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GENETICS AND ECOLOGY OF *DROSOPHILA MELANOGASTER* LARVAL FORAGING AND PUPATION BEHAVIOUR

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Abstract—In this paper we show that, (1) *Drosophila melanogaster* larvae utilize a variety of pupal microhabitats in an orchard, (2) variation in larval foraging path length, pupation distance from the food and pupal microhabitat preference (on or off the fruit) is genetically based and, (3) variation in these behaviours can be maintained in a spatially heterogenous environment since there is a reversal in pupation site suitability in wet and dry pupal microhabitats. Differences in path length in both laboratory and natural populations can be attributed to genes on the second pair of chromosomes and is under simple genetic control, whereas differences in pupal height are polygenically inherited (the second pair of chromosomes influences pupal height three times more than the third pair). Pupae collected from on-fruit sites had shorter foraging path lengths and lower pupal heights than off-fruit populations. Populations from the orchard maintained their field pupal microhabitat preferences even after 1 year of rearing them in the laboratory. Larvae with the *sitter* larval phenotype (short path lengths and low pupal heights tended to pupate more on-fruit than those with the *rover* phenotype (long path lengths and high pupal heights). To determined if these genetically based differences in microhabitat preference contributed to fitness, larval pupation behaviour was studied in a "field assay" (dish with fruit on soil) with soil water content varied. At low soil water contents, pupal survivorship was significantly better on the fruit whereas, at high soil water contents, survivorship was better in the soil. There was a reversal in which microhabitat (dry or wet) was a better site for pupation. In the field environment where soil water content fluctuates in space and time, such a reversal would explain the maintenance of genetic variation for these larval behaviours. Another selective agent acting on *D. melanogaster* larvae in our orchard is parasitization by *Asobara tabida*. This parasitoid parasitizes larvae with high locomotory scores (e.g. *rovers*) significantly more than those with low scores (*sitters*). This study relates laboratory phenotypes to field phenotypes thereby linking the ecological, behavioural and genetic components of larval habitat selection in *D. melanogaster*.

Key Word Index: Microhabitat selection, genetics, ecological, larval behaviour, *Drosophila*, polymorphism, *rover/sitter*, parasitoid

Habitat choice occurs through differences in behavioural preferences as well as selection (Powell and Taylor, 1979). Natural selection is thought to favour individuals with the most fit of alternative behaviour patterns in a particular environment. It is common to speculate about the adaptive significance of behavioural traits (Brown, 1975). Inherent to this speculation is the assumption that there is a genetic basis to differences in behaviour. The study of the genetics of behaviour is a relatively new field of investigation (Ehrman and Parsons, 1981). Behaviour, like morphology, is a phenotype. A behavioural phenotype is influenced by both genotype and environment. A genetic basis for differences in behavioural preferences must be demonstrated before the role of natural selection in habitat choice can be implicated. A genetic basis for intraspecific variation in habitat selection has not been conclusively demonstrated for any species (Parsons, 1983).

Examples of intraspecific variation in behaviours potentially important to habitat selection are numerous. Alary polymorphisms, the occurrence of several morphs differing in their ability to fly, are found in aphids (Lees, 1966; Hardie, 1980). In migratory locusts, gregarious and solitary morphs differ behaviourally and morphologically (Kennedy, 1975). In the gypsy moth, *Lymantria dispar*, the size of the hatching larva which influences dispersal tendency (Barbosa *et al.*, 1981) is proportional to the egg size (Barbosa and Carinera, 1978). Wellington (1957, 1960, 1964, 1965) has reported a foraging polymorphism in the western tent caterpillar *Malacosoma pluviale* with morphs differing in locmotory, phototactic and food location behaviour. Denno *et al.* (1980) demonstrated differences in habitat selection between the wing forms of the dimorphic planthopper, *Prokelisia marginata*. Nevertheless, studies which distinguish between genetic and environmental contributions to the components of variation between any of these morphs are lacking.

Rausher (1978) has identified a polymorphism in the searching behaviour of adult female pipevine swallowtail butterflies, *Battus philenor*. Females search for host plants in one of two modes, the broad leaf or the narrow leaf mode. Papaj and Rausher (1983) suggest that these differences in search mode can be genotypically based, environmentally induced and/or experiential. They provide preliminary evidence that

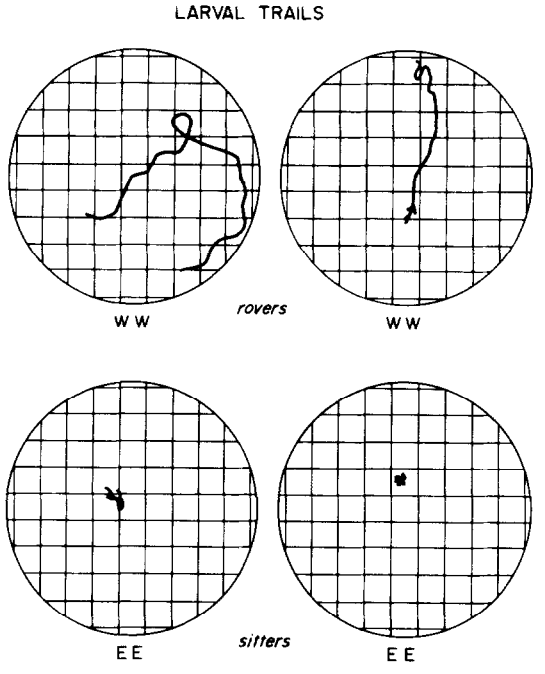

Fig. 1. Random sample of *D. melanogaster* larval trails of the WW and EE stocks superimposed on a centimeter grid. The length of the trail is termed "Path Length". WW shows the *rover* long path length behavioural phenotype whereas EE shows the *sitter*, short path length phenotype (modified from Sokolowski, 1980).

experience effects the mode of searching. Sokolowski (1980) identified a polymorphism in the locomotory component of *D. melanogaster* larval foraging behaviour. Larvae of the *rover* morph have long foraging trails and traverse a large area while foraging compared to those of the *sitter* morph (Fig. 1). *Rover* larvae also dig deeper into (Sokolowski, 1982a) and pupate higher above (Sokolowski and Hansell, 1983b) the feeding substrate than do *sitters*. This polymorphism is found in laboratory and natural populations of *D. melanogaster* (Sokolowski, 1982b, 1984). A chromosomal analysis has demonstrated that differences between the behaviour of the two morphs is genetically based and can be attributed to the second pair of chromosomes (Sokolowski, 1980). Crosses between *rovers* and *sitters* support the hypothesis that the polymorphism is under relatively simple genetic control with the *rover* phenotype showing complete dominance over the *sitter* and no significant sex-linked or maternal effects (Sokolowski, 1984; Bauer and Sokolowski, 1984). The locomotory component of foraging behaviour is rapidly determined by measuring the length of the trail a foraging larva leaves in the yeast paste during a 5-min test period. This is termed "path length" and provides the basis for quantification of the *rover/sitter* polymorphism. A sample of 100 third-instar *D. melanogaster* larvae from a single field-collected pear, showed a bimodal distribution for larval path length (Sokolowski, 1982b). Bell (p. 837 this issue, Fig. 7) has recently been able to select for adult *rover* and *sitter* foragers. They, like the larvae, differ in locomotor and turning rate.

The genetically well characterized species *D. melanogaster* is an excellent subject for studies of the genetics of behavioural preferences in the laboratory. Little is known, however, about the ecological genetics of behavioural traits in natural populations of this species (Ehrman and Parsons, 1981). Until recently, studies of the genetic and environmental determinants of behaviour in *D. melanogaster* have focused primarily on adults (Sokolowski, 1984). *Drosophila* larval behaviours that have been studied include foraging (Sokolowski, 1980; Sokolowski and Hansell, 1983a; Sokolowski, *et al.*, 1983; Bauer and Sokolowski, 1984) feeding (Bakker, 1961; Sewell *et al.*, 1975; Burnet *et al.*, 1977; Ohnishi, 1979; Sokolowski, 1980; Green *et al.*, 1983), digging (Godoy-Herrera, 1977, 1978; Sokolowski, 1982a), response to ethanol (Parsons, 1977, 1980a), response to moisture (Godoy-Hererra, *et al.*, 1984; Sokolowski *et al.*, 1984), pupation site preferences (Sokal *et al.*, 1960; Mensua, 1967; Sameoto and Miller, 1968; de Souza *et al.*, 1970; Markow, 1979; Manning and Markow, 1981; Markow, 1981; Sokolowski and Hansell, 1983b; Ringo and Wood, 1983; Bauer, 1984; Wong *et al.*, 1985; Bauer and Sokolowski, 1985). Most of the aforementioned studies utilize laboratory populations; their relevance to natural populations is unknown. Ohnishi (1979), Parsons (1980a) and Bauer and Sokolowski (1984) used the isofemale line technique to study *Drosophila* larval behaviour of natural populations. An isofemale line is initiated by a single, inseminated female from nature. Genetic variation for a trait in a natural population is indicated by significantly greater variation between than within isofemale lines (see Parsons, 1980b for an extensive review of this technique).

Variation in *D. melanogaster* larval behaviour is amenable to study in the laboratory since larvae have relatively simple behaviour patterns (Sokolowski, 1980; Green *et al.*, 1983). *Drosophila* larvae spend most of the three instars of their larval life foraging. The larval period of life history is the stage of maximal resource utilization (Bakker, 1961). Foraging behaviour can be defined as the relative amount of feeding (shovelling) and locomotory (crawling) movements performed during a test period (Sokolowski, 1980). Both feeding and locomotory components of larval foraging behaviour influence the probability of successful pupation and adult emergence. Feeding rate influences viability (Ohnishi, 1979) and developmental time (Bakker, 1961). Locomotory patterns influence a larva's ability to forage in a patchy environment (Sokolowski *et al.*, 1983). Larval movement increases its encounter rate with the parasitoid *Asobara tabida* (Sokolowski and Turlings, submitted). *D. melanogaster* larvae switch from food-related foraging activities to wandering activities that are not food-related in the mid third instar (Sokolowski *et al.*, 1984). Moisture is the most important environmental factor influencing larval behaviour throughout all three instars (Sokolowski *et al.*, 1984; Sokolowski *et al.*, 1985). Selection of a pupation site by wandering larvae may function to minimize the probability of pupal desiccation (Sokolowski *et al.*, 1985; Wong *et al.*, 1985) or drowning of pupae when water content of the medium is high (Sameoto and Miller, 1968). Larval

behaviour must certainly be an important component of fitness in the field as well as in the laboratory.

The experiments presented here investigate three questions: (1) Is there intrapopulation phenotypic variation for larval foraging and pupation behaviour? (2) Does this variation have a genetic basis? (3) Is there a relationship between the behavioural phenotype and fitness? If each of these questions can be answered affirmatively, then an evolutionary response of the behavioural traits to natural selection is possible (Lewontin, 1967).

Question 1 has been investigated by determining whether there is phenotypic variation for larval path length (the locomotory component of larval foraging behaviour), pupal height (the distance a larva pupates from the surface of the medium), and pupal microhabitat preference (the tendency for larvae to pupate on the fruit as compared to on or in the soil). Laboratory populations, isofemale lines from a natural population and stocks derived from pupae collected from four different pupal microhabitats in an orchard are used. By measuring the distance a larva pupates from the food in three test procedures it is determined that pupal height in vials is proportional to the distance a larva pupates from the fruit.

We have examined question 2 by doing a chromosomal analysis on laboratory and natural populations to determine if differences in behaviour are genetically based and if the pattern of inheritance is the same in stocks from the laboratory and the field.

Finally, question 3 was investigated by using a pupal microhabitat "field assay". This assay was used to determine whether pupae from different microhabitats differ in survivorship and whether the magnitude of any such differences was influenced by soil water content.

MATERIALS AND METHODS

Laboratory stocks

Four laboratory stocks, isogenic (homozygous at all loci) for the 2nd and 3rd pairs of chromosomes, were designated EE, EW, WE and WW. The first letter of the two letter designation denotes the 2nd pair of chromosomes whereas the second letter denotes the 3rd pair. EW has the same 2nd chromosome pair as EE but differs in having the same 3rd chromosome pair as WW. A desciption of the balanced lethal chromosome technique used to derive these stocks can be found in Sokolowski (1980).

Stocks from isofemale lines

The balanced lethal chromosome techique was also used to make isofemale lines that had long (B-15) and short (B-1) path lengths isogenic for the 2nd and 3rd pairs of chromosomes. The resultant stocks were called B1B1, B1B15, B15B1 and B15B15 with the first letter and number giving the origin of the 2nd chromosomes and the second letter and number giving the origin of the 3rd chromosomes. The isofemale lines were sampled from a dustbin outside a factory in Toronto, Ontario. Collection, testing of the lines and the crossing procedure used to derive these stocks are described in Bauer and Sokolowski (1984 and 1985).

Field stocks

In the fall of 1983, we noticed that a variety of pupal microhabitats are used by a population of *D. melanogaster* in a pear orchard in the Toronto area. Pupae were found in 4 microhabitats: (1) On the upper surface of the fruit, on the skin, (2) on the lower surface of the fruit, on the skin, (3) under the fruit, on the soil and (4) under the fruit, in the soil. The four populations of flies derived from pupae collected from these sites were called M1, M2, M3 and M4 respectively.

Larval behaviours: path length

The four laboratory stocks (EE, EW, WE and WW), isofemale line derived stocks (B1B1, B1B15, B15B1 and B15B15) and field derived stocks (M1, M2, M3 and M4) were tested for larval path length and pupal height using the techniques described in Sokolowski (1980) and Bauer and Sokolowski (1984 and 1985). One-hundred freshly hatched ($\pm$1.75 hr) larvae of each stock were grown separately under standard conditions (see Sokolowski *et al.*, 1984) until the larvae were 4 days after hatching. Path length was measured by placing a single larva in a Petri dish (8.5 cm in dia and 1.4 cm high) containing an evenly spread yeast paste. After a 5-min test period a visible trail was left in the yeast. Fifty trails were quantified for each of the isofemale and the field derived stocks and twenty-five trails for each of the laboratory stocks. Figure 1 shows a random sample of larval trails of the WW and EE laboratory stocks. The length of the trail is measured by using a digitizer and is called larval path length.

Larval behaviours: pupal height

Ten vials (2 cm in dia and 11 cm high with 5 ml of a dead yeast–agar medium) each containing 10 freshly hatched ($\pm$1.75 h) larvae were used to measure pupation height in each of the laboratory, isofemale and field derived stocks. The vials were plugged with a standard-size cotton ball and incubated under standard conditions until the larvae had pupated. The pupal height of each larva was measured as the distance from the surface of the food to the midpoint between the spiracles on the pupa.

Larval behaviours: distance from the food plug

Stocks known to differ in pupal height, were tested for the distance they pupated from the food plug to determined whether the two pupal behaviours were positively correlated. A Petri dish (8.5 cm in dia and 2.4 cm high) was filled to a depth of 0.5 cm with a hot agar solution. The agar was then flamed to eliminate bubbles, thereby ensuring a smooth surface for larval locomotion. After the agar had cooled, a food plug (1.8 cm in dia and 2.4 cm high) of dead-yeast agar medium was placed on the surface of the agar and positioned in the centre of the dish. Twenty-five first-instar larvae were placed onto the centre of each food plug for the EE and WW stocks while ten were used for each of the B1B1 and B15B15 stocks. These experiments are part of two independent studies (Wong, unpublished; Bauer, unpublished). After all the larvae had pupated the distance from the centre of the food plug to the spiracles of each larva was

measured. A more detailed description of this test procedure is given in Wong *et al.* (1985). Four replicates for each of the stocks (EE, WW, B1B1 and B15B15) were examined.

Larval behaviours: pupal microhabitat field assay I

The "field assay" was devised to simulate field conditions in the laboratory. The assay was used to determine (1) whether stocks with extreme path length and pupal height scores differed in their pupal microhabitat preference (the tendency to pupate on or off the fruit) and (2) whether flies collected from different pupal microhabitats in the orchard maintained their microhabitat preferences in the field assay. The assay was prepared by filling dishes (4.8 cm high and 8.5 cm in dia) to a depth of 1 cm with dried sifted, sterile soil. Wheat seedlings (32 pieces, 8.5 cm long) were placed randomly on the surface of the soil which was kept at 60% r.h. with the use of a sodium bisulphate solution. Purple table grapes were washed with alcohol, rinsed with distilled water and sliced in half longitudinally. Each half grape was seeded with a 1 ml yeast solution (8 g of yeast in 25 ml of water) and incubated at 24°C for 24 h. Aged larvae of the EE, WW, M1 and M4 stocks were reared under standard culture conditions. These stocks were chosen because they had the most extreme path length and pupal height scores from each of the laboratory and field derived populations (Figs 2 and 3). One-half grape was seeded with 10 larvae (aged 4 days post-hatching) and placed onto the centre of the dish containing the soil and grass. The dish was covered with a lid and incubated at 24 ± 1 C, under a 12 hr light:12 h dark, photoperiodic regime with lights on at 0800 h. Ten replicate dishes were used per stock. Each experimental set was repeated 3 times (300 larvae/stock) during March–July, 1983. Further details are in Sokolowski *et al.* (1985). After pupation, the number of pupae found on the fruit and on or in the soil and the distance the larva had pupated from the centre of the grape was scored. Survivorship to pupation was high (97%) in all replicates.

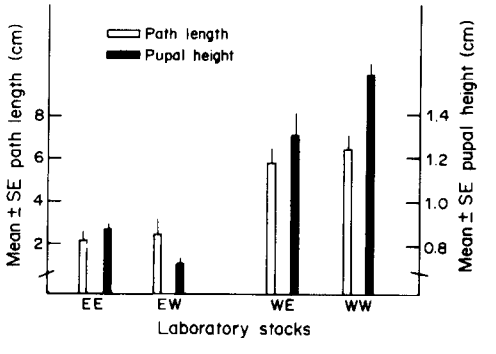

Fig. 2. Mean ± SE larval path length and pupal height for each of the four laboratory stocks. Larvae sharing an 'E' pair of 2nd chromosomes (EE and EW) have short path lengths and low pupal heights. Larvae with a 'W' pair of 2nd chromosomes have long path lengths and high pupal heights (data from Sokolowski *et al.*, 1985).

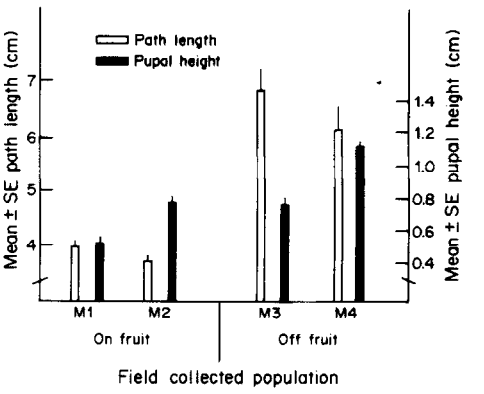

Fig. 3. Mean ± SE larval path length and pupal height for each of the four field derived stocks. Flies derived from on-fruit pupal microhabitats (M1, M2) had shorter path lengths than those from off-fruit pupal microhabitats (M3, M4). Pupation height from the on-fruit M1 stock was lower than the in soil derived M4 stock (data from Sokolowski *et al.*, 1985).

Larval behaviours: pupal microhabitat field assay II—varying soil moisture

A second field stimulation was performed to determine whether varying soil water content affected (1) pupal microhabitat preference and (2) pupal survivorship. Pupation behaviour was measured in the 2 field stocks, M1 and M4, under conditions of 0, 50, 75 and 100% soil water content. Soil water content was calculated as: [the number of grams of water] divided by [the number of grams of soil minus the number of grams of water] times 100. Ten field assays (10 larvae/assay) were prepared for each of M1 and M4 under of the 4 soil water content conditions. The assays were incubated under standard conditions. Individual pupae from M1 and M4 were categorized according to their pupal microhabitat phenotype, pupating on or off the fruit. Individual pupae of known pupal microhabitat preference were checked for adult emergence from the pupal case. After emergence the pupal cases were located. In this way the proportion of pupae found on the fruit and pupal survivorship (measured as the percent adult emergence) were scored.

RESULTS

Laboratory stocks: path length and pupal height

Chromosomal constitution has a significant effect on both path length and pupal height. Figure 2 shows the path length and pupal height scores of the laboratory stocks arranged in order of 2nd and 3rd chromosome constitution. The laboratory stocks EE and EW sharing an "E" pair of 2nd chromosomes had significantly lower path length and pupal height scores than did the WE and WW stocks which share a "W" pair of 2nd chromosomes (Mann–Whitney U-test, $P < 0.05$).

Stocks from isofemale lines: path length and pupal height

An analysis of variance by chromosomes of the

Table 1. Pupation distance (cm) from the food in 3 test procedures

Replicate set	Stock	Pupal height mean ± SE (*n*)	Distance from food plug mean ± SE (*n*)	Distance from grape in field assay mean ± SE (*n*)
1	B15B15	0.95 ± 0.05 (72)	3.57 ± 0.14 (47)	1.81 ± 0.17 (12)
	B1B1	2.32 ± 0.08 (89)	4.69 ± 0.16 (37)	2.81 ± 0.34 (17)
2	B15B15	1.29 ± 0.06 (65)	4.08 ± 0.13 (42)	—
	B1B1	2.10 ± 0.10 (75)	4.74 ± 0.16 (48)	—
1	EE	0.87 ± 0.02 (84)	4.00 ± 0.33 (89)	2.37 ± 0.66 (7)
	WW	1.60 ± 0.03 (87)	5.20 ± 0.73 (85)	3.09 ± 0.35 (22)
2	EE	1.02 ± 0.08 (97)	—	—
	WW	2.24 ± 0.09 (87)	—	—
1	M1	0.53 ± 0.06 (88)	—	2.50 ± 0.30 (15)
	M4	1.12 ± 0.04 (77)	—	3.20 ± 0.20 (40)
2	M1	—	—	2.70 ± 0.30 (29)
	M4	—	—	3.40 ± 0.20 (39)

path length data for the B1B1, B1B15, B15B1 and B15B15 stocks showed a highly significant effect of the 2nd pair of chromosomes ($F = 15.82$, $df = 1, 194$, $P < 0.0001$), no significant effect of the 3rd pair ($F = 0.16$, ns) and no 2nd by 3rd chromosome interaction ($F = 0.17$, ns). Stocks sharing the 2nd pair of "B1" chromosomes had significantly shorter path lengths than those sharing the 2nd pair of "B15" chromosomes. Reciprocal crosses of B1B1 by B15B15 and WW by EE demonstrated that the long path length phenotype was completely dominant over the short path length phenotype. No sex-linked or maternal effects on path length were found (Sokolowski, 1983; Bauer and Sokolowski, 1984). Pupal height in these stocks was also highly significantly influenced by the 2nd pair of chromosomes ($F = 43.07$, $df = 1, 334$, $P < 0.0001$), significantly influenced by the 3rd pair ($F = 6.23$, $df = 1, 334$, $P < 0.01$) and not influenced by a 2nd by 3rd chromosome interaction ($F = 0.16$, ns). B15B15 and B15B1 had significantly lower pupal heights than B1B15 which was significantly lower than B1B1. The second pair of chromosomes influenced pupation height 3 times more than the third. Reciprocal crosses of B1B1 by B15B15 yielded intermediate pupal heights (Bauer and Sokolowski, 1985). Preliminary evidence indicates that path length and pupal height are under independent genetic control with genes on the second chromosomes for these two behaviours mapping to opposite arms (Sokolowski, unpublished). In the laboratory (Sokolowski, 1980; Sokolowski and Hansell, 1983b) and orchard derived stocks (Sokolowski *et al.*, 1985; present study), larval path length and pupal height were positively correlated. Larvae with long path lengths (*rovers*) had higher pupal heights than larvae with shorter path lengths (*sitters*) (Figs 2 and 3). No correlation between these behaviours was found in the isofemale lines derived from a factory dustbin (Sokolowski, 1984).

Field stocks: path length and pupal height

An analysis of variance on larval behaviour of the progeny of pupae collected from the four pupal microhabitats (M1, M2, M3 and M4) showed significant between microhabitat variation for path length ($F = 16.85$, $df = 3, 200$, $P < 0.0001$) and pupal height ($F = 25.18$, $df = 3, 305$, $P < 0.001$). Moreover, stocks that differed in their field pupal microhabitat preference also differed in the two laboratory measures of larval behaviour (Fig. 3). Populations of flies derived from pupae found on the fruit (M1, M2) had significantly shorter path lengths than those found on or in the soil (M3, M4) (Student–Newman–Keuls test, SNK, $P < 0.05$). Pupation height showed a similar pattern although only the two extreme populations (M1, M4) differed significantly (SNK, $P < 0.05$). Pupation height in culture vials is related to the distance a larva pupates from the food (Table 1). As one moves from M1 to M4, stocks derived from on-fruit to off-fruit pupation sites, the pupation height in vials increases.

Larval behaviour: pupation distance from the food in the three test procedures

The mean distance of the pupae from the food is positively correlated in each of the three test procedures: (1) vial containing medium, (2) dish with medium plug and (3) field assay (Table 1). Extreme lines from each of the laboratory (EE, WW), isofemale derived (B1B1, B15B15) and field stocks (M1, M4) showed that stocks with low pupal height in vials tended to pupate significantly (Student's *t*-test, $P < 0.05$) closer to the plug and the fruit in test procedures 2 and 3 respectively.

Larval behaviours: pupal microhabitat field assay I

Stocks with phenotypically short larval path lengths and low pupal heights pupated more on the fruit than those with long larval path lengths and high pupal heights (Table 2). This was true for all replicate comparisons between the laboratory stocks EE and WW and the field derived stocks M1 and M4. The M1 (collected from "on-fruit" pupal site) and M4 (collected from an "in the soil" pupal site) stocks showed pupal microhabitat preference behaviour in the field assay which was consistent with that expected from the type of microhabitat from which they were collected at the orchard site: larvae of the M1 stock had a greater tendency to pupate on the fruit, and had shorter path lengths and lower pupal heights than larvae of the M4 stock (Fig. 3). M1 tends to pupate more on the fruit than M4, even when the soil water content was varied (Table 3).

Table 2. Field assay: proportion of pupae found on fruit

Replicate	Stock	Path length and pupation distance phenotype	% On fruit
A. Lab stocks			
1	EE	Short (S)	72
	WW	Long (L)	51
2	EE	S	85
	WW	L	54
3	EE	S	66
	WW	L	33
B. Field stocks			
1	M1	S	71
	M4	L	48
2	M1	S	68
	M4	L	59
3	M1	S	82
	M4	L	60

(N = 100 larvae/stock/replicate).

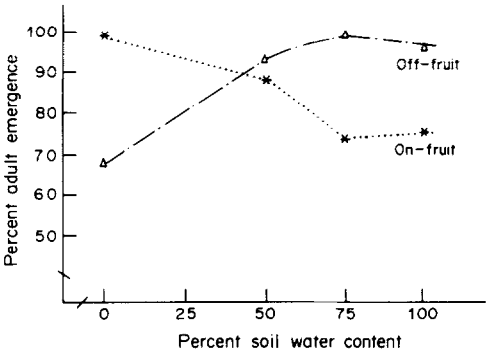

Fig. 4. Percentage adult emergence of pupae from "on and off" fruit microhabitats is plotted with percentage soil water content. In dry conditions (0%) it is advantageous to pupate in on-fruit microhabitats whereas in wet conditions (75%, 100%) off-fruit microhabitats are better (data from Sokolowski *et al.*, 1985).

Larval behaviours: pupal microhabitat field assay II—pupal survivorship

Varying the soild water content has two important effects relevant to habitat selection by the M1 and M4 stocks (Table 3). Firstly, increasing the soil water content causes a decrease in the proportion of larvae that pupate on the fruit. This is true for both stocks. The second is that M1 still tends to pupate more on the fruit than M4 in all soil water content conditions. More data is needed to investigate how the relative differences between the stocks are affected by varying the soil water content.

A third noticeable effect of varying the soil water content is on the relative suitability of the two microhabitats (on- or off-fruit) for pupation. Figure 4 illustrates the relationship between pupal microhabitat preference behaviour, the water content of the soil and the proportion of adults emerging from their pupal cases. At low soil water contents pupal survivorship is better on the fruit, whereas at high soil water contents, survivorship is better in the soil. There is a reversal in which microhabitat is a better site for pupation as the habitat changes from dry to wet.

DISCUSSION

A discussion of the adaptive significance of these differences in preadult behaviour will centre around the three questions presented in the introduction of this paper: (1) Is there phenotypic variability within a population? (2) Does this variation have a genetic component? and (3) Is there selection according to phenotype?

Table 3. Soil water content and the percentage of larvae pupating on fruit

Stock	Path length and pupation distance phenotype	Percentage soil water content	Percentage pupating on fruit
M1	S	0	89
M4	L	0	62
M1	S	50	27
M4	L	50	19
M1	S	75	45
M4	L	75	11
M1	S	100	40
M4	L	100	13

Larvae of *D. melanogaster* utilize a variety of pupation sites available to them in our orchard. *D. melanogaster* pupae were found on the upper and lower surfaces of the fruit, on the soil under the fruit, on the grass around the fruit, in the soil under the fruit, and on and in the soil at a distance from the fruit. In the present study, pupal microhabitats were categorized into "on-fruit" or "off-fruit" sites.

In answer to the first question, there was phenotypic variation for pupal microhabitat choice within the *D. melanogaster* population in the orchard. There was also phenotypic variability for larval path length and pupal height in the populations of flies derived from different pupal microhabitats (field stocks). In answer to the second question, the chromosomal contribution to differences in the two preadult behaviours was the same in laboratory and natural populations. Differences in path length are second chromosome based whereas differences in pupal height are primarily influenced by the second pair but also by the third pair of chromosomes. The phenotypic variation in pupal microhabitat preference is in large part due to underlying genetic variation in laboratory and natural populations. This conclusion follows from several results. Firstly, pupal microhabitat preference correlates well with path length and pupal height. Secondly, flies from different pupal microhabitats exhibited a preference for that microhabitat in the field assay even after 1 year of rearing them in the laboratory.

Are differences in path length and pupal height in the laboratory related to differences in behaviours in nature? It is easy to imagine that path length is related to the amount that a larva moves while foraging through the feeding substrate. It is not clear, however, how the pupal height of larvae in culture vials could be related to the prepupation behaviour of a larva in nature. The present study showed that pupation height in vials is related to the distance a larva pupates from the food (Table 1). The

four field derived stocks did not differ in larval photo- or geotaxic behaviour (Bauer, Wai-Ping, Sokolowski, unpublished). The correspondence between pupal microhabitat in the field (M1 and M4 collected from on fruit and in soil respectively) and pupation site choice in the field assay (Fig. 3) indicated the preference for pupating on or off the fruit in the field assay is related to where larvae actually do pupate in nature. The field assay is an ecologically realistic means of measuring pupal microhabitat choice. It lends ecological relevance to behaviour genetic studies in the laboratory and allows us to study the genetic architecture of traits involved in habitat selection in natural populations.

The rest of this discussion will address question 3—selection according to phenotype or how is genetic variation for larval path length and pupation site maintained in natural populations? Larvae with short path lengths and low pupal heights (EE from the lab; M1 from the field) pupated more on the fruit in the field assay than larvae with long path lengths and high pupal heights (WW from the lab: M4 from the field). This pattern was consistent in each of the three sets of replicates (Table 2). By varying an environmental parameter (soil water content) it was possible to show the potential for selection according to phenotype. Greater adult emergence of pupae on the fruit was found under dry conditions of 0% soil water content. Pupating on the fruit in a desiccating environment probably facilitates moisture retention. Under wet conditions of 100% soil water content, pupae off the fruit had greater adult emergence (Fig. 4). Adult emergence from the pupal case is a measure of the suitability (or fitness) of a larva's pupal microhabitat preference behaviour. If one makes the logical assumption that soil water content fluctuates in space and time in an orchard, then pupal microhabitat preference behaviour probably plays a role in maintaining genetic variation for these behaviours. In dry areas, larvae with short path lengths which tend to pupate close to and on the fruit would be favoured. In wet areas larvae with long path lengths which tend to pupate far from the fruit (on or in the soil) would be favoured. Why adult emergence from the pupal case is affected by soil water content is presently being investigated by measuring pupal dessication resistance in the M1 and M4 stocks. In the future, measures of larval path length and pupation behaviour of larvae from different wet/dry geographic regions will be investigated. We hypothesize that larvae from a desert region should have shorter larval path lengths and/or lower pupal heights than those from a more humid region.

Another influence on the genetic variation for larval behaviour in our orchard is the parasitoid *Asobara tabida* which finds its *D. melanogaster* larval host thorugh vibrotaxis (detecting movement). Sokolowski and Turlings (unpublished) have used larvae which differ in activity levels to demonstrate how larval behaviour can influence parasitoid encounter rates. A control test was done at 20°C in which both larval stocks (*shi*^{ts} and WW) offered to a parasitoid had equal activity levels. At the treatment temperature of 29°C the *shi*^{ts} stock becomes paralyzed whereas WW continues foraging. In the control test individual experienced parasitoids were allowed to probe larvae (15 *shi*^{ts} and 15 WW) in a patch. The total number of ovipositor probes contacting each type of larva was 186 and 189 respectively ($N = 9$ parasitoids). Alternatively, in the treatment test the parasitoid probed the moving larvae approx. 7 times more than the paralyzed ones (WW was probed 224 times; *shi*^{ts} was probed 33 times; $N = 9$ parasitoids). In our orchard, larvae with higher locomotory scores (*rovers*) may experience higher parasitization rates than do *sitters*.

The present study examined the relationship between larval behavioural phenotypes, genotypes and environment in habitat selection. Natural selection can only operate when there is phenotypic variation for a trait and a genetic basis for this variation. In the present study, a genetic basis for differences in larval behaviours involved in habitat selection was demonstrated. Differential fitness of behaviours in a varying environment leads us to the conclusion that pupal microhabitat choice behaviour is probably a trait which is acted on by natural selection. Studies of this type are necessary since they lend relevance to our speculations about the adaptive significance of behavioural traits.

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