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Larval foraging behavior in isofemale lines of *Drosophila melanogaster* and *D. pseudoobscura*

ABSTRACT: Isofemale lines of *Drosophila*, recently sampled from nature, were used to study the within- and between-line phenotypic variability of a preadult behavior. The locomotory component of larval foraging behavior was quantified by measuring the length of the path traversed by a foraging 3rd instar larva on a yeasted dish. Significant between-line variation for this behavior was found in *Drosophila melanogaster* and in *D. pseudoobscura*. Matings between lines with extreme phenotypes indicated a relatively simple genetic basis for differences in larval foraging behavior.

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IN *Drosophila*, the study of the genetic and environmental determinants of behavior has been primarily focused on adults. There has been little work done on larval behavior despite its possible importance in the life-history of the organism[21].

It is of considerable interest to determine the role of larval behavior in habitat selection[2-7,9,11-13,15-24]. The larval period of *Drosophila* is one of maximum resource utilization. Larvae spend most of their time feeding on the substrate. Larval foraging behavior involves both feeding and locomotory behaviors. Larval foraging behavior is of interest since it is an important component of fitness on which pupation and adult emergence is dependent[11].

Feeding and locomotory behavior in four strains of *D. melanogaster* larvae were investigated by Sokolowski[16]. Both feeding behavior ("shovelling") and locomotor behavior ("crawling") were tested by placing a larva on a petri dish covered with a thin layer of aqueous yeast suspension. The amount of shovelling was measured by counting the number of probes with the larval mouth hooks. The number of waves of muscular contraction passing along the body of the larva (forward and reverse movements) was used to measure the amount of crawling behavior. The larva left a visible trail in the yeast suspension while foraging and this was used as another measure of locomotory behavior. This foraging trail was measured and was called path length. The laboratory strains of flies tested by Sokolowski[16] exhibited two larval forager types: rovers (which had long path lengths and traversed large areas while feeding) and sitters (which had short path lengths and covered smaller areas while feeding). The differences in these two behavioral phenotypes were analyzed genetically using chromosomal substitutions between isogenic stocks. The rover larval phenotype was found to be dominant over the sitter and the differences in their behavior could be attributed to the second pair of chromosomes. Differences in feeding rate were found to be affected additively by the second and third chromosomes.

In a study of the larval foraging behavior of two sibling species, Sokolowski and Hansell[22] reported that *D. melanogaster* larvae showed greater intrastrain differences in mean crawling and shovelling scores than *D. simulans*. *D. simulans*, on the other hand, showed greater intrastrain differences between mean larval path length and area traversed than *D. melanogaster*. In both species, unidirectional selection for another measure of larval foraging behavior (the tendency to move towards and remain feeding on food) was successful within six generations[24]. This rapid selection indicates that this component of larval foraging behavior is under relatively simple genetic control in both of the sibling species, *D. melanogaster* and *D. simulans*.

The above studies have primarily been concerned with laboratory populations of

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Drosophila. It is of interest to determine whether the results of these studies utilizing laboratory stocks can be generalized to natural populations of *Drosophila*. The isofemale line technique can be used to study *Drosophila* larval behavior in natural populations[11-13]. Parsons[14] gives an extensive discussion of the isofemale line technique. Isofemale lines are strains that are initiated by single inseminated females from nature. Using the isofemale line technique, the amount of within- and between-line phenotypic variability can be determined for the behaviors studied. Variation between strains was found to persist over a number of generations.

In this study, the isofemale line technique was used to investigate the locomotory components of larval foraging behavior in natural populations of *Drosophila*. Crosses were made between isofemale lines with extreme phenotypes to determine whether between-line variability had a genetic basis. Two species, *D. melanogaster* and *D. pseudoobscura*, were tested to determine if there were inter- and intraspecies differences in larval foraging behavior.

Materials and Methods

The following *D. pseudoobscura* isofemale lines were used: Bryce-9F, -10F, -13F, and -21F (all caught in Bryce Canyon in the United States), BC (caught in British Columbia) and Zir-4 (caught in Mexico). These stocks were kindly supplied by Wyatt Anderson at the University of Georgia.

In the fall of 1982, flies were sampled from a natural population that consisted entirely of the species *D. melanogaster*. Fifteen single inseminated females were collected to inititate isofemale lines during September 1982. Each of the females produced viable progeny and was removed from her vial prior to the emergence of her offspring. Each line was then propagated by mass transfer. All stocks were maintained at 22 ± 1°C under standard culturing conditions. Fifteen isofemale lines of *D. melanogaster* were used in the present study. They will be referred to as B-1, B-2, B-3, . . . B-15.

The test procedure for larval foraging behavior was modified from Sokolowski[16]. One-hundred newly hatched (± 1¾ hours old) larvae were placed in a plastic petri dish (100 mm in diameter and 15 mm in height) containing at least 28 g of a dead yeast-agar medium. The dish was incubated until the larvae had reached 3rd instar (4 days post hatching for *D. melanogaster* and 6 days for *D. pseudoobscura* at 24 ± 1°C). When larvae reached the appropriate age for testing, they were removed from the culture dish by carefully separating the medium with a paint brush and picking out all the larvae that were found. A random sample of 50 larvae was then chosen. About 1 g of a yeast paste (8 g Fleischmann's active dried yeast in 25 ml of water) was spread evenly on the surface of a petri dish (100 mm in diameter and 15 mm in height). A larva was then placed, using a paint brush, in the center of the petri dish for a period of 5 minutes. A visible trail was left by the larva in the yeast. The length of the trail represented the locomotory component of foraging behavior. The trail was traced onto a sheet of paper marked with a circular (diameter = 10 cm) grid divided into cm squares. The length of the trail was measured using a Numonics Corp. digitizer and was called path length. The number of squares traversed and the number of lines crossed were counted by the use of the grid. Fifty trails for each of the 7 *D. pseudoobscura* isofemale lines and 50 trails for each of the 15 *D. melanogaster* isofemale lines were quantified in this manner. The isofemale lines were tested in a random order. The *D. melanogaster* lines were tested within 4 months of sampling the females from nature. The *D. pseudoobscura* lines were tested within 1.5 years of establishment in the laboratory.

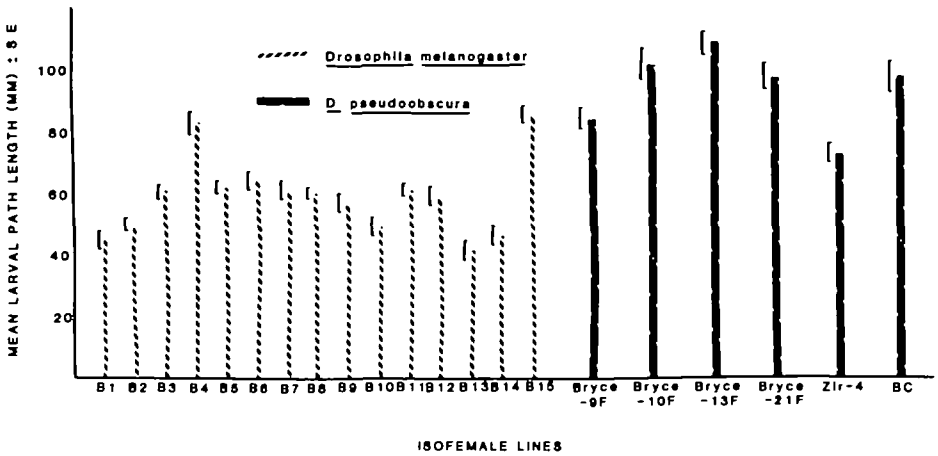

FIGURE 1 The mean larval path length (mm) ± SE for each of the isofemale lines tested for *D. melanogaster* (B-1, B-2 . . . B-15) and *D. pseudoobscura*.

Results

Path length, number of squares traversed, and number of lines crossed were found to be strongly correlated ($r > 0.90$) with each other in both *D. melanogaster* and *D. pseudoobscura*. Since there was such a strong positive correlation between the three foraging measures quantified, only path length will be used for further presentation of the results.

Figure 1 shows the mean larval path length (mm) ± SE for each of the isofemale lines tested. *D. melanogaster* is represented by dashed bars and *D. pseudoobscura* by dark bars. The following interesting observations can be drawn from Figure 1. Variability exists in the two species for larval path length. *D. pseudoobscura* tends to have longer path lengths than *D. melanogaster*. Two lines showing "extreme" behavioral phenotypes were identified in *D. melanogaster*; B-15 (high line) showed relatively long path lengths, while B-1 (low line) showed relatively short path lengths. These lines were used for genetic crosses.

An analysis of variance for path length by isofemale line showed significant between-line variation (Table I) for *D. melanogaster* and *D. pseudoobscura*. A random sample of larval

Table I. Analysis of variance of the path lengths of isofemale lines for *D. melanogaster* and *D. pseudoobscura*

Species	Source of variation	*df*	Mean squares	*F*	*P*
D. melanogaster	among lines	14	81.30	18.73	0.0001
	within lines	735	4.34		
D. pseudoobscura	among lines	6	129.14	9.53	0.0001
	within lines	368	13.54		

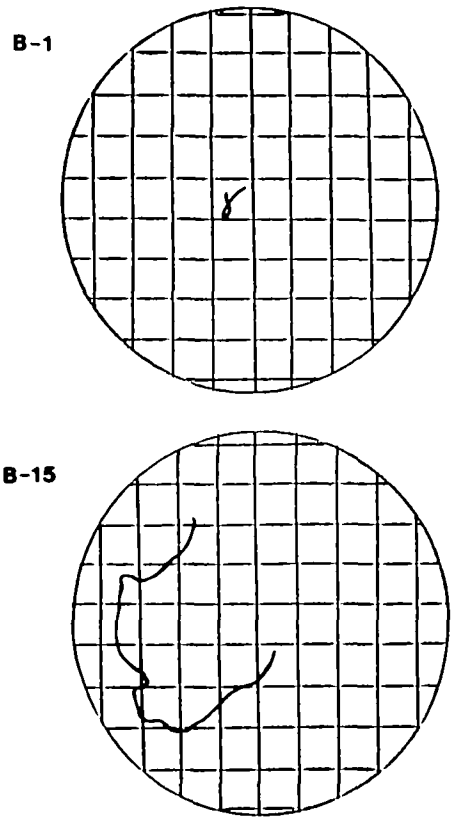

FIGURE 2 A sample of larval trails of B-1 and B-15 *D. melanogaster* isofemale lines superimposed on a centimeter grid. These lines differed in larval path length, number of squares traversed, and number of lines crossed.

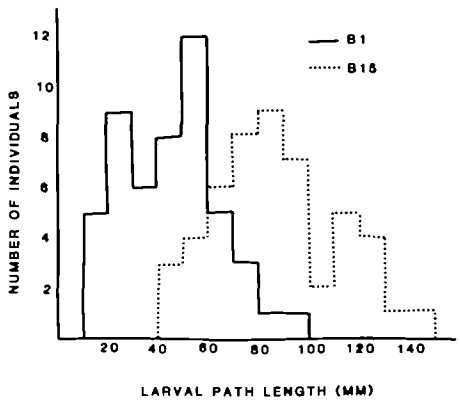

FIGURE 3 The frequency distributions of *D. melanogaster* larval path lengths for B-1 (low line) and B-15 (high line). The distributions are not from the same population, but there is some overlap.

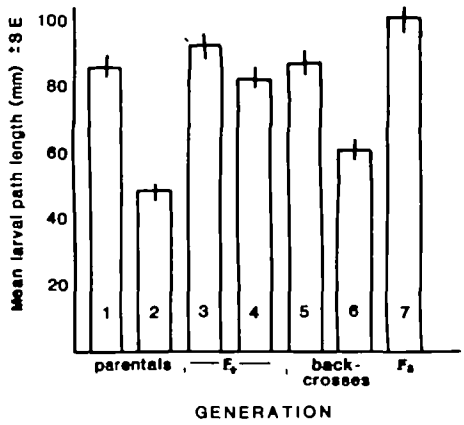

FIGURE 4 The mean (mm) ± SE path length scores for the *D. melanogaster* matings between B-15 (high line) and B-1 (low line). $N = 50$ for each bar in the histogram. Parentals: bar 1 and 2—B-15 (high line) and B-1 (low line). Reciprocal crosses to give F_1 generation: bar 3 and 4—B-15♀ × B-1♂; B-1♀ × B-15♂. Backcrosses: bar 5 and 6—F_1♂ from bar 3 above × B-15♀; F_1♂ from bar 3 above × B-1♀. F_2: bar 7—F_1 × F_1 from bar 4 above. The terms parentals, F_1, F_2, and backcrosses are not used in the Mendelian sense since parental lines are not homozygous.

foraging paths from each of the B-1 and B-15 *D. melanogaster* isofemale lines is drawn in Figure 2. B-1 was found to have a significantly ($Z = 7.02$, $P < 0.05$, Mann-Whitney U test) shorter path length than B-15. Frequency distributions were constructed enabling an examination of the variance about the mean (Figure 3). The number of individuals in each 10 mm category (out of a total of 50 animals tested per line) was plotted for B-1 (low line) and B-15 (high line). Since the sample sizes were the same, the area under the curves were equal and the two distributions could be compared. A criterion of 60 mm can be employed to discriminate between the two populations. Using a 60 mm separation point between B-1 and B-15, one could categorize B-1 larvae with 80 percent and B-15 with 86 percent accuracy.

The mean (mm) ± SE of the path length scores for the matings between B-1 (low line) and B-15 (high line) are shown in Figure 4. Fifty individuals were tested for each bar in Figure 4. The terms parentals, F_1, F_2, and backcrosses are not used in the Mendelian sense since isofemale "parental" lines are not necessarily homozygous. Bars 1 and 2 show the mean ± SE path length scores for each of the parental lines (B-15 and B-1, respectively). Larval path lengths from both reciprocal crosses (F_1 generation, bars 3 and 4) were not significantly different from each other ($Z = 1.83$, $P > 0.05$) and not significantly different from the B-15 parental. There were neither sex linkage nor maternal effects. Males and females from the F_1 generation all showed the B-15 (high line) behavioral phenotype. The results of the backcrosses (bars 5 and 6) are of greater interest. The results of the backcross (bar 5) to the high line (B-15) or dominant phenotype do not differ significantly from the B-15 parent. However, the results of the backcross (bar 6) to the low line (B-1) or recessive phenotype show an intermediate phenotype. The results of the F_2 cross (bar 7) was not significantly different from the B-15 parent.

In Table II, rather than presenting the mean ± SE path length results from the crosses, the ratio of parental types, using a discriminant criteria of 60 mm (see Figure 3), is presented. In particular, the number of larvae falling into the B-15 parental type category as compared to the B-1 category is given. For example, out of 50 progeny of the F_1 backcross to B-1 (low line), 23 larvae fell into the B-15 parental path

Table II. Categorization of path lengths in crosses between 'extreme' lines of *D. melanogaster*

	Mating	Phenotype (path length)	Forager type	Observed no. larvae out of 50 of each forager type (rover:sitter)	Observed ratio	Expected Mendelian ratio*
Parentals						
	B-15	long	rover	43:7	0.9:0.1	1:0
	B-1	short	sitter	10:40	0.2:0.8	0:1
Reciprocal crosses						
	B-15♀ × B-1♂	long	rover	44:6	0.9:0.1	1:0
	B-1♀ × B-15♂	long	rover	46:4	0.9:0.1	1:0
Backcrosses						
	F_1 × B-15	long	rover	40:10	0.8:0.2	1:0
	F_1 × B-1	medium	½ rover + ½ sitter	23:27	0.5:0.5	1:1
F_2						
	F_1 × F_1	long	rover	47:3	0.9:0.1	3:1

* These ratios are derived by assuming a one-gene, two-allele and complete dominance model of inheritance

length category and 27 into the B-1 category. A ratio of 23:27 is not significantly different from 1:1. A 1:1 ratio is expected if the genes for path length in these lines are being inherited as a unit. Moving from left to right in Table II, each of the crosses are presented, the phenotypic categorization of the progeny of the crosses, long path length (B-15 parental type or rover larvae) or short path length (B-1 parental type or sitter larvae), the actual number of larvae out of 50 observed in each parental class, the ratio and the expected Mendelian ratio given a one-gene, 2-allele model with complete dominance. The results of the crosses outlined in Table II support this model with the exception of the F_2 cross.

Discussion

The isofemale lines of two species, *Drosophila melanogaster* and *D. pseudoobscura*, established in the laboratory from natural populations were found to have significant between-line phenotypic differences for the locomotory component of larval foraging behavior (path length). These differences were found to have a genetic component.

In *D. melanogaster*, two "extreme" lines for larval foraging behavior were found. The criterion employed to distinguish between larvae of these lines (a path length of 60 mm) could separate them with 80–86 percent accuracy. This is in agreement with the accuracy of separation used to distinguish the laboratory stocks described by Sokolowski[16]. The *D. melanogaster* isofemale lines in the present study showed a similar pattern of genetic control for path lengths to that found by Sokolowski[16] in laboratory stocks. It is of interest that the genetic control of foraging behavior was similar in flies from laboratory stocks and flies from a natural population. Sokolowski et al.[24] successfully selected for decreased foraging path length in the sibling species *D. melanogaster* and *D. simulans*. Selection for increased or decreased path length in the B-15 (high line) and B-1 (low linè) proved unsuccessful (unpub. data). This may have indicated that genetic variation for path length within these two isofemale lines was minimal. A chromosomal analysis of the B-1 and B-15 lines will elucidate the chromosomal contributions for path length in the isofemale lines. Further crosses using the compound autosome technique[8] will be done to locate the regions of the chromosomes that influence path length after which further genetic analyses will be done to determine whether the resulting phenotypic ratios are closer to Mendelian expectations.

D. pseudoobscura generally had longer foraging path lengths than *D. melanogaster*. This may be related to developmental-time differences between the two species. It would be necessary to measure changes in path length during development to be able to establish a complete behavioral profile of the locomotory component of foraging behavior in these two species. *D. pseudoobscura* had significant between-line variation for path length. This may or may not be related to the isofemale lines originating from different geographic locations. To assess the significance of geographic origin, a number of lines, caught at the same time in the same place, must be tested.

Markow[10] found that *D. pseudoobscura* tended to pupate closer to the medium than did *D. melanogaster*. The differences in foraging behavior found in the present study between *D. melanogaster* and *D. pseudoobscura* illustrate another trait in which the two species diverge. The *D. pseudoobscura* stocks used in this study had been maintained in the laboratory for a longer period of time than *D. melanogaster*. One would expect a decrease in larval locomotion with increasing time spent in the laboratory due to inbreeding. Therefore, *D. pseudoobscura* may even have longer path lengths when tested within six months of sampling.

Why should genetic variability for larval foraging behavior exist in nature? The observed differences between isofemale lines within a species and between species may be related to the larval environment. Food acquisition depends on the kind of foraging behaviors and the distribution of food[16]. Sokolowski[16] hypothesized that a discontinuous food supply may give an advantage to larvae with longer path lengths whereas larvae with shorter path lengths may be at an advantage in a continuous food supply. In terms of energy budgets, a long path length larva may waste energy in locomotor activity in a continuous food environment. Another hypothesis that may help elucidate why genetic variability for path length is maintained in nature may be related to *Drosophila* larval parasitoids. Alphen[1] demonstrated that the frequency at which a larva gets parasitized is related to larval movement. In future studies we plan to investigate whether the proportion of rover and sitter larval foragers in a natural population is related to the search images used by *Drosophila* larval parasitoids.

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