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The *foraging* locus: behavioral tests for normal muscle movement in rover and sitter *Drosophila melanogaster* larvae

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Abstract

We used *Drosophila melanogaster* larvae with different alleles at the *foraging* (*for*) locus in a variety of behavioral tests to evaluate normal muscle usage of rover and sitter phenotypes. The results show that sitter and lethal sitter alleles of *for* do not affect larval behavior through a mutation which affects larval muscle usage. In general the behavior of rovers and sitters differed on food but not on non-nutritive substrates. Rovers and sitters moved equally well on non-nutritive substrates, and measures such as the time to roll over and length of forward stride showed no significant strain differences. Larvae with different alleles at *for* did not differ in body length. Rovers took more strides, not longer ones, than sitters while on foraging substrates. We conclude that differences in larval locomotion during foraging found in larvae with different alleles at *for* can not be explained on the basis of muscle usage alone. It is more likely that *for* affects larval ability to perceive or respond to the foraging environment.

Introduction

The *foraging* (*for*) locus represents one of the few genes isolated by studying larval behavior of the fruit fly *Drosophila melanogaster*. The locomotory component of larval foraging behavior is quantified by placing individual third instar larvae in petri dishes layered with a thin yeast/water paste for a set time period. The length of the visible trail left by a foraging larva is measured and called 'path length'. 'Rover' larvae have significantly longer paths than 'sitter' larvae. Two naturally occurring alleles have been found, for^R (rover) and for^s (sitter). The for^R allele is completely dominant to for^s. The *for* locus has been mapped to 24A3-5 on the second pair of autosomes (de Belle, Hilliker & Sokolowski, 1989; de Belle, Sokolowski & Hilliker, submitted; Sokolowski, 1992) using a technique which we call lethal tagging. A number of lethal alleles of *for* have been generated. Lethality acts in the pupal stage making it possible to study the foraging behavior of larvae with homozygous lethal alleles of *for* (de Belle, Hilliker & Sokolowski, 1989).

Three hypotheses address the underlying mechanistic basis of rover/sitter differences. The first is that they differ in locomotory ability due to a difference in muscle usage. The second is that they differ in their ability to sense or perceive the environment. The third is that rover and sitter responses reflect differences in decision making processes in the brain. Further behavioral studies as well as the cloning of *for* should aid in distinguishing between these hypotheses. In this paper we address the first hypothesis using behavioral experiments which were developed to test for mutations affecting larval muscle development (Ball, Ball & Sparrow, 1985).

Ball, Ball & Sparrow (1985) tested the behavior of larvae of the *lethal(2) thin* mutation, a mutation that affects larval muscle development in *D. melanogaster*. They found that crawling ('locomotory contractions') and shoveling ('number of feeding movements') were reduced in mutant larvae. In addition, locomotory stride lengths were shorter in mutants. Mutant mouth hook marks in the medium were not as deep as those of the controls, suggest-

ing inefficient mouth hook usage. Ball, Ball & Sparrow (1985) also describe the mutant's response to probing of its anterior end as being extremely sluggish. In addition, the time it took larvae to roll over after being placed on their dorsal side was greater than nine times slower in the mutants. All measures of activity were found to be much lower in the mutants as compared to wild-type larvae.

In the present study, larvae with different alleles at *for* were exposed to a variety of behavioral tests (modified from Ball, Ball & Sparrow, 1985 and from Sokolowski, 1980). They include the locomotory measurements: number of crawls, path lengths on nutritive substrates, time to roll over, stride length, number of forward and backward strides after probing with a paint brush, and the number of shovels (a feeding movement).

Materials and methods

Strains

We used three strains of *D. melanogaster* each with different alleles at *for*: for^R/for^R, for^s/for^s and $for^{1(92)}/SM1$. *SM1* is a chromosome-2 balancer. [In *(2LR)SM1*, al^2 *Cy* cn^2 sp^2]. Further details on the mutations and chromosomes mentioned in this section are given in Lindsley & Grell (1968) and Lindsley & Zimm (1985, 1987). Before *for* was mapped, for^R/for^R was called BB and for^s/for^s was called EE (de Belle & Sokolowski, 1987). During the localization of *for* (de Belle, Hilliker & Sokolowski, 1989), $for^{1(92)}/SM1$ was produced by gamma irradiation of the BB rover strain followed by a behavioral screen for lethal alleles of *for* that resulted in a sitter phenotype (for further details see de Belle, Hilliker & Sokolowski, 1989). In order to test the behavior of homozygous lethal *for* larvae we needed to distinguish between larvae of the genotypes, $for^{1(92)}/SM1$ and $for^{1(92)}/for^{1(92)}$. This was not possible without incorporating a second chromosome larval marker onto the $for^{1(92)}$ chromosome. We used the strain *Bc Elp/SM1* to produce the recombinant $for^{1(92)}$ *Bc Elp/SM1*. The *Bc* phenotype appears as black dots in the interior of the larval body. The *Elp* phenotype (asymmetrical eye cell patterns in the adult), was used to identify adults where a cross-over event had occurred. *Black cell (Bc)* is a dominant second chromosome larval marker. Homozygotes display the *Bc* phenotype earlier in development (during the egg stage) than heterozygotes (during late first larval instar). It is possible to distinguish $for^{1(92)}$ *Bc Elp*/$for^{1(92)}$ *Bc Elp* larvae from $for^{1(92)}$ *Bc Elp/SM1* larvae by examining them as they hatch out of the egg even though most of the larvae found are heterozygotes. We found that we could separate homozygotes from heterozygotes with about 80% accuracy. To be sure that we had measured the behavior of lethal $for^{1(92)}$ *Bc Elp*/$for^{1(92)}$ *Bc Elp* larvae, we used the data of individual test flies that did not emerge from their pupal cases.

Rearing and aging of larvae

Strains were maintained in plastic culture bottles on 45 ml of a dead yeast-sucrose-agar (standard culture) medium at 24 ± 1 °C, 15 ± 1 mbars vapor pressure deficit and an LD 12:12 photocycle with lights on at 0800 hours (standard conditions). Prior to behavioral testing, larvae were harvested from matings between 250 females and 125 males aged two to five days (post-eclosion), which had been provided with a Cream of Wheat and molasses egg-laying substrate. Twenty-five first instar larvae (± 1.5 h in age) were placed in petri dishes (d = 5.2 cm, h = 1.3 cm) containing 10 ml of culture medium, where they developed for 96 h to third instar larvae under our culturing conditions. The maximum expression of genetically based differences between rovers and sitters occurs during this stage of larval development (Graf & Sokolowski, 1989). Foraging third instar larvae of each genotype were randomly sampled from each dish and individually tested in petri dishes (8.5 cm × 1.4 cm) coated with a thin, homogeneous layer of aqueous yeast suspension (distilled water and Fleischmann's bakers' yeast in a 2:1 ratio by weight).

Behavioral tests

A larva was placed in the above mentioned petri dish under a dissecting microscope attached to a video monitor. The larva was given one minute to adjust to the new environment, after which the number of crawls and shovels were counted during the second minute. One crawl was defined as one muscular contraction along the body wall; one shovel was a single movement of the larval mouth

hooks into the foraging substrate (Sewell, Burnet & Connolly, 1975; Sokolowski, 1980; Green, Burnet & Connolly, 1983). During the third to fifth minute we measured stride lengths and larval lengths. Stride length was defined as the distance between successive mouth hook marks in the medium (see Ball, Ball & Sparrow, 1985). Five stride lengths were taken for each larva. The length of the larva extended to its fullest (larval length) was also measured five times. After five minutes, the petri dish was removed from the microscope stage and the path length of the foraging larva was measured during a subsequent four minute period. Path lengths were quantified using a digitizer connected to an electronic graphics calculator. After the path length measurement, the larva was gently washed in a drop of water and placed under the dissecting scope on a dish containing 1.6% agar. After one minute, the larva was gently rolled over onto its dorsal side using a paint brush. The number of seconds until the larva righted itself (onto its ventral side) was called the time to roll over (Ball, Ball & Sparrow, 1985). The roll over test was repeated three times with a 30 second rest period between each test. After this test, the larva was place in a (d = 2 cm and h = 11 cm) vial with 2.5 ml of standard medium and allowed to develop to adulthood so that emergence and sex could be recorded. All larvae were tested in the order described above. We sampled additional larvae from each strain to measure the number of forward and backward strides after probing. The larva was placed on a piece of Plexiglas (6.2 cm in length and width) that had a single lane of 6.2 cm cut into it. The lane was cut slightly deeper than the width and depth of a 96 h old larva. The lane allowed the larva to move forward or backwards in a straight line. We place a larva of one of the strains in the groove and gave it one minute to adjust to the new environment. We then gently probed the larva (with a paint brush) on its posterior end and measured the length of two sequential forward movements. This procedure was then repeated by probing the larva at the anterior end.

Statistical analysis

Analyses of variance (ANOVAs) were performed using SAS (1985) to determine whether there was significant variation in the repeated measures of each individual for stride length, larval length and roll over time. Significant variation was not found so the average of the repeated tests for each individual was used for subsequent analyses. We also found no sex differences for any of the measures so sexes were pooled. ANOVAs were performed to determine whether there were significant strain differences in any of the measures. This was followed by Student-Neuman-Keul's tests (SNK) to determine where those differences lay. Non-parametric ANOVA (Kruskal-Wallis) produced the same results as the parametric ANOVA. In the tables we present the means, standard errors and SNK results for all measures. The data for the mean lengths of forward strides after probing, from replicate experiments, done on different days, could not be pooled ($F_{1,71} = 9.68$ $P < 0.05$) and are therefore presented separately in Table 2.

Results and discussion

We had predicted that if for^{s}/for^{s} and $for^{1(92)}$ *Bc Elp*/$for^{1(92)}$ *Bc Elp* differ only in their muscle usage relative to for^{R}/for^{R} we should find consistently lower scores for these strains in all behavioral tests. We did not find such consistent differences.

The means and standard errors for all measurements are presented in Tables 1 and 2. Joined vertical lines mean that the measurements do not differ significantly according to the SNK test. The number of crawls and the path length are related to how far a larva moves while foraging on a nutritive yeast coated petri dish (Sokolowski, 1980; Sokolowski & Hansell, 1983). Path length and the number of crawls were the only behaviors found to be significantly correlated ($r^2 = 0.56$, $P < 0.001$). Sitters and homozygous lethal sitters have significantly lower mean crawling and path length measures than do rovers. This is as expected since the rover/sitter polymorphism was defined on the basis of path length. The lethal sitter allele has the same effect on the phenotype as the non-lethal sitter allele. This confirms the findings of de Belle, Hilliker & Sokolowski (1989) which suggested that sitter is an amorphic allele of *for*. The number of feeding movements (shovels) for rovers and sitters did not differ. Indeed, feeding rate is influenced by different genes than is path length (Sokolowski, 1980). The feeding rate of larvae homozygous for the lethal sitter allele is significantly higher than that of

Table 1a. Behavior (mean ± SE) of *Drosophila melanogaster* larvae differing at alleles of the *foraging* locus.*

Phenotype	Genotype	N	No. of crawls	Path lenght (cm)	No. of shovels
Rover	for^R/for^R	9	42.9 ± 4.0	5.6 ± 0.5	117.1 ± 7.7
sitter	for^s/for^s	9	19.0 ± 4.6	3.2 ± 0.6	103.7 ± 8.7
lethal sitter	$for^{l(92)}$ *Bc Elp*/$for^{l(92)}$ *BC Elp*	6	14.3 ± 5.1	2.7 ± 1.0	158.0 ± 5.8

Table 1b. Behavior (mean ± SE) of *Drosophila melanogaster* larvae differing at alleles of the *foraging* locus.*

Phenotype	Genotype	N	Stride length on yeast (mm)	Larval length (mm)	Time to roll over (s)
Rover	for^R/for^R	9	0.3 ± 0.02	3.5 ± 0.09	3.7 ± 0.8
sitter	for^s/for^s	9	0.3 ± 0.02	3.5 ± 0.09	4.9 ± 0.6
lethal sitter	$for^{l(92)}$ *Bc Elp*/$for^{l(92)}$ *Bc Elp*	6	0.3 ± 0.04	3.5 ± 0.04	9.8 ± 2.8

* Joined vertical lines mean that measurements do not differ significantly by the SNK test (P = 0.05)

larvae homozygous for the naturally occurring rover and sitter alleles. This is not likely an effect of *for*. It probably results from strain differences in third chromosome genes. Further behavior-genetic analysis of this strain and other alleles of *for* would provide a thorough test of this hypothesis. Nevertheless, sitters and lethal sitters had the same or more feeding movements than rovers, indicating that the strains do not differ because of some 'general activity factor'.

Larval length was measured to determine whether differences in path lengths or stride lengths result from strain differences in the size of 96 h post-hatch larvae. This indicates that rover paths are longer than sitter paths because rovers take more strides, not longer strides. We found that larvae of the three strains did not differ significantly in larval lengths. Graf and Sokolowski (1989) showed that the length and widths of rover and sitter larvae did not differ significantly during development.

Time to roll over, and both forward and backward stride after probing are behaviors that measure the ability of the organism to respond to experimental stimulation. There were no strain differ-

Table 2. Mean (mm) forward and backwards stride lengths after probing of *Drosophila melanogaster* larvae differing in alleles at the *foraging* locus.*

Phenotype	Genotype	Mean length of forward stride after probing (± SE) (N)	Mean length of backward stride after probing (± SE) (N)
Replicate 1			
Rover	for^R/for^R	2.6 ± 0.32 (8)	3.7 ± 0.30 (8)
sitter	for^s/for^s	2.1 ± 0.16 (8)	2.8 ± 0.32 (8)
lethal sitter	$for^{l(92)}$ *Bc Elp*/$for^{l(92)}$ *Bc Elp*	2.2 ± 0.09 (7)	2.1 ± 0.18 (7)
Replicate 2			
Rover	for^R/for^R	3.3 ± 0.12 (27)	3.9 ± 0.16 (26)
sitter	for^s/for^s	2.6 ± 0.11 (18)	3.1 ± 0.11 (17)
lethal sitter	$for^{l(92)}$ *Bc Elp*/$for^{l(92)}$ *Bc Elp*	2.6 ± 0.28 (7)	2.7 ± 0.14 (6)

* Joined vertical lines mean that measurements do not differ significantly by the SNK test (P = 0.05)

ences in time to roll over (Table 1) or the length of forward stride (Table 2). In both replicates, the length of backward stride decreased in order from rover to sitter to lethal sitter. We have no explanation for this at present and do not know whether these differences are related to *for*.

Overall, rovers, sitters and lethal sitters appear to have similar muscle usage abilities as measured by these behavioral tests and the difference in rover/sitter path lengths is conditional on the presence of the foraging environment. In the Introduction we argue that if *for* was a muscle mutant we would expect sitters and lethal sitters to do poorly (have significantly lower behavioral scores) relative to rovers in *all* behavioral tests. Indeed we found that sitters and lethal sitters are fully capable of moving but that on yeast, they perform fewer crawls which result in shorter paths.

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