Intra- and inter-specific variation in pupation behaviours of *Drosophila* from different habitats

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Isofemale strains of *Drosophila melanogaster* and *D. simulans* were collected from wet and dry habitats in Tunisia. These strains were used to study the effect of habitat (collection site) and laboratory rearing temperature on larval pupation behaviour of these species. Results showed that *D. melanogaster* isofemale strains from the wet habitat pupated significantly higher in vials than those from the drier habitat. In contrast, the pupation behaviour of *D. simulans* isofemale strains was not affected by habitat type. Significant intraspecific variation in pupation behaviour was found for both species from both habitats. The distance pupated away from food in vials was positively correlated to the distance pupated from fruit in a field-like assay. Plasticity for pupation height was found at 16 and 32°C for both species. Greater between-strain variation in pupation height was found for *D. melanogaster* at 32°C and for *D. simulans* at 16°C. The patterns of interspecific differences in larval pupation height paralleled those previously reported for adult behaviour patterns in these species.

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Des souches d'isofemelles de *Drosophila melanogaster* et de *D. simulans* ont été récoltées dans des habitats humides et des habitats secs de Tunisie. Ces souches ont servi à étudier les effets de l'habitat (site de récolte) et de la température d'élevage en laboratoire sur le comportement de nymphose larvaire chez ces espèces. Les résultats ont démontré que les souches d'isofemelles de *D. melanogaster* de l'habitat humide faisaient leur nymphose beaucoup plus haut, dans l'éprouvette que celles des habitats secs. En revanche, le comportement de nymphose des souches d'isofemelles de *D. simulans* n'était pas affecté par le type d'habitat. Une variation intraspécifique significative dans le comportement de nymphose a été trouvée chez les deux espèces dans les deux habitats. La distance entre le site de nymphose et la source de nourriture dans les éprouvettes était en corrélation positive avec la distance entre des fruits et la distance du site de nymphose au cours d'expériences de simulation en nature. La hauteur du site de la nymphose s'est avérée assez souple à 16 et à 32°C chez les deux espèces. C'est chez *D. melanogaster* à 32°C et chez *D. simulans* à 16°C que la variation entre les souches s'est avérée la plus grande. Les variations des différences interspécifiques quant à la hauteur du site de nymphose correspondent aux variations enregistrées au cours d'études du comportement des adultes chez ces espèces.

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Introduction

Pupation behaviour of two cosmopolitan species, *Drosophila melanogaster* and its sibling species *D. simulans* from the subgenus *Sopohophora*, species group *melanogaster*, are used in the present study to gain a better understanding of how larvae choose pupal microhabitats. These sibling species are similar morphologically and can be distinguished by comparing the male genital tergite (Sturtevant 1919). Genetically, *D. melanogaster* exhibits more polymorphism than *D. simulans* (Parsons 1975). Seasonal differences occur in their respective population sizes with *D. melanogaster* dominating in early summer and *D. simulans* in fall (Parsons 1975). *Drosophila melanogaster* has greater adult dispersal activity than does *D. simulans*. Dispersal and mating activity are more light dependent for *D. simulans* than for *D. melanogaster* (McDonald and Parsons 1973). When temperatures fluctuate, the relative number of adults of *D. simulans* compared with *D. melanogaster* decreases. Long periods of high temperature are tolerated by adults of both species when relative humidity is high; however, when relative humidity is low, both species die quickly, especially *D. simulans* (Parsons 1979). Adults of *D. melanogaster* are more tolerant of alcohol than *D. simulans* (McKenzie and Parsons 1972; Parsons 1977). Little information, however, is available on interspecific comparisons of larval behaviour and tolerance of environmental stress. These results imply that (*i*) *D. melanogaster* is better able to withstand environmental extremes and (*ii*) *D. melanogaster* uses a wider range of resources than *D. simulans*. This may account for its wider distribution.

In this study isofemale strains of *D. melanogaster* and *D. simulans* from two habitats in Tunisia (wet and dry) are used to test the hypothesis that larvae from dry environments pupate more on the fruit than those from wetter environments. Intra- and inter-specific variations in pupation height at three temperatures are also investigated.

Materials and methods

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. 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 has a higher average cactus height than site 2 (4.2 ± 0.9 m (±SD) as compared with 1.6 ± 0.6 m). In addition, the higher density of cacti at site 1 provides greater shade for the larval and pupal habitat (the

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fallen *Opuntia* fruit). The illumination, air temperature, and humidity also differ. Site 1 is near a freshwater spring and site 2 is on the edge of the oasis close to the desert.

Isofemale strains of *D. melanogaster* and its sibling species *D. simulans* were collected from two habitats in Tunisia designated site 1 and site 2. 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 of populations taken from different sites is tested using a nested ANOVA that tests for a significant difference between sites taking into account the within-site, between-line variation, and the within-site, within-line variation.

Sixty *D. melanogaster* and 46 *D. simulans* isofemale strains were collected from the two sites. The species was verified by examining male progeny from each strain. 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. Twenty-nine *D. melanogaster* and 25 *D. simulans* isofemale strains were collected from site 1. Thirty-one *D. melanogaster* and 21 *D. simulans* isofemale strains were collected from site 2. All behavioral assays were done within 8 months of establishing the isofemale strains in the laboratory.

Files were maintained in 180-mL glass culture bottles on 45 mL of a standard dead yeast – sucrose – agar medium (culture medium) and incubated under standard conditions (24 ± 1°C, 60% RH with a 12 h L : 12 h D photoperiod; lights on at 08:00) following Sokolowski *et al.* (1984).

The pupation height assay is described in detail in Bauer and Sokolowski (1985, 1988). Ten randomly collected first instar larvae ±1.75 h in age were placed in a vial (2 cm in diameter and 11 cm in height) containing 5 mL of a yeast–agar–sucrose medium. Five replicate vials were used for each of the 106 Tunisian isofemale strains for a total of 5300 larvae. Vials were randomly placed into an incubator under standard conditions until all larvae had pupated (7–8 days after hatching). Pupation height was determined by measuring the distance from the surface of the food to the same fixed point on each pupa.

The isofemale strains of both species that showed "extreme" behavioural phenotypes (low and high pupation height) were identified from both sites so that their pupation behaviour could be examined in a field-like assay (Sokolowski *et al.* 1986). The assay consisted of glass dishes (8.5 cm in diameter and 4.8 cm in height) containing sterile sifted soil with approximately 50% RH, grass, and half a grape containing a yeast and water paste (1.5 water to 1.0 yeast by weight) placed into the centre of each dish. Ten randomly collected third instar larvae were placed onto the yeast paste. Ten larvae were used per dish per strain for a total of 100 larvae per strain. Dishes were incubated under standard conditions until all larvae had pupated. The proportions of pupae found in two microhabitats, on the fruit and off the fruit, were scored. The pupation heights in vials of these isofemale strains were also investigated at 16, 25, and 32°C (±1°C; 60% RH with a 12 h L : 12 h D photoperiod; lights on at 08:00). Ten replicate vials were used per strain for a total of 100 larvae per strain.

Prior to statistical analysis, all pupation height measurements and percentage data were transformed using natural logarithm + 1 and arcsine square root transformations, respectively (Sokal and Rolf 1969; Zar 1984). These transformations normalized the data.

Results

Nested analyses of variance (ANOVA) were performed on the pupation height data of all isofemale strains to determine whether habitat (site 1 or site 2) had a significant effect. Significant variation in pupation height between habitats was found for *D. melanogaster* strains but not for the *D. simulans* isofemale strains (Table 1). Pupation heights (cm) of the *D. melanogaster* site 1 strains were significantly higher than the site 2 isofemale strains (site 1, $\bar{x} \pm SE = 1.11 \pm 0.03$, $n = 1256$; site 2, $\bar{x} \pm SE = 0.90 \pm 0.03$, $n = 1342$). Pupation heights (cm) of the *D. simulans* isofemale strains were significantly lower than *D. melanogaster* strains (site 1, $\bar{x} \pm SE = 0.46 \pm 0.01$, $n = 958$; site 2, $\bar{x} \pm SE = 0.39 \pm 0.01$, $n = 811$). Table 2 shows that there was significant between-isofemale strain variation for pupation height within each site indicating genetic variation for pupation height in each sampled population for each species.

TABLE 1. Nested analyses of variance of log pupation height (cm) of *D. melanogaster* and *D. simulans* isofemale strains collected from different habitats in Tunisia

Source	df	SS	MS	*F*	*P*
		Drosophila melanogaster			
Habitat	1	6.65	6.65	4.41	*
Strain (habitat)	58	87.43	1.51	7.19	***
Vial (strain)	239	50.32	0.21		
		Drosophila simulans			
Habitat	1	1.14	1.14	1.66	ns
Strain (habitat)	45	30.99	0.69	5.75	***
Vial (strain)	161	19.90	0.12		

NOTE: ns, not significant.
$*p < 0.05$.
$***p < 0.0001$.

TABLE 2. Analyses of variance of log pupation height (cm) for isofemale strains of *D. melanogaster* and *D. simulans* collected from different habitats in Tunisia

Habitat	Source of variation	df	SS	MS	*F*	*P*
		Drosophila melanogaster				
Site 1	Between strains	28	42.17	1.51	10.57	***
	Within strains	1227	174.84	0.14		
Site 2	Between strains	30	45.26	1.51	9.34	***
	Within strains	1312	211.87	0.16		
		Drosophila simulans				
Site 1	Between strains	25	68.14	2.73	7.95	***
	Within strains	934	320.37	0.34		
Site 2	Between strains	20	40.24	2.01	7.48	***
	Within strains	788	211.95	0.27		

$***p < 0.0001$.

The pupation heights of the extreme isofemale strains were designated as follows: Dm or Ds designates the species either *D. melanogaster* or *D. simulans*, L or H designates a low or high pupating strain, and 1 or 2 designates site 1 or 2. For example, DsH1 is for *D. simulans* high pupating strain from site 1. DmL1 and DsL1 pupated significantly lower than DmH1 and DsH1, and DmL2 and DsL2 pupated significantly higher than DmH2 and DsH2 (Table 3). For both species a positive relation was found between the tendency to pupate low in vials and the tendency to pupate on the fruit in the field-like assay (Table 3). Strains that pupated low in the vials pupated significantly more on the fruit than those that pupated high in the vials (Student–Newman–Keuls, $p < 0.05$).

The pupation heights of each species were affected by

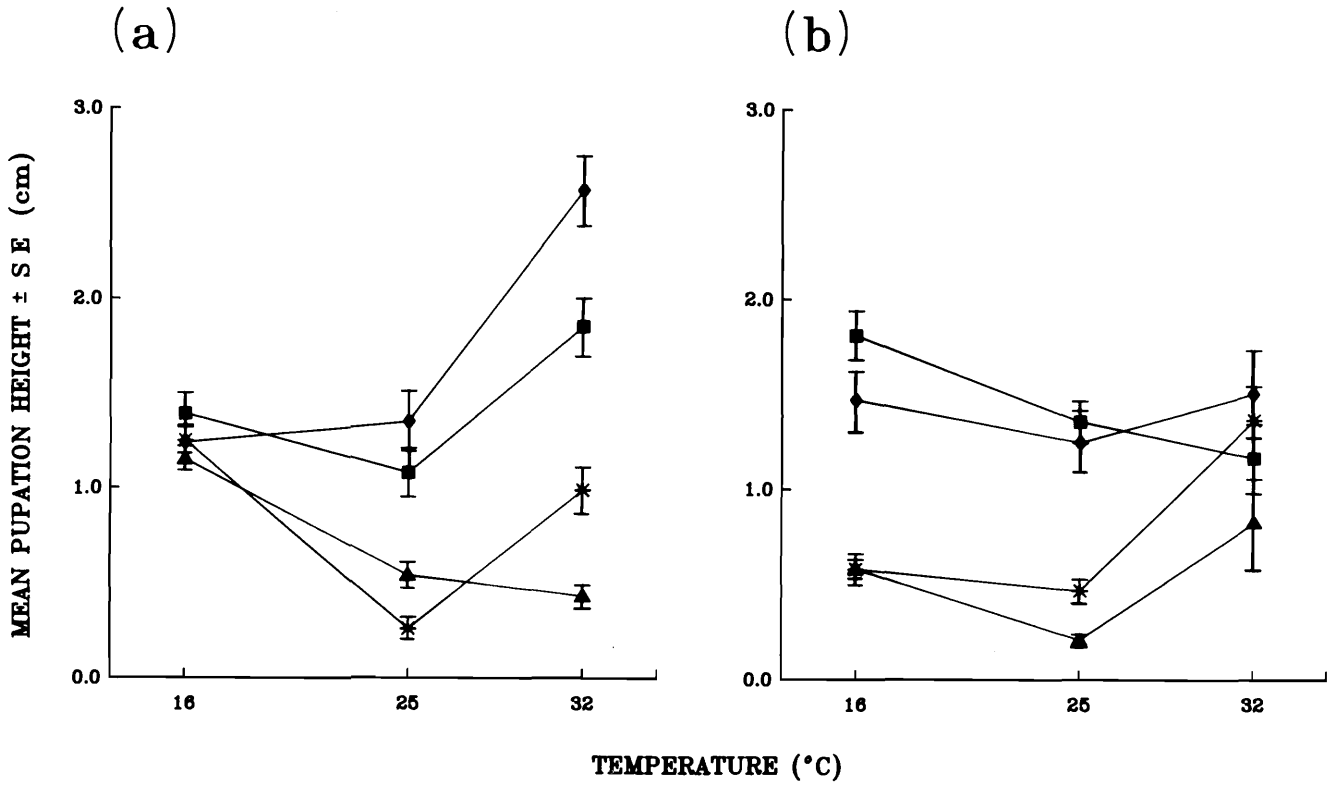

FIG. 1. The mean pupation heights of the extreme strains are shown for (*a*) *D. melanogaster* (*, DmL1; ◆, DmH1; ▲, DmL2; ■, DmH2) and (*b*) *D. simulans* (*, DsL1; ◆, DsH1; ▲, DsL2; ■, DsH2) at three temperatures (± 1°C).

TABLE 3. Pupation behaviour (in vials and in the field-like assay) of isofemale strains of *D. melanogaster* and *D. simulans* collected from different habitats in Tunisia

Strain	Pupation height (cm)	% pupated on fruit
DmL1	0.26±0.06 (57)	61.96±3.54 (95)
DmH1	1.35±0.16 (56)	38.89±3.44 (92)
DmL2	0.54±0.07 (57)	75.97±4.83 (95)
DmH2	1.08±0.13 (60)	31.30±5.30 (93)
DsL1	0.47±0.06 (87)	71.57±5.64 (90)
DsH1	1.25±0.17 (97)	47.61±2.41 (77)
DsL2	0.21±0.03 (87)	76.50±4.83 (89)
DsH2	1.36±0.11 (90)	49.98±3.21 (89)

NOTE: Values are means ± SE, with the number of pupae measured in parentheses. The proportion pupating on the fruit was arcsine square root transformed.

TABLE 4. Analyses of variance of log pupation height (cm) at different air temperatures for isofemale strains of *D. melanogaster* and *D. simulans* from two habitats in Tunisia

Source	df	SS	*F*	*P*
	Drosophila melanogaster			
Strain	3	28.62	58.68	***
Temperature	2	10.48	32.24	***
Strain × temperature	6	18.98	19.46	***
Error	859	139.63		
	Drosophila simulans			
Strain	3	28.57	41.90	***
Temperature	2	6.71	14.77	***
Strain × temperature	6	6.72	4.93	***
Error	885	201.15		

***$p < 0.0001$.

temperature. The effects of strain, temperature, and the interaction between strain and temperature were all highly significant for both species (ANOVA, $p < 0.001$; Table 4). On average, the low isofemale strains of both species tended to pupate closer to the food in vials at all three temperatures than the high strains (Fig. 1). Student–Newman–Keuls tests ($p < 0.05$) showed significant differences between means for *D. melanogaster* at 32°C but not at 16°C. In contrast, *D. simulans* pupation heights showed significant differences between means at 16°C but not at 32°C.

Discussion

Indeed, *D. melanogaster* larvae from a dry environment pupate more on the fruit (and closer to food in vials) than those from a wet environment. This was not found for *D. simulans* strains. Pupation heights of *D. simulans* were on average lower than those of *D. melanogaster*. This is in agreement with previous studies that report significantly lower pupation heights for *D. simulans* than for *D. melanogaster* (Sameoto and Miller 1968; Markow 1979; 1981; Kidwell 1981; Ringo and Wood 1982, 1983). In addition, *D. simulans* adults are less resistant to desiccation than *D. melanogaster* adults (Parsons 1979). It is possible that *D. simulans* larvae chose more moist microenvironments for pupation (closer to food in vials) because of their greater sensitivity to water loss.

Previous work on *D. melanogaster* pupation height in vials and pupation site choice in a field-like assay showed that (*i*) both pupation height in vials and pupation distance in the field-like

assay measure how far larvae pupate away from a food source (Sokolowski and Bauer 1989) and (*ii*) both soil moisture and air temperature affect larval pupal microhabitat choice and pupal survivorship. Pupal survivorship was higher in dry environments when larvae pupated close to or on the food source. In contrast, pupal survivorship was higher in wet environments when larvae pupated far from the food source, on or in soil (L. Rodriguez and M. B. Sokolowski, manuscript submitted for publication). The hypothesis that larvae from a dry habitat pupate more on the fruit than those from a wet one arose from these results.

Even after many generations in the laboratory, extreme isofemale strains of both species tested from both habitats showed a positive relation between the tendency to pupate low in vials and more on the fruit at 25°C in the field-like assay. This indicates that (*i*) these behaviours are common to both species and (*ii*) they have a heritable component.

Parsons (1979) has shown that *D. melanogaster* tolerate higher temperatures than *D. simulans* and that the frequency of *D. simulans* is higher than *D. melanogaster* when temperatures are low (18–21°C). In a population of adult *D. melanogaster* from Japan, emigration activity increased when air temperatures were extreme (16 and 31°C) (Mikasa and Narise 1983). Larvae, like adults, may increase their wandering activity at extreme temperatures; this may result in greater pupation heights.

The present study showed that variation for pupation behaviour exists between and within *Drosophila* species from different habitats. This variation in pupation behaviour was due to the interaction between isofemale strain (genotypes) and the environment. Differences in larval tolerance of different temperature and desiccation conditions likely resulted in variation in pupal microhabitat choice. These findings (*i*) help to explain the heterogeneity in pupal microhabit choice between and within sibling species of *Drosophila* and (*ii*) help us understand how variation for behavioral traits in natural populations of *Drosophila* may arise.

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