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Drosophila Larval Foraging Behavior. I. The Sibling Species, *D. melanogaster* and *D. simulans*

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Phenotypic variation in foraging patterns of laboratory strains of Drosophila melanogaster *and* D. simulans *is reported in this paper. Mean scores of several replicate samples of each strain are compared to determine if both species show similar amounts of variation in each of the measures of foraging behavior. The mean scores for crawling and shoveling behavior performed by each species were similar. However,* D. melanogaster *showed greater intrastrain differences in mean crawling and mean shoveling scores than did* D. simulans. *In contrast,* D. simulans *showed greater intrastrain differences between the mean path lengths and the average areas traversed than did* D. melanogaster.

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

INTRODUCTION

Two possible evolutionary strategies that permit species to attain widespread distributions are (1) to have a broad niche breadth, that is, an ability to survive different environmental conditions, and/or (2) to be able to adapt rapidly to changes in environmental conditions. Niche breadth and adaptability are two independent but not mutually exclusive concepts. Niche breadth is usually measured by exposing animals to a range of environmental conditions and then measuring the animal's response, e.g.,

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survivorship and fecundity. Generally each environmental factor is tested separately, [see Parsons (1975, 1978) for review]. For example, McKenzie and Parsons (1972), and Parsons (1977, 1980) measured the comparative ethanol tolerance of the sibling species *Drosophila melanogaster* and *D. simulans* by exposing animals from various populations to a range of ethanol concentrations and then measuring their survivorship. Since, *D. melanogaster* tolerated a wider range of ethanol concentrations, it was concluded that *D. melanogaster* had a greater niche breadth, with respect to ethanol tolerance, than *D. simulans*. Similar experiments have been performed by exposing these sibling species to a range of temperatures (Tantawy and Mallah, 1961), desiccation conditions (Parsons, 1970), photoperiods (Kawanishi and Watanabe, 1978), and carbon dioxide concentrations (Matheson and Parsons, 1975).

This paper is the first of two devoted to larval foraging behavior in the sibling species *D. melanogaster* and *D. simulans*. Foraging behavior is defined as the relative amounts of feeding (shoveling) and locomotor (crawling) behavior performed by a larva. Burnet *et al.* (1977) and Sewell *et al.* (1975) studied the feeding rate (the number of probes or shovels with the mouth hooks per unit time) of larvae placed in an aqueous yeast suspension. *Drosophila* larvae tend to crawl along the feeding substrate while feeding. Larval feeding and locomotor behavior can be examined simultaneously when larvae are placed in a petri dish covered with a yeast paste. Sokolowski (1980, 1982) identified a behavioral polymorphism in *D. melanogaster* larval foraging trails. *Rover* larvae had long path lengths, whereas *sitter* larvae had significantly shorter ones, while foraging on a yeasted petri dish. Differences in these forager types were attributed to genes on the second pair of chromosomes.

In this paper the intrastrain phenotypic variation and correlations among various larval foraging behavior measures in *D. simulans* and *D. melanogaster* are studied. The mean ± SE scores of several intrastrain samples are compared, to determine whether both species show similar amounts of variation in each of the measures of foraging behavior. [The second paper in this series (Sokolowski *et al.*, 1983) compares the response to selection of foraging behavior in these species.]

METHODS

The *D. melanogaster* and *D. simulans* stocks used in these experiments were caught 15 years ago near the University of California, Riverside, by Timothy Prout. The *D. melanogaster* strain carries a sepia-eyed mutation, while *D. simulans* has wild-type red eyes. These differences in phenotype facilitated the separation of these species, which are otherwise morphologically very similar. Both stocks were obtained from Gibo (1969), who showed that the *D. melanogaster* sepia-eyed mutation had no appreciable affect on fitness and that females of these two stocks oviposit comparable numbers of eggs per unit time.

Flies were housed at 22 ± 1°C under a light cycle of 12 h of light followed by 12 h of darkness. Three replicates of both the *D. melanogaster* strain and the *D. simulans* strains were used in this experiment; they are called M(1), M(2), and M(3) and S(1), S(2), and S(3), respectively. All replicates were tested in pairs within a period of, 3 weeks during 1979: M(1) and S(1) on May 31, M(2) and S(2) on June 7, and M(3) and S(3) on June 15.

Ten pairs of flies (5–10 days old) were placed in a petri dish, 4.5 cm in diameter and 0.5 cm high, which was filled with a standard sucrose, brewer's yeast–agar medium, to a depth of 0.5 cm. The flies were allowed to lay eggs for a period of 5 h. Ninety-six hours after oviposition, 25 third-instar larvae (for each replicate) of approximately the same size were removed with a paintbrush and tested as described below.

A petri dish, 8.5 cm in diameter and 1.4 cm high, was covered with a thin layer of aqueous yeast suspension (8 g of Fleischman's fast-rising active yeast in 25 ml of distilled water). It was necessary for the yeast layer to be thin and pasty so that a moving larva would leave a visible trail. The test dish was then placed under a dissecting microscope. A paintbrush was used to transfer a third-instar larva of either *D. melanogaster* or *D. simulans* to the test dish. The test dish was covered with a petri dish lid which was marked with a centimeter grid. Both the animal and the grid were visible under the microscope.

Two behaviors, shoveling and crawling, are defined operationally. A bout of shoveling is a single probe or extension with the mouth hooks. A bout of crawling is a wave of muscular contraction passing along the body of the larva. The number of bouts of crawling and shoveling was recorded with a counter during the 5-min period beginning immediately after the larva was introduced (Sokolowski, 1983). After each test period, a copy of the foraging trail was drawn on a data sheet marked with grids. 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 in millimeters. The number of squares traversed also was recorded. All measurements were performed by the same experimenter.

RESULTS

The mean crawling scores (± SE) for the three replicates of *D. melanogaster* and *D. simulans* were 194.5 ± 12.5 for M1, 125.6 ± 11.4 for

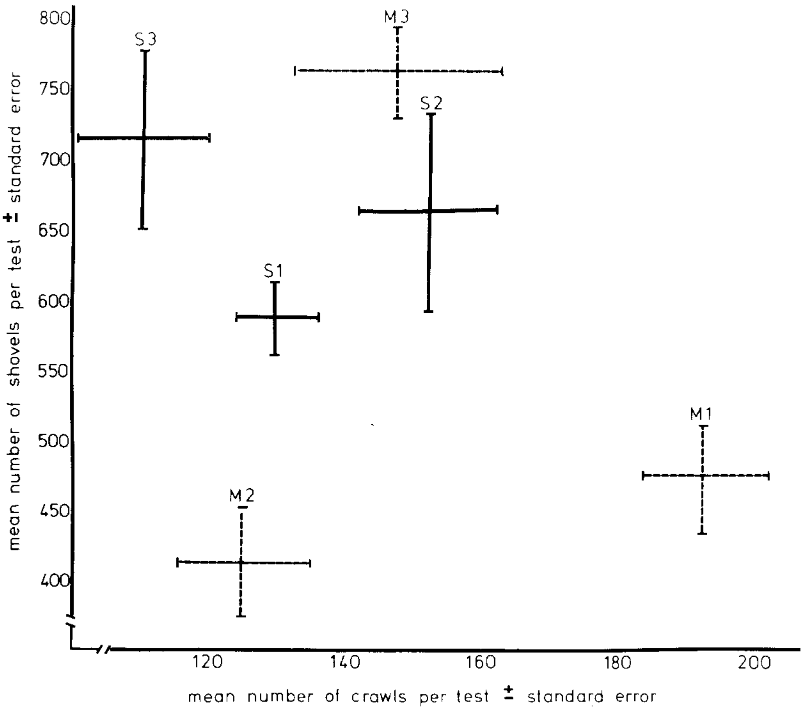

Fig. 1. Mean crawling and shoveling scores (±SE) plotted against the mean crawling scores (±SE) for each of the three replicates per species.

M2, and 148.2 ± 13.2 for M3 and 130.0 ± 11.5 for S1, 152.2 ± 10.1 for S2, and 111.0 ± 11.5 for S3. The mean shoveling scores were 477.7 ± 43.5 for M1, 419.0 ± 31.3 for M2, and 759.7 ± 43.8 for M3 and 582.6 ± 28.9 for S1, 672.45 ± 54.22 for S2, and 718.8 ± 53.0 for S3. In general, *D. melanogaster* had greater intrastrain differences in both mean crawling and mean shoveling scores than did *D. simulans*. However, this conclusion is made cautiously, because only 60 larvae per species, 20 larvae per replicate, were examined, and a comparison of only three mean scores per species was utilized. The mean crawling score of M1 was significantly higher than the scores of both M2 ($t = 7.4$, $P = 0.005$, Student's t test) and M3 ($t = 2.6$, $P = 0.01$), whereas M2 and M3 comparisons exhibited no significant differences. The only significant difference in the crawling scores of *D. simulans* was in the comparisons of S2 and S3 ($t = 2.8$, $P < 0.01$). The mean shoveling score of M3 was significantly higher than those of both M1 ($t = 4.6$, $P = 0.005$) and M2 ($t = 6.4$, $P = 0.005$); S3 shoveled more than S1 ($t = 2.3$, $P < 0.02$).

Differences in the variability of these behaviors in *D. melanogaster* and *D. simulans* are more clearly illustrated in Fig. 1, which shows the mean shoveling scores plotted against the mean crawling scores for each of the three replicates. The crawling and shoveling scores of *D. simulans* are less variable than in *D. melanogaster*, and within-species correlations in these behaviors are small for both species. There is little correlation (−0.03) between these behaviors in *D. melanogaster*, and there is a low negative correlation between crawling and shoveling in *D. simulans* (−0.4).

Figure 2 shows the mean path length scores plotted against the mean number of squares traversed for each replicate in *D. melanogaster* and

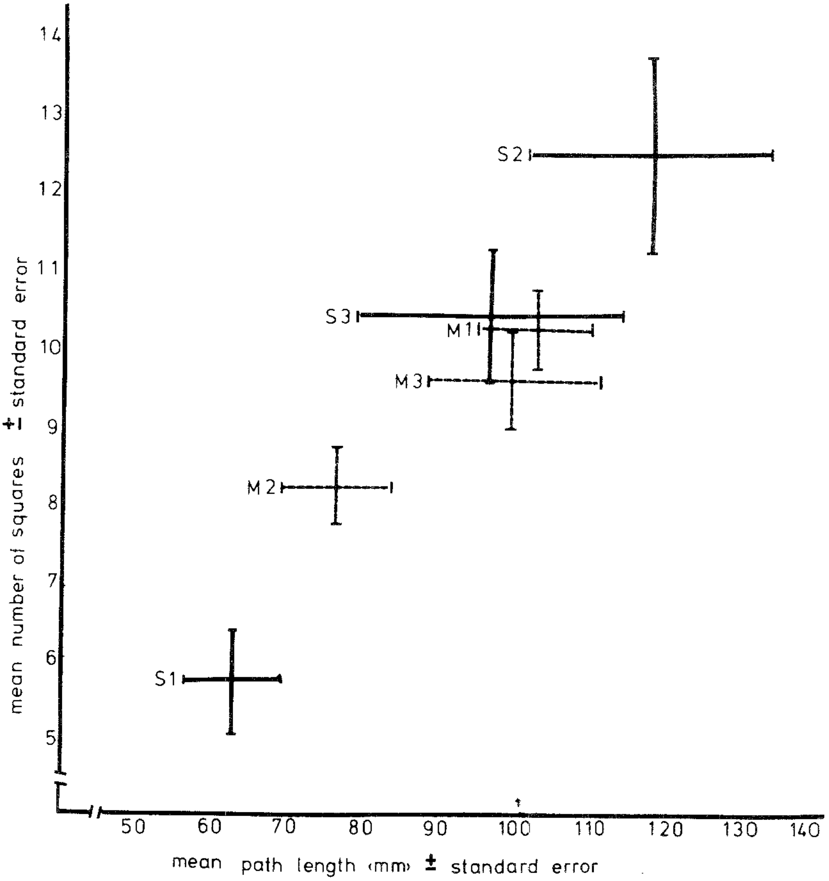

Fig. 2. Mean path length scores (±SE) plotted against the mean number of squares traversed (±SE) for each replicate in *D. melanogaster* and *D. simulans*.

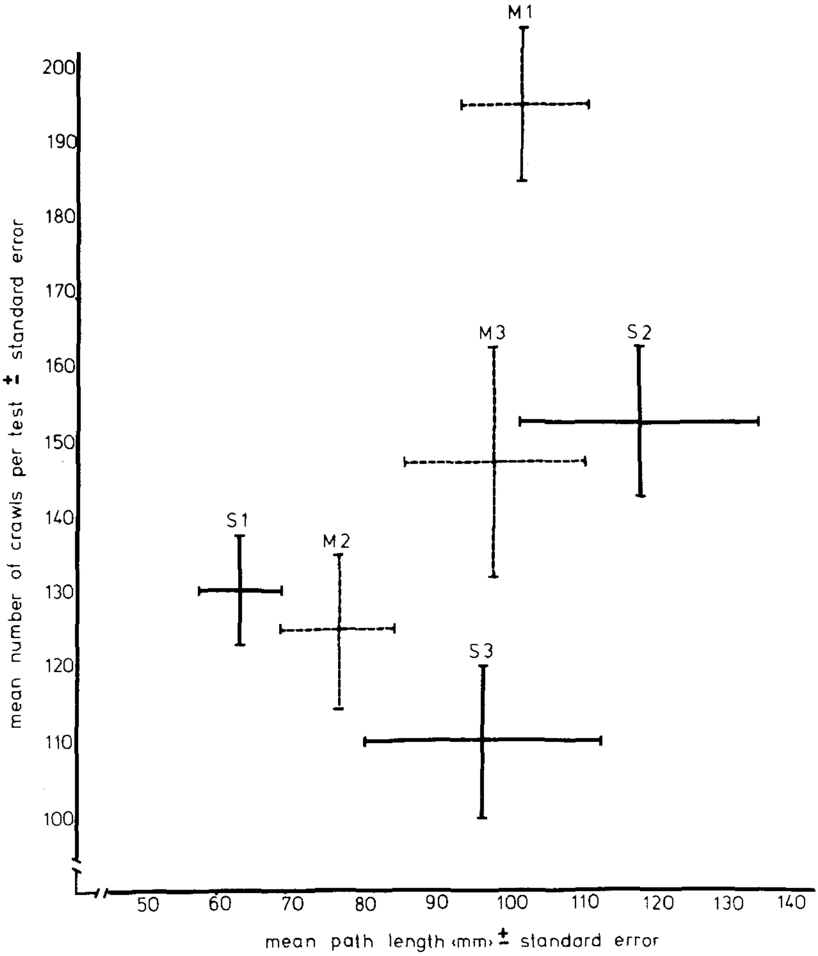

Fig. 3. The mean (±SE) of each of the replicates in *D. melanogaster* and *D. simulans*, for path length and number of crawls.

D. simulans. Unlike crawling and shoveling, these two characters show a strong positive correlation. In both species the correlation coefficient is +0.95. There are two obvious differences between the graphs of these species: (1) the means of *D. simulans* are more variable than those of *D. melanogaster*, and (2) the variances about the mean (shown as the standard error) are also greater in *D. simulans*. *D. simulans* also appeared to have a larger but not significantly different number of sitters than did *D. melanogaster*. Twenty larvae were tested in each replicate for each species. The replicates and their respective numbers of sitters were as follows: S(1) = 3, S(2) = 1, S(3) = 3, M(1) = 1, M(2) = 1, and M(3) = 2. Twelve percent of the *D. simulans* and 6% of the *D. melanogaster* larvae were sitters.

Figure 3 shows the mean behavioral score of each of the replicates in *D. melanogaster* and *D. simulans*, for path length and the number of crawls. Path length correlates positively with crawling but not as strongly as with the number of squares traversed. Crawling and path length correlate more highly in *D. melanogaster* (+0.83) than in *D. simulans* (+0.41). There is greater variability in crawling behavior in the mean scores of *D. melanogaster* than in those of *D. simulans*.

DISCUSSION

D. melanogaster has always been more abundant in regions where the two species coexist. During the last 10–20 years *D. simulans* appears to be replacing *D. melanogaster* in California (Gibo, personal communication), Egypt (Tantawy *et al.*, 1970), and Japan (Watanabe and Kawanishi, 1976). One hypothesis which may help explain the increasing ratio of *D. simulans* to *D. melanogaster* is that *D. simulans* has a greater niche breadth than *D. melanogaster*. However, the studies discussed below support the reverse hypothesis, that is, *D. melanogaster* has a greater niche breadth than *D. simulans*.

An abundance of information is available showing differences in adult preferences for a large range of environmental factors in *D. melanogaster* and *D. simulans* (McKenzie and McKechie, 1979; McKenzie and Parsons, 1974). However, little is known about preferences in the larval stage. McDonald and Parsons (1973) showed that adult dispersal activity was greater in *D. melanogaster* than in *D. simulans*, especially toward a light source. Parsons (unpublished work) observed that *D. simulans* larvae dig deeper into the media than *D. melanogaster*. He hypothesized that increased digging behavior in *D. simulans* may be a behavioral adaptation to dryness. Moore (1952) showed that *D. simulans* tends to pupate on dryer areas of the medium (toward the center rather than the periphery). In a pilot study Sokolowski (unpublished work) found no difference in the central as opposed to peripheral pupation sites of *D. melanogaster* compared to *D. simulans*. Ayala (1969) has shown coexistence in *D. melanogaster* and *D. simulans* when temperatures were intermediate to the preferred temperatures of each species [*D. melanogaster* prefer temperatures of approximately 25°C, whereas *D. simulans* prefer temperatures of approximately 18°C (Moore, 1952)]. Kawanishi and Watanabe (1978) hypothesized that coexistence in these species may result from differences in photopreferences. *D. melanogaster* showed a greater tolerance to a wider range of temperatures (Tantawy and Mallah, 1961; Parsons, 1973) and a preference for ethanol (McKenzie and Parsons, 1972) than *D. simulans*. At constant larval densities, *D. melanogaster* pupated on the walls of the vial and *D. simulans* on the medium (Sameoto and Miller, 1968). Finally, Barker (1973), Barker and Podger (1970a, b), Sameoto and Miller (1966), Miller (1964a, b), and Ohnishi (1979) reported differences in fecundity, adult and larval viability, egg hatchability, and various kinds of survivorship in these species.

The majority of researchers has concluded that since *D. melanogaster* can tolerate a wider range of environmental conditions, it has a larger niche breadth and hence must be phenotypically more variable than *D. simulans*. *D. melanogaster* may appear to show more phenotypic variability than *D. simulans* because of the specific set of environmental conditions used in the aforementioned experiments. For example, most laboratories maintain their stocks of 25°C, and this temperature facilitates growth in *D. melanogaster* but not in *D. simulans*. In the present study, an alternative approach to determining niche breath was used. The variability in larval foraging behavior of *D. melanogaster* and *D. simulans* was measured. The hypothesis that *D. melanogaster* is always more variable than *D. simulans* was not supported.

In our laboratory these stocks have been maintained for 6 years at a temperature intermediate to the temperatures preferred by *D. melanogaster* and *D. simulans* (22 ± 1°C). Replicates for each species were run in parallel so as to minimize daily environmental fluctuations. Differential responses of *D. melanogaster* and *D. simulans* to the same environmental fluctuations were expected to contribute to the between group variability.

While the overall amount of foraging behavior performed by each species was similar, the intrastrain variability differed between the species, depending on the subunit of foraging activity considered. *D. melanogaster* showed greater intrastrain differences in mean crawling and shoveling scores and *D. simulans* showed greater intrastrain differences in mean path lengths and the number of squares traversed. Patterns of correlation between behaviors also reflected differences in these species. Both species showed strong correlations between path length and number of squares traversed (+ 0.95). No correlation between crawling and shoveling behaviors was found in *D. melanogaster*, whereas a low negative correlation (− 0.4) was found in *D. simulans*. From a behavioral viewpoint, this difference means that, as feeding increases in *D. simulans*, locomotor movements decreases, and vice versa. *D. simulans* showed a greater tendency toward stationary feeding patterns compared with *D. melanogaster*, which exhibited both behaviors (crawling and shoveling) independently of one another. Crawling and path length correlated more highly (+0.83) in *D. melanogaster* than in *D. simulans* (+0.41). Correlations among the four behavioral measures elucidate differences in the patterns of movement during foraging.

The mean ± SE scores of several intrastrain samples of *D. simulans* and *D. melanogaster* were compared to determine whether both species show similar amounts of variation in four measures of larval foraging behavior. It was concluded that (1) *D. simulans* and *D. melanogaster* showed similar mean behavioral scores for each measure of foraging behavior, (2) the intrastrain variability differed between the species depending on the measure of foraging behavior, and (3) the patterns of correlation between behavior reflected differences in the species patterns of movement.

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