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Drosophila Larval Foraging Behavior. II. Selection in the Sibling Species, *D. melanogaster* and *D. simulans*

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A laboratory study is presented which shows that larval foraging behavior in the sibling species Drosophila melanogaster *and* D. simulans *can respond rapidly (in six generations) to unidirectional selection. An apparatus was designed which selected for larvae which moved from nonnutritive agar medium to plugs of nutritive medium and remained feeding there. Larvae of the selected lines showed a correlated decrease in foraging path length which mirrored the* sitter *larval forager behavior type previously defined by Sokolowski [(1980).* Behav. Genet. **10**:*291–302]. This supported the hypothesis that sitter larvae moved toward, and remained feeding on, a food source when they were not already utilizing one, whereas* rover *larvae foraged from food patch to food patch.*

KEY WORDS: foraging behavior; larval; selection; *Drosophila melanogaster*; *D. simulans*.

INTRODUCTION

Sokolowski (1980) identified a behavioral polymorphism, *rover* versus *sitter*, in *Drosophila melanogaster* larval foraging traits. Rover larvae had long path lengths, whereas sitter larvae had significantly shorter ones, while foraging on a yeast-covered petri dish. Genetic analysis using chro-

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mosomal manipulations of isogenic stocks showed that differences in these forager types could be attributed to genes on the second pair of chromosomes. No sex-linked differences were found in these behaviors, and a hypothesis of overdominance was supported, the rover strategy being dominant over the sitter strategy. Since the path lengths of larval trails in yeasted petri dishes were highly correlated (+0.8) with the crawling behavior (the original behavior used to identify these differences), path length measurements were used to determine the behavioral phenotype rapidly. Rover larvae had significantly longer paths than did sitter larvae. A separating criterion of 35–40 mm in path length separated rovers from sitters, with a 20% overlap in the stocks used.

In the preceding paper (Sokolowski and Hansell, 1983), a study of the phenotypic variability of foraging patterns in *D. melanogaster* and *D. simulans* is presented. *D. melanogaster* was found to be more variable than *D. simulans* in some measures of larval foraging behavior, whereas the reverse was true for other measures of foraging behavior. It was of interest to determine whether this intraspecific variability in mean foraging scores reflected genetic components of foraging behavior in these species. If larval foraging behavior in these strains of *D. melanogaster* and *D. simulans* can be made to respond to a selection regime, this would support the hypothesis that there is a genetic component to this behavior.

Foraging behavior reflects the relative amounts of feeding (shoveling) and locomotor (crawling) behavior performed. The effectiveness of foraging behavior would be expected to vary with the distribution of food. When food is distributed discontinuously, animals that are genetically predisposed to move toward food would be favored. However, when food is evenly distributed, foragers that wander would waste energy in locomotion. Variation in foraging patterns may be an important factor in determining success in exploiting food resources and, hence, competitive ability (Bakker, 1961, 1969).

In this paper, an experiment involving selection for larval foraging behavior on a discontinuous food supply is reported. In particular, it is determined that larval foraging strategies in these species are responsive to rapid selection.

METHODS

The *D. melanogaster* and *D. simulans* stocks used in these experiments have been cultured by mass mating in the laboratory for approximately 15 years. The stocks of rovers and sitters which were used by Sokolowski (1980) are poor subjects for selection. A heterogeneous population of each of the *D. melanogaster* and *D. simulans* species, with a

mixed percentage of foragers, was required. These stocks are described by Sokolowski and Hansell (1983).

An apparatus to select for a specific larval forager type was designed in the following manner: a large petri dish (13.5 cm in diameter and 2.2 cm high) was filled to a depth of 0.5 cm with hot liquid Difco agar (prepared by combining 2 g of agar with 100 ml of water and then autoclaving the solution for 5 min). The petri dish was then refrigerated and the agar was allowed to set overnight. The next day, three plugs of brewer's yeast–agar medium (4.5 cm in diameter and 0.5 cm high) were placed on the agar layer in the positions diagrammed in Fig. 1. The apparatus was then allowed 1 h to reach room temperature.

Ten pairs of flies (5–10 days old) were allowed to mate and lay eggs on a large petri dish (13.5 cm in diameter and 2.2 cm high) which was filled to a depth of 0.5 cm with brewer's yeast–agar medium. Eggs were laid for a period of 72 h in order to facilitate the collection of a large number of larvae for testing and selection. Eggs were laid at 22 ± 1°C and under constant light.

When most of the larvae were in their early third instar (approxi-

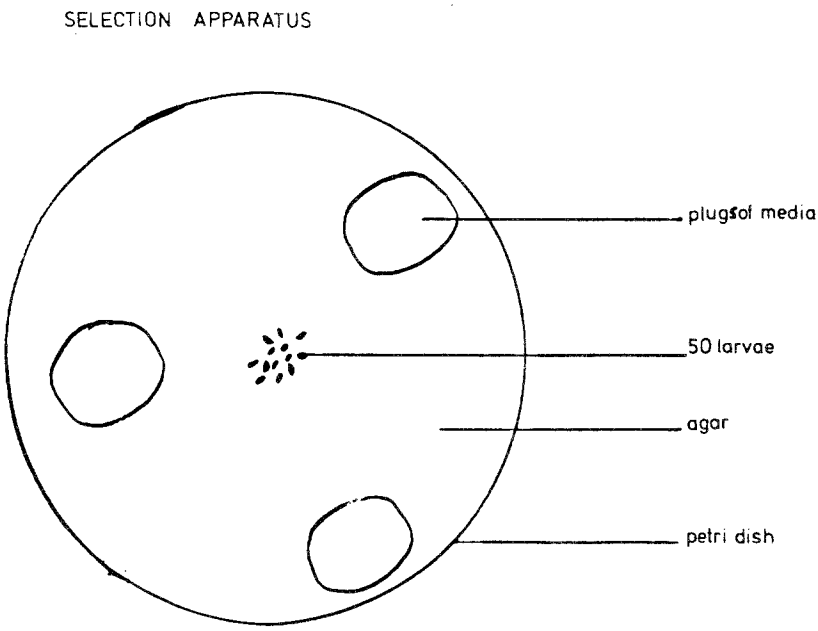

Fig. 1. A selection apparatus designed to select for larval foraging behavior, constructed of a petri dish (13.5 cm in diameter) which was coated with nonnutritive agar and three nutritive plugs of medium.

mately 96 h from the placement of the flies in the "grow" dish), the medium was carefully dissected with a small paintbrush. All the larvae were removed and washed in distilled water. One hundred early third-instar larvae of either *D. melanogaster* or *D. simulans* were sampled at random. Fifty larvae were tested in a yeast-covered petri dish as follows: a petri dish (8.5 cm in diameter and 1.4 cm high) was covered with a thin layer of an aqueous yeast suspension (8 g of Fleischmann's fast-rising active dry yeast in 25 ml of distilled water). It was necessary for the yeast to be thin and pasty so that a moving larva would leave a visible trail. Each larva was tested separately. A paintbrush was used to transfer one larva to the test dish. The animal was allowed to forage in the dish for a 5-min period. After the test period, a copy of the foraging trail was drawn onto a data sheet marked with a centimeter grid. The length of the trail was measured by superimposing a string, 2 mm in diameter, over the trail and then measuring the length of the string. This was repeated at each generation of selection, for each larva (50 larvae/replica, 6 replica/species).

The other 50 larvae of the original 100 were then placed in the center of the selection apparatus (see Fig. 1), and the selection apparatus was covered with a petri dish lid. After 20 min the three plugs of medium, and the larvae that had migrated and remained on these plugs, were lifted and placed into a single large culture bottle (a standard 180 ml capacity urine specimen bottle, 12 cm high and 5 cm in diameter at the base) which contained 60 ml of medium. These culture bottles were incubated at 22 $\pm$ 1°C until the progeny emerged. The larvae that remained within a 2-cm radius of the center of the selection apparatus were gently lifted with a paintbrush, counted, and placed in a medium-supplemented small vial (9.5 cm high and 1.8 cm in diameter), which contained 5 ml of medium. Larvae that were neither on the medium plugs nor in the center of the vial were counted and called "unclassifiable"; they were discarded.

When the progeny emerged from the culture bottles they were counted and sexed. On the appropriate day (schedule for replicates described below), all of the emerging flies were allowed to lay eggs on the "grow" dish and the cycle was then repeated. If the total number of flies was less than 10, a smaller grow dish (8.5 cm in diameter and 1.4 cm high) was used. The selection program continued for five or six generations. In lines ML(2), ML(6), and SL(6) selection had to be discontinued before the sixth generation because less than 100 third-instar larvae were found in the grow dishes of these lines.

Six replicate lines, from *D. melanogaster* and *D. simulans*, were used. They were labeled ML(1) to ML(6) and SL(1) to SL(6), respectively. ML(1) to ML(3) and SL(1) to SL(3) were examined on week 1, and ML(4)

to ML(6) and SL(4) to SL(6) were examined on week 2. The pattern for examining the stocks was as follows: ML(1) and SL(1) on day 1 of week 1, ML(2) and SL(2) on day 2 of week 1, ML(3) and SL(3) on day 3 of week 1, ML(4) on day 1 of week 2, etc.

In addition, before and after the period of selection, 100 larvae from each original stock culture were tested in the selection apparatus and the yeasted petri dishes. The behavioral scores of these larvae were used as the controls for the selected lines. This ensured that the changes seen in the selected lines were not due simply to fluctuations occurring in the stock cultures.

RESULTS

Figure 2 shows the results of selection on six replicate lines of *D. melanogaster* and *D. simulans*. By the sixth generation there were significant increases in the percentage of the larvae at the food plugs in all of the selected lines compared to the control ($P < 0.05$, sign test). The stars show the results of the control study which were performed both before and after selection. The 95% confidence intervals in all of the selected lines do not overlap with those of the control.

The larvae that were found neither on the food plugs nor in the center of the selection apparatus after the 20-min test period were termed unclassifiable larvae. In all cases this group included larvae that had moved rapidly to the food plugs but did not remain feeding on them. A pilot study had previously shown that if larvae were left in the selection apparatus for greater than 20 min, the number of larvae in the percentage unclassifiable category would not vary greatly. Thus it is unlikely that sooner or later all larvae would wander from a good food supply. As the percentage of the larvae remaining at food plugs increased with selection, there was a significant decrease in the percentage of unclassifiables. In *D. melanogaster* the mean percentage of unclassifiable larvae ± SE decreased from 6.0 ± 1.6 to 0.0 ± 0.0, and in *D. simulans* from 5.0 ± 1.2 to 1.0 ± 0.7.

Larvae that remained in the center of the selection apparatus were collected in the first generation to select for individuals which did not move toward the food. These lines were lost by the second or third generation because of low viability. The low viability may have been the result of inbreeding, since the numbers in the parental generations were small. An alternate, but not necessarily contradictory hypothesis is that the larvae remaining in the center were "sick" (genetically and or environmentally damaged). Certainly their phenotype (remaining on nonnutritive medium) was maladaptive.

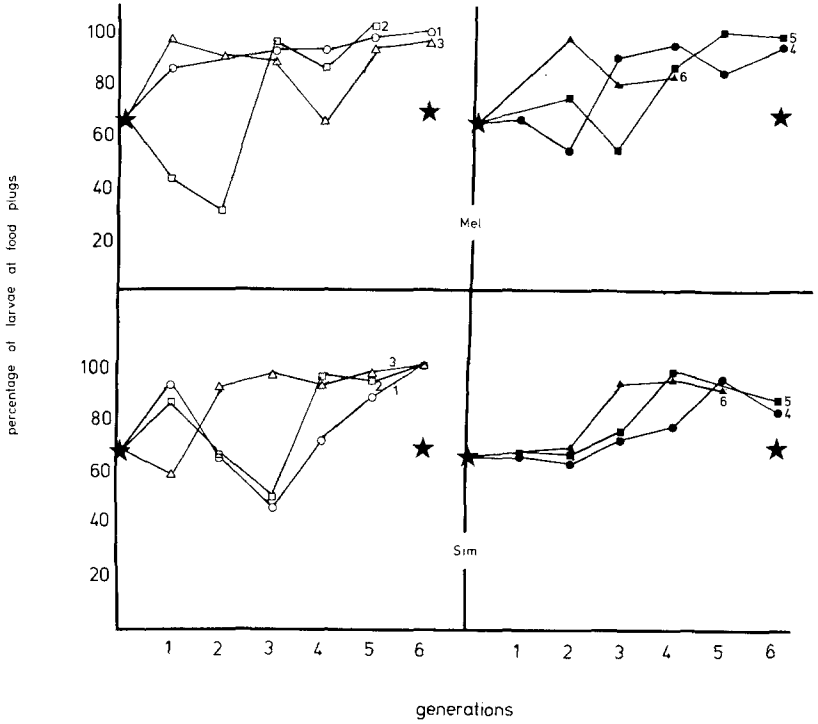

Fig. 2. The six selected lines, L1–L6, and the control values from the stock bottle populations (stars) of *D. melanogaster* and *D. simulans*. The percentage of the larvae found on the food plugs increased significantly from that of the control in all lines by five or six generations of selection.

Figure 3 shows the effect of selection on the original rover/sitter behaviors. This figure shows the mean path length of the 50 larvae tested at each generation of selection in the yeast-covered petri dish. In both species the general tendency was for the path length to decrease. The final path lengths were significantly less than the control in *D. melanogaster* lines 1, 2, 4, and 5 and in *D. simulans* lines 3, 5, and 6. There were no significant increases in any of the mean path lengths when comparing them to the controls (shown by the stars). In over one-half of the lines there is a correlation between decreasing path length and increasing selection for the percentage of the larvae at the food plugs.

DISCUSSION

Although many authors have found *D. melanogaster* to be competitively superior in the laboratory and to have a larger "potential" niche

breadth than *D. simulans* (see references in Parsons, 1975, 1977; Kawanishi and Watanabe, 1978; Moth and Barker, 1977; McKenzie and McKechnie, 1979), a rapid response to selection for at least one factor (foraging behavior) affecting niche utilization was true for both species in this study. Measuring the relative niche breadths of these species may not be the only approach to determining why *D. simulans* is extending its range (Ohnishi, 1979; Tantawy *et al.*, 1970; Watanabe and Kawanishi, 1976). Studies of niche breadths in these species should be coupled with a comparative study of the response to selection for a particular trait. In particular, it should be determined whether a trait is responsive to selection in both species and, if so, whether the magnitude and rates of response are similar. While niche breadth is thought to be important in studying

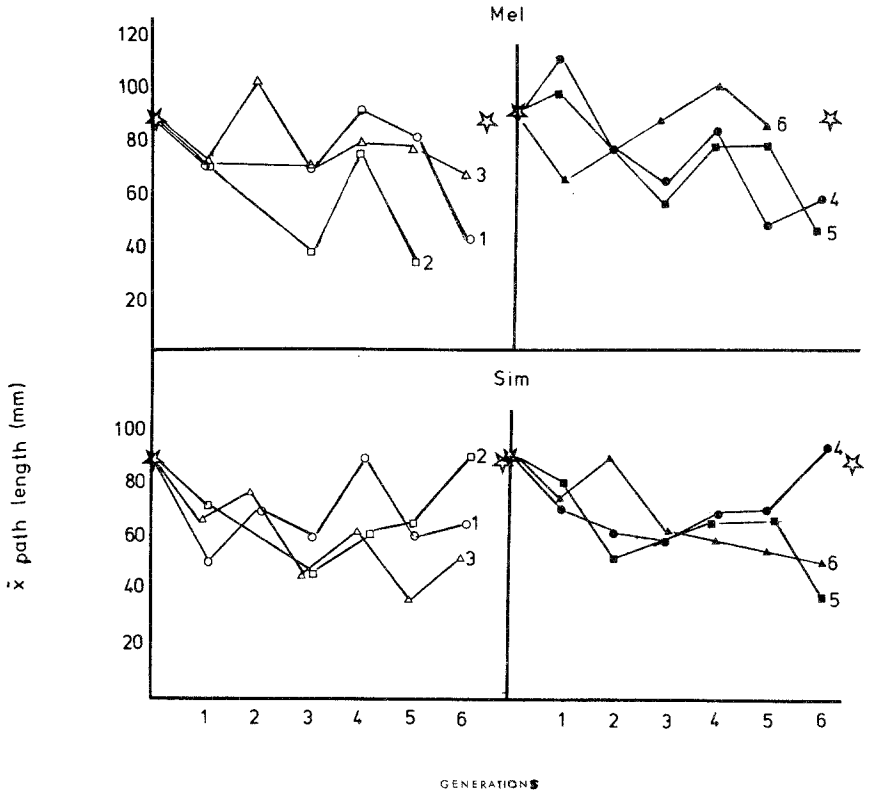

Fig. 3. The mean larval path length in the yeasted petri dish decreases with increased generations of selection. By the sixth generation there were significant decreases in mean path length in many of the selected lines of *D. melanogaster* and *D. simulans* compared to the control population (stars).

long-term competitive interactions between sibling species, a study of the competitive process should probably also involve an examination of the specific behavioral traits that are changing, their rate of change, and the genetic and environmentally determined interrelationships between them.

In the present study, stocks of sibling species, *D. melanogaster* and *D. simulans*, both showed a rapid response to selection for a foraging behavior. Larvae of both species did not move randomly in the selection apparatus. They oriented and moved directly toward the food plugs. This observation provided evidence that the character that was selected for was not simply a locomotory one; rather it was some combination of a larva's ability to "sense" food (visually and/or by olfaction), move toward it, and then remain foraging on the food source. The correlation between this trait and the shorter path lengths found in the larvae of many of the selected lines suggests that a sitter larva will move toward food when it is initially placed on nonnutritive food supply. However, the larvae will not wander away from a rich food source. In contrast, a rover larva probably tends to forage over a large area, moving from one food patch to another. Further evidence for the hypothesis that rover larvae would leave one food supply for another may have been provided if selection for the percentage of unclassifiable larvae had been studied. Experiments performed on the original rover and sitter stocks support this hypothesis (unpublished results).

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REFERENCES

Bakker, K. (1961). An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. *Arch. Neerl. Zool.* **14**:200–281.

Bakker, K. (1969). Selection for rate of growth and its influence on competitive ability of *Drosophila melanogaster*. *Neth. J. Zool.* **19**:541–595.

Kawanishi, M., and Watanabe, T. K. (1978). Difference in photo-preferences as a cause of coexistence of *Drosophila simulans* and *D. melanogaster* in nature. *Jap. J. Genet.* **53**:209–214.

McKenzie, J. A., and McKechnie, S. W. (1979). A comparative study of resource utilization in natural populations of *Drosophila melanogaster* and *D. simulans*. *Oecologia* **40**:299–309.

Moth, J. J., and Barker, J. S. F. (1977). Interspecific competition between *Drosophila melanogaster* and *Drosophila simulans*: Effects of adult density on adult viability. *Genetics* **47**:203–218.

Ohnishi, S. (1979). Relationship between larval feeding behavior and viability in *Drosophila melanogaster* and *Drosophila simulans*. *Behav. Genet.* **9**:129–134.

Parsons, P. A. (1975). The comparitive evolutionary biology of the sibling species, *Drosophila melanogaster* and *D. simulans*. *Q. Rev. Biol.* **50**:151–161.
Parsons, P. A. (1977). Genes, behavior, and evolutionary processes: The genus *Drosophila*. *Adv. Genet.* **19**:1–32.
Sokolowski, M. B. (1980). Foraging strategies of *Drosophila melanogaster*: A chromosomal analysis. *Behav. Genet.* **10**:291–302.
Sokolowski, M. B., and Hansell, R. I. C. (1983). *Drosophila* larval foraging behavior. I. The sibling species, *D. melanogaster* and *D. simulans*. *Behav. Genet.* **13**:159–168.
Tantawy, A. O., Mourad, A. M., and Masry, A. M. (1970). Studies on natural populations of *Drosophila*. VII. A note on the directional changes over a long period of time in the structure of *Drosophila* near Alexandria, Egypt. *Am. Nat.* **104**:105–109.
Watanabe, T. K., and Kawanishi, M. (1976). Colonization of *Drosophila simulans* in Japan. *Proc. Jap. Acad.* **52**:191–194.

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