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Genetics

Mutations in the larval foraging gene affect adult locomotory behavior after feeding in *Drosophila melanogaster*

(behavioral genetics/polymorphism)

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ABSTRACT **Previous studies have shown a correlation between the locomotory component of larval and adult foraging behavior in the fruit fly. Here we show that this relationship is far more than mere correlation. It can be attributable to different alleles at the same genetic locus of the behavioral gene foraging (*for*). The *for* gene offers us the unique opportunity to study the genetic basis and evolutionary significance of a naturally occurring behavioral polymorphism. Until now, only the effect of *for* on *Drosophila melanogaster* larval behavior was studied. Larvae with the rover allele (for^R) move significantly more while eating during a set time period than those homozygous for the sitter alleles (for^s). Here, we show that rover and sitter larval strains derived from nature differ in the distance adults walk after feeding per unit time and that this variation results from different alleles at the foraging locus, the very gene originally defined on the basis of larval behavior. We hypothesize that *for* may be involved in the way flies evaluate a food resource.**

Little is known about how genes affect an animal's behavior throughout development; here we address how a single gene can have multiple but similar behavioral effects in very different life history stages. Larval and adult fruit flies can, for argument's sake, be considered to be different organisms. Complex reorganization of the larva's body, brain, and behavior occurs during metamorphosis—as in, for example, the mushroom body, a major neuropil area thought to be involved in adult insect odor assessment (1). The brain structure mutant mushroom-body-miniature shows defective olfactory learning in both larvae and adults (2). The behavior and ecology of larval and adult *Drosophila* show little similarity. The larva has a limited behavioral repertoire, feeding on yeast while moving through foraging substrates such as fruit. In contrast, the adult fly shows complex mating, egg-laying, and visually oriented behavior patterns. Despite these differences in life style and brain organization, we have found that the foraging gene alters the amount of locomotion during foraging in both the larval and adult fruit fly, *Drosophila melanogaster*. Larvae with the rover allele, for^R, have long larval trails while feeding in a yeast paste, whereas those homozygous for the sitter allele, for^s, have noticeably shorter trails (3). This difference in larval locomotion is not observed on an agar (nonnutritive) substrate. Therefore, we have described rover/sitter behavior as a difference in locomotion while foraging (3); it is expressed only when food is present in the environment and when larvae are feeding (4).

In the present paper, we examine adult fly walking behavior after feeding to determine whether the foraging locus affects adult behavior in a similar fashion to larval behavior. We found that adults homozygous for the for^R allele walk farther from a drop of sucrose after feeding per unit time than do adults homozygous for the for^s alleles. This difference in adult behavior is not found when flies are walking on a nonnutritive substrate. Neither larvae nor flies of the two morphs differ in their general activity or in muscle usage (4). We think that the foraging gene affects how larvae and adult flies perceive and/or evaluate food in their environment. Thus, understanding foraging may help elucidate processes important to the development of complex behavior in insects.

Here we use a genetic approach to show (*i*) that the foraging gene of *D. melanogaster*, originally identified through its effect on larval behavior, influences adult behavior, and (*ii*) that induced mutations in *for* affect both larval and adult locomotion when food is present in the environment.

METHODS

Strains. Strains were maintained in 250-ml plastic culture bottles on 45 ml of a dead yeast/sucrose/agar medium at 25°C ± 1°C, 15 ± 1 mbar (1 bar = 100 kPa) vapor pressure deficit, and a 12-h light/12-h dark photocycle with lights on at 0800 h.

Rover-larval (R) and sitter-larval (S) behaving *D. melanogaster* strains were obtained by collecting 500 adult flies from an orchard in the Toronto area. The population was reared in the laboratory and not allowed to go through bottle necks. After 1 year, the lengths of the foraging trails of 500 third-instar larvae were measured (as described in ref. 5; also see below). Individual rover and sitter behaving male larvae were used to produce homozygous for^R/for^R and for^s/for^s strains. Since *for* had been localized to chromosome 2 at cytological position 24A3-5 (6, 7), we crossed the sampled flies to a chromosome-2 balancer stock [$In(2LR)SM1,al^2\ Cy\ cn^2\ sp^2/In(2LR)bw^{V1},\ ds^{33k}\ bw^{V1}$; described in ref. 8] that had been repeatedly backcrossed (10 times) to the orchard population. The resulting lines had heterogeneous genetic backgrounds from the orchard population and were homozygous for either the for^R or the for^s allele. We verified this with crosses to laboratory rover and sitter larval strains and to the deficiency $Df(2L)ed^{Sz}$, which uncovers *for* (6).

de Belle *et al.* (6) produced two strains, $for^{s(R)136}$ and $for^{s(R)164}$, by irradiating a for^R/for^R strain (called BB) and selecting for sitter behaving larvae. These strains carried second-site lethal chromosome-2 mutations. We used genetic recombination with a lethal *for* mutation (made on a BB genetic background) to cross off these second-site lethal mutations. This resulted in two new sitter larval mutant strains, for^{s1}/for^{s1} and for^{s2}/for^{s2}, which were homozygous for their sitter mutations. In our behavioral studies, we use the BB strain as a control for the genetic background of these mutant strains.

Larval Behavior. The locomotory component of foraging behavior in third-instar larvae was quantified by a procedure described in ref. 5, which we briefly outline here. One

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hundred synchronous first-instar larvae (±1.5 h posthatch) were placed in Petri dishes containing 35 ml of culture medium, where they developed to third-instar larvae under standard conditions in 96 h (9). The maximum expression of genetically based differences between rovers and sitters occurs during this stage of larval development (10). At 120 h posthatch, larvae leave the food to pupate. Locomotory differences between rovers and sitters are not expressed during prepupation behavior (9). Foraging third-instar larvae were randomly sampled and individually tested in Petri dishes (8.5 × 1.4 cm) coated with a thin homogeneous layer of aqueous yeast suspension [distilled water and Fleischmann's bakers' yeast in a 2:1 (wt/wt) ratio]. The path length made by each larva while feeding during a 5-min test period was measured and recorded with a digitizer connected to an electronic graphics calculator.

Adult Behavior. The locomotory component of adult foraging behavior was quantified as described (11). Briefly, a 4- to 6-day-old fly that was starved for 4 ± 0.5 h was put on a 0.2-μl drop of 0.25 M sucrose in the center of an arena (1-m^2 glass sheet on a 60-cm concentric circular array with 30-cm-high white walls and a 40-W incandescent bulb hung 50 cm above center). We measured the distance (cm) the fly walked from the drop within 30 sec after feeding. The farthest concentric circle (in cm) from the drop that the fly reached was used as our distance measure. We chose to use a distance measure (rather than velocity) since a distance measure (path length) has been used as the norm in our measurements of larval behavior. Flies were never anesthetized. Data from flies that flew prior to the end of the 30-sec test were not included. There was no difference in the numbers of rovers and sitters that flew away or in the amount of time they spent ingesting the sucrose drop. Only 1 of ≈300 flies tested returned to the drop and began feeding during the test period. Visual inspection of the drop after the fly fed indicated that both rover and sitter flies appeared to ingest all of the sucrose drop.

To determine whether the differences in adult behavior were conditional on the fly being in a feeding environment, we measured adult walking behavior of all strains in a nonnutritive environment (modified from ref. 12). Briefly, 4- to 6-day-old flies were placed in individual vials with moist cotton wool 1 h prior to the test. Walking behavior was measured in a circular dish (diameter, 10 cm; height, 0.5 cm) whose lid had a 1-cm grid inscribed with a razor. Flies were never anesthetized. They were allowed to walk onto the lid of the dish, after which the lid was replaced. After a 1.5-min adjustment period, the number of squares that a fly walked into during the subsequent 1-min period was counted.

We measured the distance adult flies walked after eating for the R and S strains and their 14 reciprocal crosses. We measured larval and adult behavior for the BB rover laboratory strain for^R/for^R and the mutant strains for^{s1}/for^{s1} and for^{s2}/for^{s2}. All cross progeny were tested on 2 consecutive days. The effects of test day and fly sex on distance walked were not statistically significant, so the data were pooled. The reciprocal cross data were analyzed by using a contrast analysis of variance (see ref. 13 for an application of this technique and an explanation of the contrasts used). We also counted the number of squares the fly walked into in the nonfeeding environment for all strains. The effect of fly sex on the number of squares entered was not statistically significant, so the data were pooled. We also measured the path lengths of for^R/for^R and for^s/for^s on a 3% agar substrate.

RESULTS

Rover and sitter larvae can be found in the descendants of a wild population after collecting and rearing flies en masse in the laboratory. Adults from the R larval strain exhibited more rover-like adult behavior; they walked farther from the food source than adults from the S larval strain (Table 1). Reciprocal F_1 adult phenotypes showed intermediate behavior. The 16 reciprocal cross analysis between R and S strains showed that differences in adult behavior did not deviate from a simple autosomal pattern of inheritance ($F_{(1,236)}$ = 0.20; not significant) and that maternal factors, cytoplasmic factors, and sex chromosomes had no significant effect (Table 2) on this difference in behavior.

The larval and adult behavioral phenotypes of the rover-induced (for^R/for^R) and sitter-induced (for^{s1}/for^{s1} and for^{s2}/for^{s2}) mutant strains are shown in Table 3. Larvae of the rover strain have significantly longer larval paths than either of the sitter mutants (Student–Newman–Keuls test, $P < 0.05$). Similarly, adults of the rover strain walk significantly farther from the food source after eating than do those of the sitter mutant strains (Student–Newman–Keuls test, $P < 0.05$).

Table 1. Mean distance walked by *D. melanogaster* adults after feeding: Reciprocal crosses between rover (R) and sitter (S) strains

Female	Male	Sample size	Distance walked, cm $\bar{x}$	SE
Parent strains				
S	S	14	15.32	2.39
R	R	16	37.56	3.26
Reciprocal F_1 hybrids				
S	R	15	24.28	3.73
R	S	16	26.30	3.23
Reciprocal backcrosses				
S	S × R	15	26.73	4.00
S	R × S	16	29.12	2.50
R	S × R	15	26.27	3.91
R	R × S	16	28.00	4.00
S × R	S	16	34.38	4.15
R × S	S	16	24.62	3.04
S × R	R	16	32.44	3.45
R × S	R	16	26.53	3.86
Reciprocal F_2 hybrids				
S × R	S × R	16	22.47	3.44
S × R	R × S	16	33.09	3.57
R × S	S × R	16	21.72	3.64
R × S	R × S	16	31.38	4.11

Table 2. Analysis of variance of distance (cm) *D. melanogaster* adults walk after feeding: Reciprocal crosses between rover (R) and sitter (S) strains

Source	df	MS	F	*P*
Model (between crosses)	15	462.26	2.34	*
Error	236	197.41		
Comparisons				
Parental strains	1	3693.50	18.71	***
Dominance	1	20.48	0.10	NS
Reciprocal F_1s	1	32.60	0.17	NS
Y chromosome†	1	230.51	1.21	NS
X chromosome‡	1	3.75	0.02	NS
Cytoplasmic effects	1	24.38	0.12	NS
Maternal effects				
S ♀ compared to F_1 ♀	1	9.81	0.05	NS
R ♀ compared to F_1 ♀	1	0.00	0.00	NS
Deviation from an autosomal model				
All crosses	1	39.47	0.20	NS

*, $P < 0.01$; ***, $P < 0.0001$; NS, not significant; df, degrees of freedom; MS, mean square; MSE, mean square error; F, F statistic.
†Male data only; df = 15, 92; MSE = 190.61.
‡Female data only; df = 15, 128; MSE = 214.26.

Table 3. Behavioral phenotypes of *D. melanogaster* for^R/for^R, for^{s1}/for^{s1}, and for^{s2}/for^{s2} strains in the presence of food

	Larval behavior: Path length during 5 min, cm	Adult behavior: Distance walked during 30 sec postfeeding, cm
Strain from which mutants were derived		
for^R/for^R	17.83 ± 0.28 (197) A	36.17 ± 3.49 (12) A
Mutants		
for^{s1}/for^{s1}	10.00 ± 0.46 (50) B	17.38 ± 3.43 (12) B
for^{s2}/for^{s2}	10.89 ± 0.47 (50) B	22.38 ± 3.74 (12) B

Results are means ± SE (n). Means with different letters within larval and adult test data differ significantly ($P < 0.05$) by Student–Newman–Keuls test.

There were no strain differences in larval path lengths on agar, a nonnutritive substrate (for^R/for^R:$\bar{x}$ = 16.97, SE = 1.20, n = 25; for^s/for^s:$\bar{x}$ = 16.91, SE = 1.04, n = 25) as compared to yeast paste, a nutritive substrate (for^R/for^R:$\bar{x}$ = 17.80, SE = 0.35, n = 148; for^s/for^s:$\bar{x}$ = 6.33, SE = 0.24, n = 151). Similarly, Table 4 shows that there were no strain differences in adult walking behavior in the absence of food (analysis of variance; $F_{(4,72)} = 0.62$; $P = 0.65$).

DISCUSSION

Nagle and Bell (11) artificially selected for *D. melanogaster* adult rover and sitter behavior and found a correlated response in larval rover and sitter behavior, respectively. In addition, they showed that larvae previously shown to differ in rover and sitter behavior (3) also differed in adult foraging behavior. Although suggestive, these correlations did not provide evidence that *for* is involved in adult behavior. To address this, we mutagenized (6) BB, the for^R/for^R larval laboratory strain and isolated two new sitter larval mutant strains, for^{s1}/for^{s1} and for^{s2}/for^{s2}. We then tested the adult behavior of these mutant strains. Thus, if mutagenesis resulted in a change in both larval and adult foraging behavior, this could only be due to a change in alleles at *for*. Indeed, for^{s1}/for^{s1} and for^{s2}/for^{s2} showed a significant reduction in the distance adults walked after feeding from that of for^R/for^R flies. The adult behavior of the sitter mutants provides strong evidence that *for*, originally defined through its effect on larval behavior, also influences adult behavior. The findings of intermediate F_1s in adult behavior (14) and complete dominance of the rover phenotype in larval behavior (5) do not conflict with this conclusion since the same allele can have different dosage effects on different phenotypes (15).

Table 4. Adult walking behavior in the absence of food: Mean number of squares entered in 1 min by *D. melanogaster* strains that differ in alleles at foraging

Strains	Squares entered during 1-min test, mean ± SE (n)	SNK grouping
Derived from nature		
Rover (R)	20.19 ± 3.78 (16)	A
Sitter (S)	16.60 ± 3.07 (15)	A
Laboratory		
for^R/for^R	17.53 ± 2.02 (15)	A
for^{s1}/for^{s1}	17.73 ± 3.61 (15)	A
for^{s2}/for^{s2}	13.56 ± 2.58 (16)	A

Student–Newman–Keuls test (SNK) groupings show no significant difference between strains. Means with the same letter do not differ significantly.

What is the underlying mechanistic basis of the differences between rovers and sitters? As larvae, rovers and sitters move equally well on nonnutritive substrates, grow at the same rate, reach the same size at pupation (10), and perform normally in tests for muscular defects (4).

The difference in behavior that we report here involves how far the fly walks away from a food resource after feeding on it. Sitters spend more time circling around the sucrose drop after feeding compared with rovers, which walk away from it. If a fly spends more time circling around the resource, as sitters do, then it is more likely to find food resources that are tightly packed. In contrast, if a fly walks in a straight line away from the resource, then it is more likely to find resources that are in dispersed patches (16, 17). In nature, rovers may be better able to exploit environments where food is distributed in patches, whereas sitters may be better able to exploit environments where food is homogeneously distributed, without wasting energy in locomotion.

This difference in walking behavior after feeding may indicate how flies perceive the quality of the resource. In our experiment, adult rovers and sitters ingest the same concentration of sucrose but appear to differ in their evaluation of resource quality. Sitters stay close to the resource, whereas rovers move on to "better" drops elsewhere. One hypothesis is that rover and sitter responses reflect differences in how flies evaluate the environment. Examining the larval and adult behavior of flies with various central brain mutations may help correlate differences in locomotion during feeding behavior with changes in specific structures of the fly brain during metamorphosis (18). Interestingly, most neuron cell bodies and the shape of certain neuropil regions are conserved in the central brain, thought to be the control center for behavior (19). Further behavioral and molecular analysis of *for* will help us understand how it exerts pleiotropic effects on both larval and adult behavior.

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1. Technau, B. & Heisenberg, M. (1982) *Nature (London)* **295**, 405–407.
2. Heisenberg, M., Borst, A., Wagner, S. & Byers, D. (1985) *J. Neurogenet.* **2**, 1–30.
3. Sokolowski, M. B. (1980) *Behav. Genet.* **10**, 291–302.
4. Sokolowski, M. B. & Hansell, K. P. (1992) *Genetica* **85**, 205–209.
5. de Belle, J. S. & Sokolowski, M. B. (1987) *Heredity* **59**, 73–83.
6. de Belle, J. S., Hilliker, A. J. & Sokolowski, M. B. (1989) *Genetics* **123**, 157–163.
7. de Belle, J. S., Sokolowski, M. B. & Hilliker, A. J. (1992) *Genome* **36**, 94-101.
8. Lindsley, D. L. & Grell, E. H. (1968) *Genetic Variations of Drosophila melanogaster* (Carnegie Inst. of Washington, Washington, DC), Publ. 627.
9. Sokolowski, M. B., Kent, C. & Wong, J. (1984) *Anim. Behav.* **32**, 645–651.
10. Graf, S. & Sokolowski, M. B. (1989) *J. Insect Behav.* **2**, 301–313.
11. Nagle, K. J. & Bell, W. J. (1987) *Behav. Genet.* **17**, 385–408.
12. Connolly, K. (1966) *Anim. Behav.* **14**, 444–449.
13. Sokolowski, M. B. & Bauer, S. J. (1989) *Heredity* **62**, 177–183.
14. Bell, W. J. & Nagle, K. (1987) *Drosoph. Inf. Serv.* **66**, 22–23.
15. Kyriacou, C. P., Burnet, B. & Connolly, K. (1978) *Anim. Behav.* **26**, 1195–1206.
16. Tortorici, C. & Bell, W. J. (1988) *J. Insect Behav.* **1**, 209–223.
17. Bell, W. J. (1990) *Annu. Rev. Entomol.* **35**, 447–467.
18. Heisenberg, M. (1989) *Prog. Zool.* **37**, 3–45.
19. White, K. & Kankel, D. R. (1978) *Dev. Biol.* **65**, 296–321.