (Arlian and Eckstrand, 1975). Alternatively, when the foraging substrate is liquefied, pupae can be drowned by actively foraging larvae (Chiang and Hodson, 1950; Mueller and Sweet, 1986) or they can die from disease caused by microorganisms.

D. melanogaster larvae showed variation and strain differences in their choice of pupation site and survival on and off of the fruit. Differences in survival can occur at several stages during development wandering, pupation and adult emergence. Larvae are most vulnerable during the wandering stage because they are exposed to a range of stresses which may result in desiccation or rotting. Therefore, wandering behavior influences the probability of adult emergence, not merely the actual site of

pupation. A controlled laboratory study in which pupae of different strains are placed in dry or wet soil at different temperatures would not adequately mirror the present study or for that matter the fitness consequences of pupation site choice in nature.

The field-like nature of our assay resulted in large variances in the habitat chioce and suitability measures. We have used the field-like assay to enable us to measure these traits under more natural conditions (than in vials with culture medium, for example). Despite the field-like nature of this assay, we find significant effects that can be attributed to strain, environmental treatments and their interactions as well as significant correlations between habitat choice behavior and habitat suitability. Our results provide us with a fitness related interpretation of the strain-by-environment interactions found in Sokolowski and Bauer (1989).

Here we have shown that the microenvironment (soil moisture and air temperature) that a larva experiences during wandering and pupation is important to pupal survivorship which is a component of fitness. These findings describe a strain's survivorship only with respect to this portion of its life history. Certainly, selection may act at other times in the life cycle, for example during oviposition, larval foraging, courtship and mating. Strain differences in behavior at these times may significantly affect overall fitness. However, due to the complexity of *Drosophila* life history stages and their interaction with varying abiotic and biotic environmental factors, we find it necessary and worthwhile to "take a window of time" during a life-history stage and ask specific questions about habitat selection. Consequently, we conclude that 1) larvae show habitat choice behavior, 2) there is a genetic component to habitat choice and to fitness differences in the varied environments, and 3) habitat choice is positively correlated with survivorship. Thus genetic variability for pupation behavior may be maintained through habitat selection in heterogeneous environments.

Acknowledgments

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