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Foraging Strategies of *Drosophila melanogaster*: A Chromosomal Analysis

Marla B. Sokolowski[1]

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Two larval foraging strategies in Drosophila melanogaster *were identified, "rover" and "sitter." "Rovers" traverse a large area while feeding whereas "sitters" cover a small area. The difference between "rovers" and "sitters" was analyzed genetically by chromosomal substitutions between isogenic stocks. Differences in larval locomotor behavior ("crawling behavior") can be attributed to the second chromosome, the "rover" strategy being dominant over the "sitter" strategy. Differences in feeding rate ("shoveling behavior") are affected additively by both the second and third chromosomes. Natural populations of* Drosophila *larvae were sampled three times over a 2-month period; "rovers" and "sitters" were at constant frequencies in these populations. The two foraging strategies are discussed in the light of resource utilization in environments where food is distributed continuously or discontinuously.*

KEY WORDS: foraging strategies; chromosomal analysis; *Drosophila melanogaster*; larvae; feeding-locomotor behavior.

INTRODUCTION

A foraging strategy reflects the relative amounts of feeding and locomotor behavior. The utility of any one strategy is a function of the environment. A *Drosophila* larva feeds by shoveling food with its mouth hooks, and moves by alternately extending its anterior end and retracting its posterior end. Variation in foraging patterns may be an important factor in determining success in exploiting different kinds of food resources.

Preliminary observation suggests two distinctive types of foraging pat-

[1] Department of Zoology, University of Toronto, Toronto M5S 1A1, Ontario, Canada.

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terns in *D. melanogaster* larvae. One type of larva ("rover") traverses a large area while feeding, whereas the other type ("sitter") covers a small area. If the food distribution is discontinuous, a "rover" larval strategy would have an advantage over a "sitter" larval one. In an environment with food evenly distributed, the advantage would be reversed. The success of a species' exploiting a given set of resources may be related to the types of foragers in its population.

The outcome of competition between different species of *Drosophila* is unpredictable (Miller, 1964*a*,*b*; Ayala, 1970; Gibo, 1972; Hedrick, 1972; Parsons, 1975, 1977). Furthermore, factors involved in competitive ability are at present poorly understood. Sewell, Burnet, and Connolly (1975) have shown that *D. melanogaster* larvae feed continuously throughout development but that feeding rate is age related. Feeding rate is thought to be important because it affects the rate of larval development. It increases during the first and second larval instars, reaching a maximum during the first half of the third larval instar, and then decreases as the larva searches for a pupation site. Bakker (1961, 1969) showed that pupation time is dependent on feeding rate since a minimum larval weight is necessary for pupation and emergence. Ohnishi (1979) showed that larval feeding behavior measurably affects egg-to-adult viability. Since feeding rate is intimately associated with food acquisition, feeding-locomotor behavior is important to study in the light of resource utilization and its possible effects on competitive ability.

This study was performed to describe larval foraging strategies and to analyze the strategies genetically.

MATERIALS AND METHODS

Laboratory Study

Cultures of *D. melanogaster* were maintained on a standard yeast–agar medium at 25 ± 1°C. Isogenic stocks homozygous for the second and third chromosomes were obtained from Dr. E. Rapport (Rapport and Sing, 1971).

To measure the contribution of autosomal genes, the two large autosomes, chromosomes 2 and 3, were manipulated; the tiny fourth chromosomes were not controlled. Substitution of the second or third chromosomes of these stocks was accomplished with a breeding scheme that utilized the presence of crossover suppressors. The technique was that of Muller and Oster (1963). The inversions that most effectively prevent crossing over between homologous chromosomes were used; they contain Curly (*Cy*) and Sternopleural (*Sp*) on the second chromosomes and Moire (*Me*) and Dichaete (D^3) on the third chromosomes. These balancers enable one to

keep desired pairs of second or third chromosomes intact during successive generations. The balanced lethal stock j172 (Bowling Green *Drosophila* stock center designation) was used to make the chromosome substitutions. Lindsley and Grell (1967) describe the mutants.

Four stocks were used in these experiments, two original stocks and two chromosomally manipulated ones. The original stocks were e^{11} (*ebony*, dark body color) and w^{bl} (*white-blood*, wild-type body color); w^{bl} is a sex-linked mutation that was eliminated in the process of making the stock isogenic. The original stocks will be called E_2E_3 and W_2W_3. The chromosomally manipulated stocks will be called E_2W_3 and W_2E_3. The subscript denotes the chromosome number. The E_2W_3 stock contains second chromosomes from the original e^{11} stock and third chromosomes from the original w^{bl} stock.

Figure 1 describes the crosses used in preparing the chromosomally manipulated stocks. Flies from each of the original stocks were crossed with flies from the j172 (*Cy Me; Sp* D^3) stock. The F_1 heterozygote was backcrossed to the j172 balancer stock. Stocks containing the desired homologues and marker chromosomes were then crossed with those containing reciprocal homologues and marker chromosomes. Heterozygotes containing *Me* and *Cy* markers were crossed, and the F_2 flies containing no markers were selected. The first and fourth chromosomes in the chro-

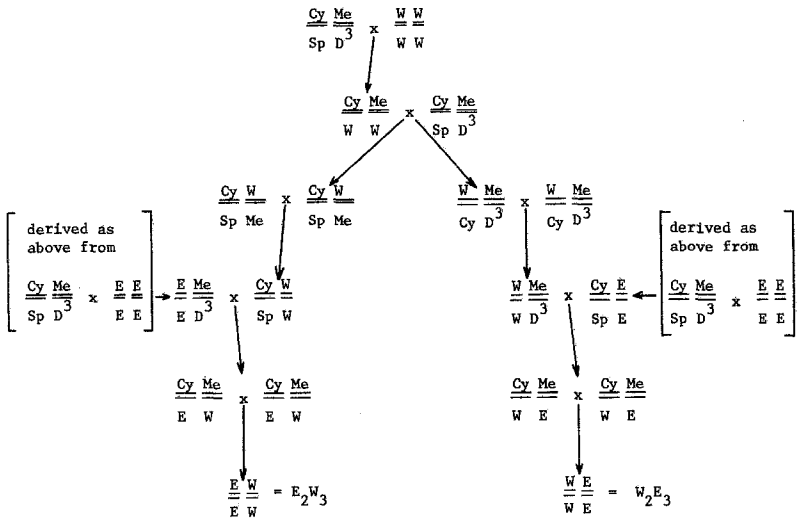

Fig. 1. Breeding scheme employed to obtain chromosome-substituted stocks from the original stocks. This figure illustrates how E_2W_3 and W_2E_3 were derived from E_2E_3 and W_2W_3. Only second and third chromosome pairs are illustrated.

mosomally manipulated lines were an unknown mixture of the original stocks and the balanced lethal chromosome stock. This unknown mixture from the three stocks (j172, W_2W_3, E_2E_3) added "background noise" to the reconstructed stocks (E_2W_3, W_2E_3). The X chromosome was further studied by performing reciprocal crosses between the original stocks.

In order to test for feeding-locomotor behavior, early third instar larvae (48–50 hr from hatching) were obtained as follows: Forty 5- to 10-day-old flies were placed in a 0.25-liter bottle. Females were allowed to lay eggs on a plastic teaspoon containing *Drosophila* medium. Two hours later, the spoon and eggs were transferred to a petri dish containing medium seeded with a yeast solution and then incubated.

Test Procedure

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 Fleishmann's fast-rising active dry 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 third instar larva, either E_2E_3, W_2W_3, E_2W_3, or W_2E_3, was transferred to the test dish using a paintbrush. The test dish was covered with a petri dish lid which had a centimeter grid marked on it. Both the animal and the grid were visible under the microscope.

Two behaviors, shoveling and crawling, were defined operationally as follows. A bout of shoveling was a single probe with the mouth hooks. A bout of crawling was a wave of muscular contraction passing along the body of the larva. The number of bouts of crawling and shoveling was recorded on a counter over a 6-min period. After the test period a copy of the foraging trail was drawn onto a data sheet marked with grids (see Fig. 2). 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 was also recorded.

In order to test large numbers of animals, a more rapid determination of the behavioral phenotype was required. Since the number of crawls was directly correlated with path length, animals could be scored as "rovers" or "sitters." Two criteria, path length and the number of squares traversed, were used (see Fig. 5). Larvae crawling farther than 35 mm or crossing five or more squares were classified as "rovers"; others were classified as "sitters."

In order to investigate possible effects of the X chromosome, reciprocal crosses of E_2E_3 by W_2W_3 were performed. The F_1 progeny from these

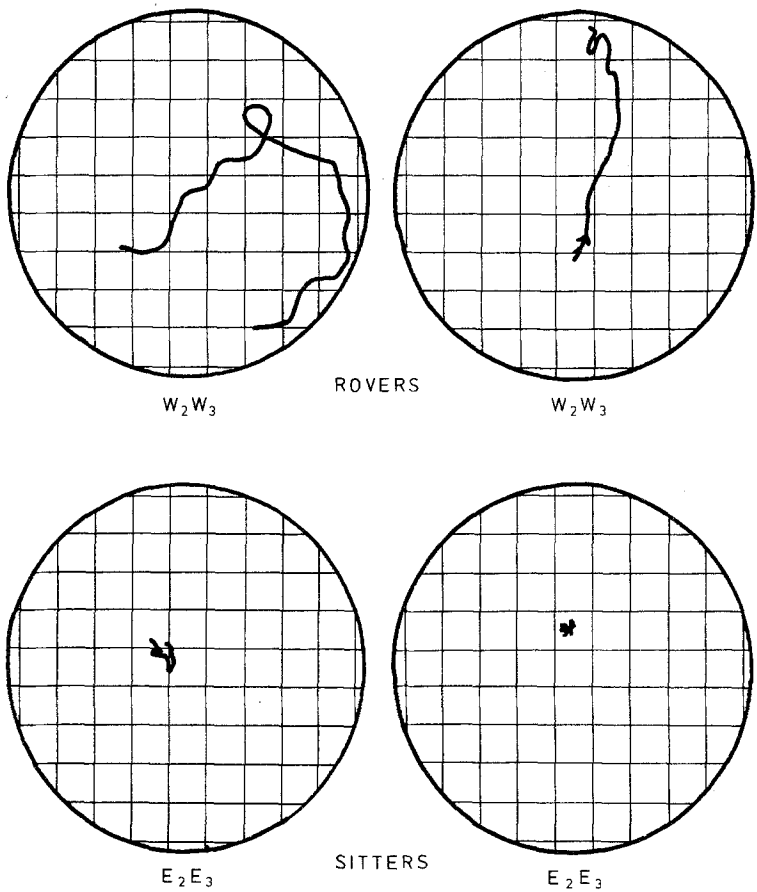

Fig. 2. Larval trails of E_2E_3 and W_2W_3 superimposed on a centimeter grid. The length of the trail and the number of squares traversed are clearly different. W_2W_3 shows the "rover" strategy whereas E_2E_3 shows the "sitter" strategy.

crosses were reared, sexed (Demerec, 1950), and tested by scoring path length and the number of squares traversed.

Natural Population

Samples of *Drosophila* larvae were obtained from pear trees found in an unkept backyard in downtown Toronto. Each sample contained eight rotting pears taken from a 1-m^2 area. This area was sampled three times during the fall of 1977, at 3-week intervals, so that at least three successive generations could be tested. Early third instar larvae of approximately the

same size were removed from the pears with a paint brush and tested within 48 hr of bringing the pears into the laboratory. The larvae were simply scored as being "rovers" or "sitters" by examining their foraging trail over a 5-min period.

RESULTS

Laboratory Study

Figure 2 shows a random sample of the trails made by each original stock. The W_2W_3 stock foraged over a much larger area than did the E_2E_3 stock. The length of the W_2W_3 trail was longer, as might be expected in view of their high crawling score (see Fig. 2). I have called the W_2W_3 type forager the "rover" larval and the E_2E_3 type forager the "sitter" larval strategy.

Figure 3 illustrates that the feeding-locomotor behaviors of the W_2W_3 and E_2E_3 stocks were phenotypically different, especially crawling behavior. W_2W_3 crawled significantly more than E_2E_3 ($z = 5.0$, $p < 0.0001$, Mann-Whitney U test). There was a much smaller difference between the amount of shoveling performed by the two stocks ($z = 1.6$, $p \cong 0.1$, Mann-Whitney U test).

The chromosomally manipulated stocks were employed to determine

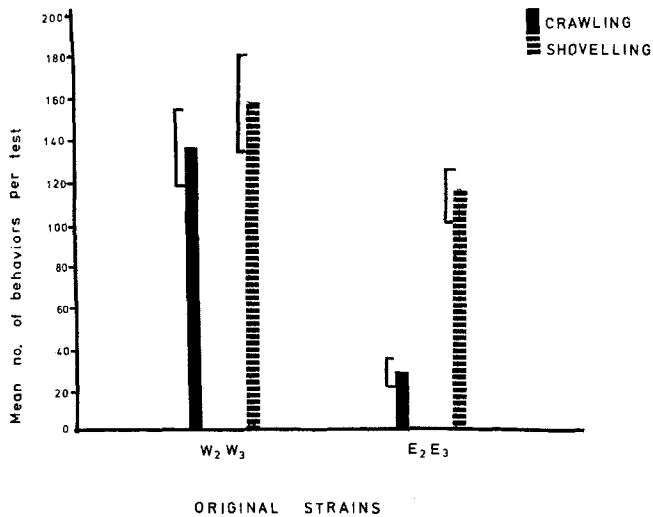

Fig. 3. Shoveling and crawling scores of W_2W_3 and E_2E_3. Each histogram represents the mean number of behaviors ± the standard error per test. Twenty-five animals of each stock were tested.

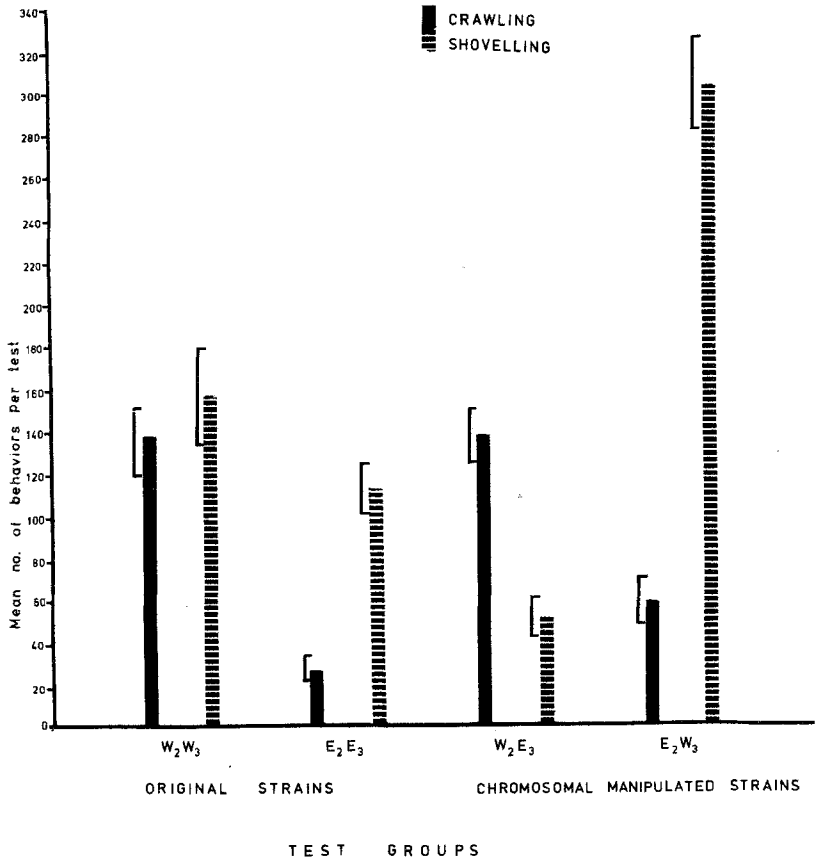

Fig. 4. Shoveling and crawling scores of the original stocks (W_2W_3 and E_2E_3) compared to the scores of the chromosomally manipulated stocks (W_2E_3 and E_2W_3). Each histogram represents the mean number of behaviors ± standard error per test. Twenty-five animals of each stock were tested.

the contribution of chromosomes 2 and 3 to shoveling and crawling. Figure 4 shows the mean scores and standard errors of each behavior as performed by each of the four stocks tested. W_2W_3 and W_2E_3 had high crawling scores and were not significantly different from each other ($z = 1.7$, $p \cong 0.1$, Mann-Whitney U test). The E_2E_3 and E_2W_3 had low crawling scores and were not significantly different ($z = 1.2$, $p \cong 0.2$, Mann-Whitney U test). A comparison of the amount of crawling performed by the two chromosomally manipulated stocks E_2W_3 and W_2E_3 showed a significant difference ($z = 4.0$, $p < 0.0001$, Mann-Whitney U test). The difference in crawling behavior therefore appears to be attributable to the second chromosomes (see Table I). The shoveling behavior does not fit the same pattern

as the crawling behavior. There were significant differences ($p < 0.0001$, Mann-Whitney U test) between shoveling performed in all two-way comparisons except E_2E_3 by W_2W_3 ($z = 1.6$, $p \cong 0.1$, Mann-Whitney U test). Table I illustrates the chromosomal contributions to each behavior.

Table I shows the mean number of behaviors performed per test broken down by chromosome. An E_2E_3 larva has isogenic chromosomes 2 and 3 from the original ebony population. The mean number of crawls performed by E_2E_3 was 27.7 ± 5.8. The mean number of crawls performed by E_2W_3 was 58.2 ± 11.5. As stated previously, there was no significant difference between the number of crawls performed by E_2E_3 and E_2W_3 and by W_2W_3 and W_2E_3. Differences in crawling behavior therefore appear to be attributable to the second chromosomes. Shoveling (Table IB) cannot be localized on one chromosome. The second and third chromosomes affect this behavior additively. The presence of W/W third chromosomes triples the amount of shoveling, and the presence of W/W second chromosomes halves the amount of shoveling behavior performed. The chromosomal contributions to each behavior are clear.

Figure 5 shows the distribution of path length in W_2W_3 and E_2E_3, the "rover" and "sitter" foragers. The path length scores were first divided into categories of 20 mm. The number of larvae in each category was then plotted on the vertical axis for each stock. A test for the equality of variances showed that the variances in path length in the two stocks were significantly different ($F = 72.02$, $p < 0.01$). In E_2E_3 the distribution of path length was skewed to the right, whereas in W_2W_3 the path length distribution was platykurtic. A single data transformation was not appropriate.

Indeed, the larvae of W_2W_3 and E_2E_3 have different path length distributions. The "rover" larva (W_2W_3) has a longer path length than the "sitter" larva (E_2E_3). The point that separates "rovers" and "sitters" lies at

Table I. Chromosomal Contribution to Each Strain: Means and Standard Error of Behaviors

Second chromosome	Third chromosome E/E	Third chromosome W/W
	A. Crawls	
E/E	27.7 ± 5.8 (E_2E_3)	58.2 ± 11.5 (E_2W_3)
W/W	137.1 ± 12.6 (W_2E_3)	137.2 ± 17.8 (W_2W_3)
	B. Shovels	
E/E	112.9 ± 11.2 (E_2E_3)	301.7 ± 22.2 (E_2W_3)
W/W	50.7 ± 9.1 (W_2E_3)	157.2 ± 22.0 (W_2W_3)

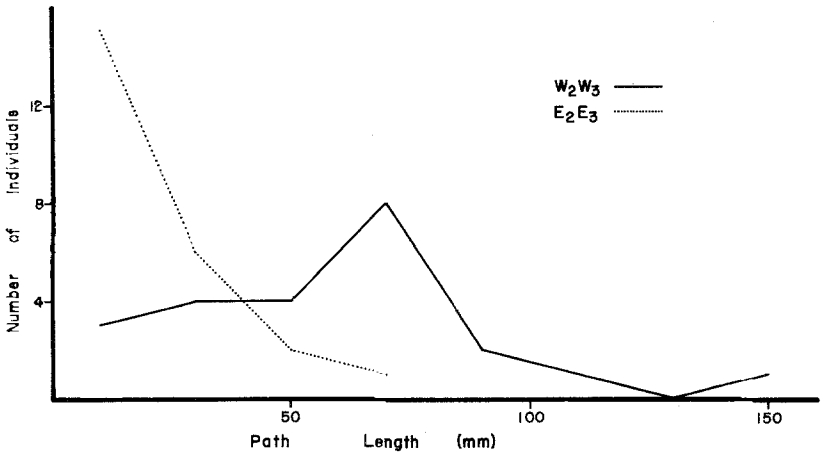

Fig. 5. Distribution of path lengths in W_2W_3 and E_2E_3, the "rover" and "sitter" foragers. "Rover" larvae show significantly longer paths than do "sitters." The distributions are clearly not from the same population, although there is some overlap.

about 40 mm. There is, however, some overlap in path length scores. The number of squares traversed can also be used to provide information about the area covered by a foraging larva. This correlates highly with path length (+0.8). The following criteria are used to distinguish the behavioral types. If a larva crawled farther than 35 mm or crossed five or more squares, it can be classified as a "rover." If neither of these criteria are met, the larva is a "sitter." By testing a larva's foraging trail, its genotype (W_2W_3 or E_2E_3) can be predicted with 80% accuracy.

A discriminant function analysis produced similar criteria with the same accuracy. However, the failure of the statistical assumptions of this method makes its inclusion here inappropriate.

Reciprocal crosses were performed in order to determine if the X chromosome had any effect on the crawling behavior. Reciprocal crosses of E_2E_3 by W_2W_3 showed no sex-linked differences in the crawling behavior. The "rover" strategy was dominant over the "sitter" strategy. In each cross 80% of the larvae performed "rover" paths whereas 20% performed "sitter" paths. It was concluded that the X chromosomes had no significant effect on crawling.

Natural Population

Table II demonstrates that the "rover" and "sitter" foraging types exist in nature as well as among laboratory stocks. Seventy-two percent of the 250 larvae tested were "rovers" while 28% were "sitters." There were no

Table II. Natural Population

Date	*N*	Phenotypic Frequencies	
		ROVERS	SITTERS
22/9/77	100	80	20
13/10/77	100	68	32
3/11/77	50	34	16

significant differences between the samples taken ($\chi_4^2 = 4.36$, $p \cong 0.5$) over the 9-week period.

DISCUSSION

The differences between the two isogenic stocks in the amount of crawling and shoveling have a genetic component. Chromosomal analysis demonstrated that differences in crawling are attributable to the second chromosome, whereas differences in shoveling are affected additively by both second and third chromosomes.

Burnet *et al.* (1977) performed a genetic analysis of larval feeding behavior in *D. melanogaster*, using lines selected for fast and for slow feeding rates, and an unselected control line. They found that larval feeding rate is affected by genes on the three major chromosomes, whereas the effect of the fourth chromosome is negligible. The authors reported epistatic interactions between the second and third chromosomes of their fast-feeding lines; I found no interaction between second and third chromosomes in the original and chromosone-manipulated stocks.

Path length and the number of squares traversed were used to quantify the larval foraging trail. Animals with the genotypes W_2W_3 and E_2E_3 can be categorized as "rovers" and "sitters." The "rover" strategy larvae traverse a large area while feeding; the "sitters" cover a small area. The criteria employed separate W_2W_3 and E_2E_3 with 80% accuracy. When natural populations of mixed strategies are tested, a large number of larvae must be scored. The unchanged ratio of "rovers" to "sitters" in rotting pears over the 2-month sampling period warrants an investigation into the population genetics of these foraging strategies. The "rover" and "sitter" strategies may exist in nature as a balanced behavioral polymorphism. The "rovers'" mixing the medium may facilitate the development of the "sitter" larvae and younger larvae of both types.

Food acquisition depends on the kind of foraging behavior and the distribution of food. A single pear can be viewed as an uneven environment, since different sections of the pear rot at different rates. The degree of dis-

continuity of the feeding substrate varies with the abiotic factors over space and time. It is therefore not surprising to find two different approaches to foraging (the "rover" and "sitter" strategies). It is hypothesized that the "rover" strategy would be advantageous when food is distributed discontinuously, whereas the "sitter" strategy would be advantageous when food is distributed continuously. Each strategy would provide a larva with a competitive advantage when the environment is consistent with the strategy.

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