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Ecological genetics and behaviour of *Drosophila melanogaster* larvae in nature

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Abstract. *Drosophila melanogaster* populations derived from pupae collected from four microhabitats (on and under fruit, on and in soil) differed in their proportion of rover and sitter larval forager morphs, a second chromosome based genetic polymorphism (Sokolowski 1980). 'On-fruit' populations had shorter foraging trails and lower pupal heights (sitters) than 'off-fruit' populations (rovers). In a field assay, sitters pupated significantly more on fruit than rovers. When soil water content was increased, the proportion of larvae pupating on fruit decreased. At 0% soil water content, on-fruit pupae (sitters) showed a higher percentage of adults emerging than off-fruit pupae (rovers). The reverse was true at 100% soil water content. The rover/sitter polymorphism may be maintained in an environment where the soil water content fluctuates. In this study, we demonstrate a direct relationship between laboratory and field phenotypes, thereby linking the ecology, behaviour and genetics of *D. melanogaster*.

Habitat choice depends on behavioural preferences as well as selection (Powell & Taylor 1979). A genetic basis for differences in behavioural preferences must be demonstrated before the role of natural selection can be implicated in habitat choice. Although the genetics of behavioural preferences are well known for *D. melanogaster* in the laboratory, little is understood about the ecological genetics of behavioural traits in natural populations of this species (Ehrman & Parsons 1981). A genetic basis for intraspecific variation in habitat selection has not yet been conclusively demonstrated for any species (Parsons 1983).

We have previously established a chromosomal basis for differences in larval locomotory (path length) and pre-pupation (pupal height) behaviour in laboratory stocks (Sokolowski 1980; Sokolowski & Hansell 1983). 'Path length' is defined as the length of the trail traversed by a larva in a moist yeasted dish during a test period (Sokolowski 1980; Sokolowski & Hansell 1983). We call larvae with long path lengths 'rovers', and those with short path lengths, 'sitters'. 'Pupal height' is defined as the distance from the surface of the food to a fixed point on the pupa (Sokolowski & Hansell 1983; Bauer 1984). Preliminary evidence indicates that path-length and pupal-height genes map to opposite arms of the second pair of chromosomes. Genetic variation for both these traits exists in three natural populations (Sokolowski 1982b; Bauer & Sokolowski 1984; this study). Differences in path length and pupal height are also second chromosome based in natural populations (Bauer & Sokolowski 1985).

Lewontin (1967) has given three conditions for an evolutionary response to natural selection: (1) there must be phenotypic variability within a population; (2) the variation must have a genetic component; and (3) there must be differential selection according to phenotype. In the present study we show that: (1) *Drosophila melanogaster* uses a variety of pupal microhabitats in nature; (2) there is a genetic component to differences in pupal microhabitat preferences; and (3) we provide preliminary evidence for selection according to phenotype.

The present report is divided into three parts. In part I we show that stocks originating from pupae collected from different microhabitats differ in our two measures of larval behaviour. The behaviour of genetically-characterized laboratory stocks is compared with the field stocks. In part II, a field assay measures the tendency of laboratory and field stocks to pupate on fruit. In part III, soil water content is varied to demonstrate the effect of pupal microhabitat variation on adult emergence.

PART I

Field Stocks

In the fall of 1983, we noticed that a variety of pupal microhabitats were used by a population of

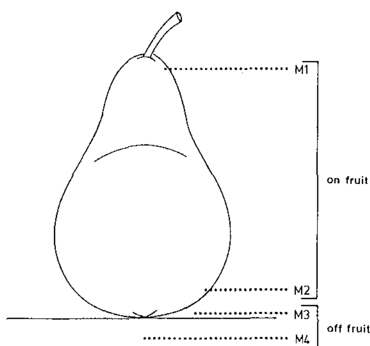

Figure 1. A schematic representation of the four microhabitats, M1–M4, from which *D. melanogaster* pupae were collected. M1 is on the upper surface of the fruit on the skin, M2 is on the lower surface of the fruit on the skin, M3 is under the fruit on the ground and M4 is under the fruit in the soil.

D. melanogaster in a pear orchard in the Toronto area. Pupae were found in four 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 that we derived from pupae collected from these sites were called M1, M2, M3 and M4, respectively (Fig. 1).

Laboratory Stocks

The four laboratory stocks, isogenic for the second and third chromosomes, were designated EE, EW, WE and WW. The first letter of the two-letter designation denotes the second pair of chromosomes whereas the second letter denotes the third pair. EW has the same second chromosome pair as EE and the same third chromosome pair as WW. A description of the technique used to derive these stocks can be found in Sokolowski (1980).

Larval Behaviour: Path Length and Pupal Height

The four field stocks (M1, M2, M3 and M4) and the four laboratory stocks (EE, EW, WE and WW) were tested for larval path length and pupal height. The field stocks were tested within 1 month of their collection. In order to test larval path length, culture dishes (8·5 cm in diameter and 1·4 cm high), which contained no less than 28 g of a standard dead yeast–agar medium, were seeded with 100 freshly hatched ($\pm 1{\cdot}75$ h) larvae of each of the laboratory and field stocks. The dishes were incubated under standard conditions of 24 ± 1°C, 60% RH with a 12 h L: 12 h D photoperiod, lights on at 0800 hours. When the larvae were 3·5 days post-hatching they were removed from the culture dish and lightly rinsed in distilled water. A random sample of larvae was tested. Path length was measured by placing a single larva in a culture dish containing an evenly spread yeast paste (prepared by combining 8 g Fleischmann's active dried yeast with 25 ml distilled water). A visible trail was left in the yeast after a 5-min test period and was measured to obtain 'path length'. Fifty trails were measured for each of the field-derived stocks, 25 trails for each of the laboratory stocks.

The following method was used to measure pupal height in each of the laboratory and field-derived stocks. Ten newly-hatched larvae ($\pm 1{\cdot}75$ h) were placed in each of 10 randomly chosen vials (2 cm in diameter and 11 cm in height) using a dissecting needle. Each contained 5 ml of a 2-day-old dead-yeast–agar medium. The vials were plugged with a standard size cotton ball and incubated until the larvae had pupated. The pupal height of each larva was measured as the distance from the surface of the food to the point between the spiracles on the pupa.

Results indicated significant between-microhabitat variation for path length (ANOVA: $F = 16{\cdot}85$; $df = 3{,}200$; $P < 0{\cdot}0001$) and pupal height (ANOVA: $F = 25{\cdot}18$; $df = 3{,}305$; $P < 0{\cdot}001$) in the field-derived stocks. A simple relationship between field pupal microhabitat and the two laboratory measures of larval behaviour is shown in Fig. 2a. Populations of flies derived from on-fruit pupae (M1,M2) had significantly shorter path lengths (Student Newman–Keuls test (SNK): $P < 0{\cdot}05$) than those found on or in the soil (M3, M4). Pupal height showed a similar pattern although only the two extreme populations (M1, M4) differed significantly (SNK: $P < 0{\cdot}05$). As one moves from M1 to M4 (stocks derived from on-fruit to off-fruit pupation sites) the pupal height in vials increases. Pupal height in culture vials appears to be related to the distance a larva pupates from the food. Figure 2b shows the results for the laboratory stocks in order of second and third chromosome constitution. The laboratory stocks EE and EW, which share an E pair of second chromosomes, had significantly lower path-length and pupal-height scores than did the WE and WW stocks, which share a W pair of second chromosomes (Mann–Whitney U-test, $P < 0{\cdot}05$). We have called larvae with longer path lengths

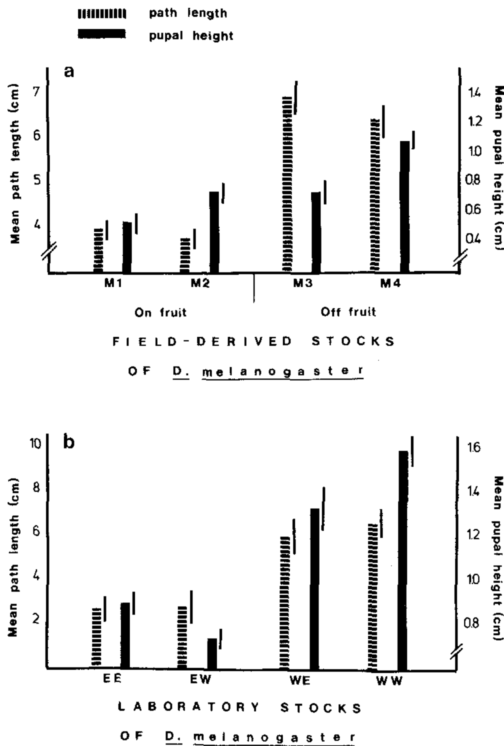

Figure 2. Mean larval path length and pupal height for each of the four laboratory and field-derived populations of *D. melanogaster*. Vertical lines represent SE. (a) Flies derived from 'on-fruit' pupal microhabitats had shorter path lengths and higher pupal heights than flies derived from 'off-fruit' pupal microhabitats. Populations of flies derived from on-fruit microhabitats show the sitter larval phenotype while flies derived from off-fruit microhabitats show the rover larval phenotype. (b) In the laboratory stocks, larvae sharing an E pair of second chromosomes (EE and EW) show the sitter larval phenotype. Larvae which share a W pair of second chromosomes (WE and WW) show the rover larval phenotype.

'rovers' and those with relatively shorter path lengths 'sitters'. Reciprocal crosses have indicated that the rover phenotype is completely dominant over the sitter and that no sex-linked or maternal effects were evident (Sokolowski, in press).

We wanted to determine (1) whether our laboratory sitter stock (EE) pupated more on the fruit than our laboratory rover stock (WW) and (2) whether our field sitter stock (M1) pupated more on the fruit than the field rover stock (M4), even after 23 generations of rearing them in the laboratory. A field assay was devised to answer these questions.

PART II

The field assay was developed to simulate field conditions in the laboratory. The field assay for pupation site preference was prepared by filling dishes (4·8 cm high and 8·5 cm in diameter) to a depth of 1 cm with dried, sifted, sterile soil. Thirty-two pieces (2·5 cm long) of wheat seedlings were placed at random on the surface of the soil, which was kept at approximately 50% RH by using a sodium bisulphate solution. Purple grapes, with seeds, were washed with alcohol and rinsed with distilled water. The grapes were sliced in half lengthwise and the seeds were removed. Each half was seeded with 1 ml yeast paste and incubated at 24 ± 1°C for 24 h. Aged larvae of the EE, WW, M1 and M4 stocks were raised under standard culture conditions as described above. One half grape was seeded with 10 larvae (4 days post-hatching) and placed in a dish with soil and grass, covered with a lid and incubated under standard conditions. Ten replicate dishes were used per stock (100 larvae/stock). The experiment was repeated three times (300 larvae/stock) from March to July, 1984. After pupation, the number of pupae found on the fruit and on or in the soil, was counted. Survivorship to pupation was high (97%) in all replicates.

Table I shows the proportion of larvae pupating

Table I. Field assay: proportion of *D. melanogaster* pupae found on fruit*

Replicate	Stock	Phenotype	% On fruit
Laboratory stock			
1	EE	S	72
	WW	R	51
2	EE	S	85
	WW	R	54
3	EE	S	66
	WW	R	33
Field stock			
1	M1	S	71
	M4	R	48
2	M1	S	68
	M4	R	59
3	M1	S	82
	M4	R	60

* $N = 100$ larvae/stock/replicate.
S: sitter; R: rover.

on fruit in our field assay. In all replicates, populations with the sitter (S) short-path-length phenotype (from laboratory: EE; from field: M1, on-fruit), show significantly higher proportions of larvae pupating on the fruit than populations with the rover (R) long-path-length phenotype (from laboratory: WW, from field: M4, in soil).

PART III

The field assay described above was also used to study the tendency of larvae to pupate on fruit when soil water content was varied. In this series of field assays, pupation behaviour was measured in two field stocks, M1 and M4, under conditions of 0, 50, 75 and 100% soil water content. Soil water content was calculated and defined using the formula:

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

Ten field assays (10 larvae/assay) were prepared for each of the two stocks under each of the four soil-water-content conditions. Both the proportion of pupae found on the fruit and the percentage of adult emergence were measured and differences were tested with a Mann–Whitney *U*-test.

The effect on pupation behaviour of varying soil water content in the on-fruit (M1) and off-fruit (M4) stocks is presented in Table II. Two important observations can be drawn from this table. The first is that the sitter stock, M1, tends to pupate more on the fruit than the rover stock, M4. This is true for all soil water contents. The second observation is that, as soil water content increases, the number of larvae pupating on the fruit decreases.

Table II. Soil water content and percentage of *D. melanogaster* larvae pupating on fruit*

Strain	Phenotype	% Soil water content	% Pupating on fruit
M1	S	0	89
M4	R	0	62
M1	S	50	27
M4	R	50	19
M1	S	75	45
M4	R	75	11
M1	S	100	40
M4	R	100	13

* $N = 100$ larvae/stock/soil water condition.
S: sitter; R: rover.

Table III. Percentage adult emergence in *D. melanogaster*

% Soil water content	On-fruit pupae	Off-fruit (soil) pupae
0	99	69
50	89	94
75	75	100
100	77	97

In Table III we see that there is a relationship between pupation behaviour, soil water content and adult emergence. The adult emergence of pupae on the fruit is highest at 0% soil water content. In contrast, the adult emergence of pupae off the fruit is highest at 100% soil water content.

DISCUSSION

Different pupation sites are available to *D. melanogaster* in the field. Larvae of *D. melanogaster* use these different microhabitats for pupation. Populations of flies derived from pupae collected from different pupal microhabitats differ in laboratory measures of larval behaviour, path length and pupal height. These laboratory measures of larval behaviour have been shown to have a genetic basis in both laboratory and field-derived stocks (Sokolowski 1980; Bauer & Sokolowski 1984). Path length and pupal height correlate well with field pupation site. We believe that flies from different pupal microhabitats differ at the genetic level in their habitat choice.

The relations reported in part I allowed us to make two sets of predictions about larval behaviour in the field assay presented in parts II and III. Populations chosen a priori from the laboratory to represent particular locomotory phenotypes should express particular field pupal microhabitat phenotypes. Indeed, the laboratory EE sitter stock with short path lengths and low pupal heights had significantly more larvae pupating on the fruit than the WW rover stock, which has longer path lengths and higher pupal heights. Populations chosen a priori to represent particular pupal microhabitats in the field, for example M1 and M4, should

continue to express these differences after many generations in the laboratory. To date, after one year in the laboratory, the M1 stock (from pupae found on fruit) still pupates significantly more often on the fruit than the M4 stock (from pupae found in soil).

These results show that our field assay is related to larval pupation sites in nature. The assay presented here is a more ecologically realistic means of measuring pupation preferences than pupal height in culture vials. The present study lends ecological relevance to our measures of larval behaviour in the laboratory. Pupal height is related to the distance a larva pupates from the food, not to differences in larval photo- or geotaxic behaviour (Bauer, Wai-Ping & Sokolowski, unpublished data).

We now know of a suite of correlated larval behavioural traits from the first instar to prepupation behaviour. First and second instar larvae express the rover/sitter phenotypes previously shown in the third instar (Sokolowski, unpublished data). This trait is strongly influenced by the second pair of chromosomes in both laboratory and field-collected populations and seems to be a common feature of natural populations. The foraging path length of rovers and sitters measures the horizontal component of larval foraging behaviour. Downward digging into the medium measures another component of larval foraging behaviour which also has a strong second chromosome basis (Sokolowski 1982a). During the third instar, larvae shift from food-related foraging activities to wandering. Wandering is hypothesized to be a pre-pupation site choice behaviour. During the wandering phase, larval behaviour is strongly affected by the degree of moisture in the feeding substrate (Sokolowski et al. 1984).

The present study deals with microhabitat variation through genetically-based behavioural preferences. We can now begin to assess the role of natural selection in habitat choice because we have demonstrated a genetic basis for differences in behavioural preferences. Intraspecific variation in pupal microhabitat choice is related to variation in pre-adult behaviour which is known to be under the control of the second pair of autosomes.

Reviewing Lewontin's three conditions for an evolutionary response to natural selection we see that (1) there is phenotypic variability within a population of *D. melanogaster* for field pupal microhabitats, larval path length and pupal height; (2) this variation has a genetic component; and (3) there is evidence for selection according to phenotype.

How is the rover/sitter polymorphism maintained? When soil water content is low, larvae that pupate on fruit have significantly higher numbers of adults emerging than those pupating on or in soil. In contrast, when soil water content is high, larvae pupating on or in soil are more fit than those pupating on fruit. In nature, soil water content fluctuates in space and time. One could imagine that when the soil is dry the sitter phenotype (pupating on fruit) would confer an advantage. In contrast, when soil is wet the rover phenotype (pupating off fruit) would be advantageous. Habitat selection occurs in the orchard environment which is heterogeneous in time and space. Genotypes choose microhabitats in which they have the greatest fitness. The rover/sitter behaviour genetic polymorphism which we find in nature may be maintained through this environmental heterogeneity. At present we are investigating why soil water content affects adult emergence by measuring pupal desiccation resistance in these stocks. In addition, we plan to test the prediction that the proportion of sitters should be higher in dryer areas.

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