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Short Communication

Microgeographic Variation in a *Drosophila melanogaster* Larval Behavior

Marla B. Sokolowski[1,3] and Yves Carton[2]

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Parsons (1983) hypothesizes that heritability measures for ecological traits of direct importance in determining distribution and abundance should be low in populations from stressful habitats and higher in those from benign habitats. The implication here is that selection has historically acted to reduce the additive genetic variance in stressful environments to a greater extent than in benign ones. In this paper we address this prediction at the microgeographic level by sampling *Drosophila melanogaster* from two sites (1500 m apart) in an oasis in Tunisia. These sites differ in temperature, light and moisture, three abiotic factors known to affect preadult fitness (Ehrman and Parsons, 1981).

Drosophila larvae are amenable to behavioral, genetic and ecological analyses (Sokolowski, 1985). Larval locomotion (path length) is measured as the distance traveled by a foraging larva during a fixed time. A genetic basis to path length differences in *D. melanogaster* third-instar larvae is found in laboratory and natural populations (Sokolowski, 1980, 1985; Bauer and Sokolowski, 1984, 1985). Some larvae forage as rovers (long paths), while others forage as sitters (shorter paths). Specifically, a major gene called *foraging* (*for*) has been localized to the left arm of the second chromosome at 24A3-C5 on the *D. melanogaster* polytene chromosome map; the rover allele (for^R) is genetically dominant to the sitter (for^S) (de Belle and Sokolowski, 1989; de Belle *et al.*, 1989). Phenotypic expression of *foraging* is modified by minor genes, some of which are X linked (de Belle and Sokolowski, 1987).

[1] Department of Biology, York University, North York, Ontario, Canada M3J 1P3.
[2] Centre National De La Recherche Scientifique, Laboratoire de Biologie et Genetique Evolutives, 91190, Gif-Sur-Yvette, France.
[3] To whom correspondence should be addressed.

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Preadult *Drosophila* are vulnerable to (1) desiccation at high temperatures and low humidities, (2) rotting by microorganisms at high temperatures and high humidities, and (3) drowning by actively foraging larvae when substrates are liquefied (Chiang and Hodson, 1950; Sokolowski, 1985). Successful pupation and emergence are dependent on food acquisition during foraging, larval pupation site choice, and the probability of parasitization by Drosophilid parasitoids (Bakker, 1961; Sokolowski, 1985; Sokolowski and Turlings, 1987). Food patch size and interpatch distance may influence the relative success of rover and sitter foragers, since unlike sitters, rovers move from patch to patch while foraging (Sokolowski *et al.*, 1983). In desiccating environments, sitters which pupate more on the fruit have greater pupal survivorship than rovers which pupate more off fruit (Sokolowski, 1985). Sitters are parasitized less than rovers by parasitoids such as *Asobara tabida* which use vibrotaxis (larval movement as a cue) to detect their second instar larval hosts (Sokolowski and Turlings, 1987; MacDonald *et al.*, 1988).

The approximately 100-ha circular-shaped oasis of Nasrallah is located in central Tunisia, about 60 km from Kairouan. The oasis contains orchards of olive trees fringed with hedges of the cactus *Opuntia ficus indica* (Cactaceae). The *Drosophila* community, *D. melanogaster*, *D. simulans*, and *D. buzzattii*, lay their eggs in fallen fruit of *Opuntia* cacti (Carton *et al.*, 1986). The sites (1500 m apart) differ in a number of abiotic parameters important to *Drosophila* larval growth, development, and pupation. Site 1 (Mafia) has a higher average cactus height than site 2 (Bout du Monde), 4.2 ± 0.9 m (SD) as compared to 1.6 ± 0.6 m. The higher density of cacti at site 1 provides greater shade for the larval and pupal habitat (the fallen *Opuntia* fruit). The illumination, air temperature, and humidity also differ. For example, on an October morning in 1984 when the isofemale lines were collected the air temperature at site 2 was 4°C higher, the humidity was 19% lower, and the illumination was 38,000 lux higher than in site 1. Site 1 is near a freshwater spring and site 2 is on the edge of the oasis close to the desert.

An isofemale line is established by randomly sampling inseminated females from nature and placing them individually into vials so that families of flies can be produced each originating from the same mother. This approach can be used to estimate relative amounts of genetic variation for a quantitative trait within and between populations (Parsons, 1980). Genetic variation within a site is estimated using analysis of variance (ANOVA) by determining if there is significant between-isofemale line (between-family) variation relative to the within-isofemale line (or within-family) variation. Genetic variation between sites is tested using a nested ANOVA which tests for a significant difference between sites taking into account the within-site, between-line variation and the within-site, within-line variation.

We sampled approximately 30 isofemale lines of *D. melanogaster* from

each of two sites. Each founder female was collected from a different fruit and fruits were chosen randomly along a transect of 10 m for site 1 and 30 m for site 2.

To study larval behavior, synchronous first-instar larvae were harvested within 3 h of hatching (Sokolowski *et al.*, 1984) and 100 larvae from each isofemale line were placed on a spoon containing 5 ml of a yeast–agar–sucrose culture medium. Larvae were maintained under standard conditions for 48 h, at which time they had developed to second instar. Each larva was then tested individually by placing it in a petri dish (8.5 × 1.4 cm) coated with a thin homogeneous layer of aqueous yeast suspension (distilled water and Fleishman's bakers' yeast at a 2:1 ratio by weight) applied with a glass spreading rod in a petri dish spinner. Each larva was allowed to forage for a 5-min test, after which the length of the foraging trail (path length) was measured using a Numonics Corp. digitizer. All tests were performed within a 6-h interval starting at 1200 h, at 22 ± 1°C, 14 ± 1 mbar vapor pressure deficit under homogeneous overhead illumination. Twenty-five second-instar larval paths were measured for each isofemale line (29 isofemale lines were used for site 1 and 27 for site 2). Path lengths were measured 20 months after founder female collection. The long-term stability (4 years to date) of isofemale line differences for larval behavioral traits has been repeatedly demonstrated (Bauer and Sokolowski 1984; 1985).

Figure 1 shows the distributions of mean path length scores for each isofemale line in each site. No difference in mean path length scores was found (Table Ic), however, a significant difference in variance was found between the two sites [$F(29, 27) = 2.81$, $P < 0.01$]. The variance in path length scores was greater in site 1 than in site 2.

The isofemale line genotypic variance [$V_{G(i)}$] is estimated by subtracting the mean square within lines from the mean square between lines and dividing by the number of replicates per isofemale line (in this case $n = 25$). The isofemale line environmental variance [$V_{E(i)}$] is estimated from the mean square within lines. These estimates (variance components) are used to calculate intraclass correlations, which Parsons (1983) calls isofemale heritabilities $h^2_{B(i)} = V_{G(i)}/[V_{G(i)} + V_{E(i)}]$. [See Mitchell-Olds and Waller (1985) for a discussion of the lack of standard tests for comparisons of variance components of isofemale lines or families.]

Parsons (1983) predicts that isofemale line heritabilities should be low in populations from stressful or extreme habitats and higher in those from more benign habitats. We suggest that site 2 is a more extreme larval habitat than site 1 and therefore predict that isofemale line heritability should be higher in the oasis than the desert-like site. Indeed this prediction is met for larval path length; $h^2_{B(i)}$ is 0.224 for site 1 and 0.069 for site 2. Their 95% confidence intervals [calculated as by Neter and Wasserman (1974)] do not overlap.

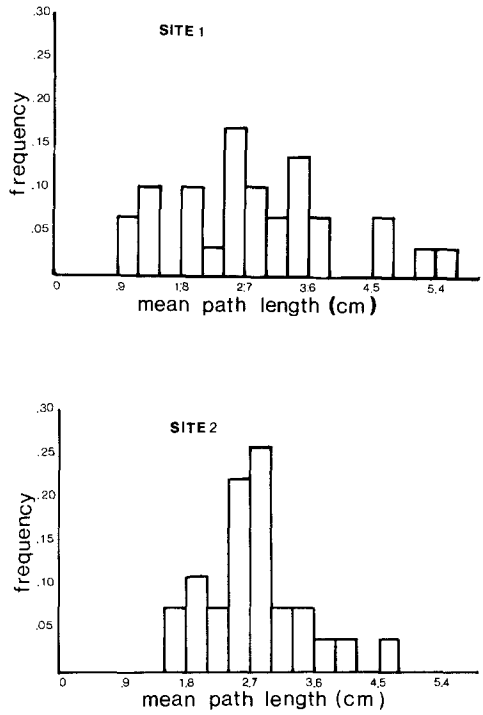

Fig. 1. The distribution of the mean isofemale line path lengths for the two sites. Isofemale line paths are more variable in Site 1 than in Site 2 [$F(29, 27) = 2.81$, $P < 0.01$].

Table I. Analyses of Variance of *Drosophila melanogaster* Second-Instar Larval Path Lengths from Sites 1 and 2

Source	df	MS	*F*	*P*
(a) Test for between-line variation in site 1				
Between isofemale line	28	34.50	8.23	<0.0001
Within isofemale line	696	4.19		
(b) Test for between-line variation in site 2				
Between isofemale line	26	12.22	2.86	<0.0001
Within isofemale line	648	4.28		
(c) Test for a site effect: Nested ANOVA				
Between site	1	8.43	0.35	NS
Within isofemale line (between site)	54	23.77		

Overall, the results of this preliminary study support the notion that isofemale line heritabilities are lower in more stressful habitats. Future studies will identify factors which have played a historical role in selection at these sites. Of special interest are factors influencing larval desiccation and parasitization.

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