Chaser (*Csr*), a New Gene Affecting Larval Foraging Behavior in *Drosophila melanogaster*

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ABSTRACT

Chaser (*Csr*) was uncovered in a gamma mutagenesis screen to identify genes that modify the larval foraging behavior of sitters to rovers. Rover larvae have significantly longer path lengths than sitters while foraging on a yeast and water paste. This difference is influenced by one major gene, *foraging* (*for*), which has two naturally occurring alleles, for^R (rover) and for^s (sitter). In a mutagenesis screen for modifiers of *for*, we identified three lines with viable mutations on chromosome *3* that alter foraging behavior. Each of these mutations increased larval path lengths in for^s/for^s larvae in a dominant fashion, and were not separable by recombination. These mutations are therefore probably allelic and define a new gene that we have called *Csr*. *Csr* was genetically localized using the lethal-tagging technique. This technique resulted in seven lines with a significant decrease in larval path-length and recessive lethal mutations on chromosome *3*. We refer to these as reverted Csr (Csr^{rv}) lines. Deficiencies that uncovered cytologically visible chromosome rearrangements in three of the seven reverted lines were used in a complementation analysis. In this way we mapped the lethal mutations in the Csr^{rv} lines to cytological region 95F7–96A1 on the right arm of chromosome *3*.

DROSOPHILA melanogaster has been extensively used as a model to investigate the biological processes involved in an organisms response to environmental stimuli (SOKOLOWSKI 1992). *D. melanogaster* is an ideal model for behavioral genetic analysis. It displays a large number of both complex (reviewed in HALL 1985; KYRIACOU 1990) and simple behaviors (for example SAWIN *et al.* 1994). The genetic basis of a number of complex behaviors, including male and female specific behaviors during courtship and mating, biological rhythms, and learning and memory, have been partially characterized in *D. melanogaster* (for a comprehensive review, see HALL 1994). Generally, the approach used to determine the genes involved in such behaviors includes altering the normal or wild-type behavior by mutagenesis and then determining which genes were affected (BENZER 1967, 1973).

In our laboratory, we are interested in characterizing the underlying genetical, molecular and physiological processes involved in the larval and adult foraging behavior of *D. melanogaster*. Larval foraging behavior is measured as the distance individual larvae travel on a yeast and water paste over a 5-min period. We call this distance path length. Unlike most behavioral phenotypes studied thus far, there are two distinct naturally occurring phenotypic variants in foraging behavior (SOKOLOWSKI 1980). One variant, the "rovers," travel significantly further than the "sitters" while foraging on the yeast and water paste (SOKOLOWSKI 1980; DE BELLE *et al.* 1989, 1993). Interestingly, there are also differences in the way adult flies forage. Adult rovers move significantly further away from the food source in the 30-sec period after eating than sitters (PEREIRA and SOKOLOWSKI 1993). There are no significant differences between rovers and sitters in larval or adult locomotion on nonnutrient substrates (SOKOLOWSKI and HANSELL 1992; PEREIRA and SOKOLOWSKI 1993), or in developmental time and growth rates (GRAF and SOKOLOWSKI 1989). Also, both rovers and sitters are found in nature in the Toronto area and in a number of geographically dispersed populations (SOKOLOWSKI 1980; CARTON and SOKOLOWSKI 1992).

When exposed to an equal amount of yeast and water paste of the same concentration, sitter larvae reduce the locomotory component of foraging to a greater extent than do rovers. The rate of shoveling with the larval mouth-hooks (feeding rate) does not significantly differ between rover and sitter strains (SOKOLOWSKI and HANSELL 1992). Rover larvae move between food patches while foraging, whereas sitters go to the nearest food patch and remain feeding there (SOKOLOWSKI *et al.* 1983). Furthermore, rover adults spend less time in local searching (near the recently injested sucrose drop) than sitter adults (NAGLE and BELL 1987; PEREIRA and SOKOLOWSKI 1993). We speculate that the behavioral difference between rovers and sitters results from differences in some aspect of the sensorimotor transformation processes by which they perceive and respond to foraging substrates.

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The behavioral difference between rovers and sitters in nature is attributable to one major gene, *foraging* (*for*), and a number of minor genes (DE BELLE and SOKOLOWSKI 1987, 1989). There are two alleles of *for* in nature, for^R (rover) and for^s (sitter). In larvae, for^R shows complete genetic dominance to for^s (DE BELLE *et al.* 1989). Because of the difficulty in mapping quantitative behavioral phenotypes that do not have discrete distributions, we developed a technique called "lethal tagging," which enabled us to genetically map *for* to cytological region 24A3–5 on chromosome *2* (DE BELLE *et al.* 1989, 1993). In this way, a number of lethal alleles of *for* were generated that were localized using deletion mapping.

We want to characterize other genes that modify the foraging behavior phenotype as they may be involved in the same or a related biological pathway as *for*. In this paper, we show how we induced and genetically characterized three viable mutations that alter foraging behavior in a dominant fashion. Each of these mutations increased larval path lengths in for^s/for^s larvae and were not separable by recombination. These three mutations are therefore probably allelic and define a new gene, which we have called *Chaser* (*Csr*). Here, we describe how *Csr-1, -2* and *-3* alleles were initially recovered in an attempt to mutate the for^s allele and cause an increase in path length; how seven recessive lethal revertants (Csr^{rv}) of Csr-3 were generated and genetically characterized; and how we localized *Csr-3* to a small region on the right arm of chromosome *3* at cytological position 95F7–96A1 using deletion mapping of the recessive lethals associated with the Csr^{rv} lines.

MATERIALS AND METHODS

Strains and chromosomes: The standard sitter and rover *D. melanogaster* strains EE and BB are isogenic for chromosomes *2* and *3* and homozygous for the for^s and the for^R alleles, respectively (SOKOLOWSKI 1980; DE BELLE and SOKOLOWSKI 1987). The standard sitter strain (EE) is also homozygous for a recessive marker $ebony^{11}$ on the third chromosome, resulting in a dark body color in homozygous adult flies. The *ebony* marker does not affect larval locomotory or foraging behaviors. The second chromosome balancer strain *In(2LR)SM1,* al^2 *Cy* cn^2 $sp^2/In(2LR)bw^{V1}$, ds^{33k} bw^{V1} (hereafter referred to as $SM1/bw^{V1}$) was used in the first mutagenesis screen. The second and third chromosome balancer strain $Pu^2/In(2LR)SM5$, al^2 *Cy* lt^w sn^2 sp^2; *Ly/In(3LR)TM3,* y^+ *ri* p^p *sep l(3)89Aa* bx^{34e} *e* (hereafter referred to as $Pu^2/SM5$, *Cy*; *Ly/TM3, Sb*) was used in all further chromosome manipulations. A for^s/for^s; *Ly/TM3, Sb* stock was made and used as a third chromosome balancer strain. The chromosome *3* deficiencies *Df(3L)*81K19, *Df(3L)*Pc-MK, *Df(3R)*e-N19, *Df(3R)*crb87–4, *Df(3R)*crb87–5, and *Df(3R)*XS were obtained from the Bloomington Stock Center. All the above mutations and chromosomal rearrangements are described in LINDSLEY and ZIMM (1992). Cytological analysis was done to confirm the reported break points of the deficiencies shown in Figure 6. All strains were maintained in plastic culture bottles on 45 ml of a dead yeast, sucrose and agar (culture) medium at 25 ± 1°, 15 ± 1 mbar vapor pressure deficit and an L:D 12:12 photocycle with lights on at 0800 hours.

Larval foraging behavior: The locomotory component of foraging third instar larvae was quantified using procedures modified from DE BELLE and SOKOLOWSKI (1987) and DE BELLE *et al.* (1989, 1993); these procedures will be described here. Black rectangular plexiglass plates (25 cm width, 37 cm length, 0.5 cm height) with six circular 0.5-mm deep circular wells of standard petri-dish dimensions (4.25-cm radius) were used to record larval path-lengths. The six circular wells are arranged in a 2 × 3 ($x \times y$ axis) fashion on the plexiglass plates. A homogeneous yeast suspension (distilled water and Fleischmann's bakers' yeast in a 2:1 ratio by weight) was spread evenly over the plates, thereby filling the circular wells. Foraging third instar larvae (96 ± 1.5 h posthatching) were individually placed in the center of the coated wells, and the wells were covered with petri-dish lids. After 5 min, the path lengths of the foraging larvae were traced onto the petri-dish lids for further analyses. Four plates (each with six wells) were used for each run, so that a maximum of 24 path lengths were traced per 5-min run. The distance each larva travelled in a 5-min period was measured, and the path lengths of each strain were statistically analyzed in comparison with concurrently tested standard sitter and rover strains. This allowed us to classify each strain as rover or sitter behaving.

Larval activity: The general locomotion of rover, sitter and Csr larvae was measured as the total distance individual larvae travelled on agar during a 5-min period. To measure this component of larval locomotion, we coated the bottoms of standard petri dishes with a homogeneous agar solution (1.6 g agar:100 ml H_2O) and allowed it to harden. Foraging third instar larvae (96 ± 1.5 h posthatching) were individually placed in the center of the coated petri dishes, covered with petri-dish lids, and allowed to move for a 5-min period [sample size (n) was 25 larvae per strain].

Statistics: All path lengths were analyzed using one-way analysis of variance (one-way ANOVA). To determine which strains differed significantly in path length, a student-Newman-Keuls (SNK) was used as an *a posteriori* test. Statistical analyses were done using SAS (SAS INSTITUTE 1990).

RESULTS

Initial mutagenesis that led to uncovering *Chaser*: A mutagenesis screen was performed to induce a lethal mutation in or adjacent to the for^s allele and screen for an associated change in behavior from sitters to rovers and to identify other autosomal genes that interact with *for*. We screened 5000 progeny from a cross between gamma irradiated (2000 rads) for^s/for^s males and for^s/for^s virgin females for a change in behavior from sitter to rover. Individual male progeny from this cross with rover-like path lengths were used to establish separate lines with balanced second chromosomes (see Figure 1). Each line was then screened for recessive lethal mutations on chromosome *2*, by selecting lines in which only adults with a balanced second chromosome (*Curly, Cy*) emerged. Lines without recessive lethals were discarded. To determine if the induced lethal mutations on chromosome *2* mapped to the *for* gene, complementation analyses were done between each of lines and the previously characterized lethal alleles of *for* (DE BELLE *et al.* 1989, 1993).

We recovered three independently isolated lines with significantly longer path lengths than for^s/for^s, which had recessive lethal mutations on chromosome *2*. Based

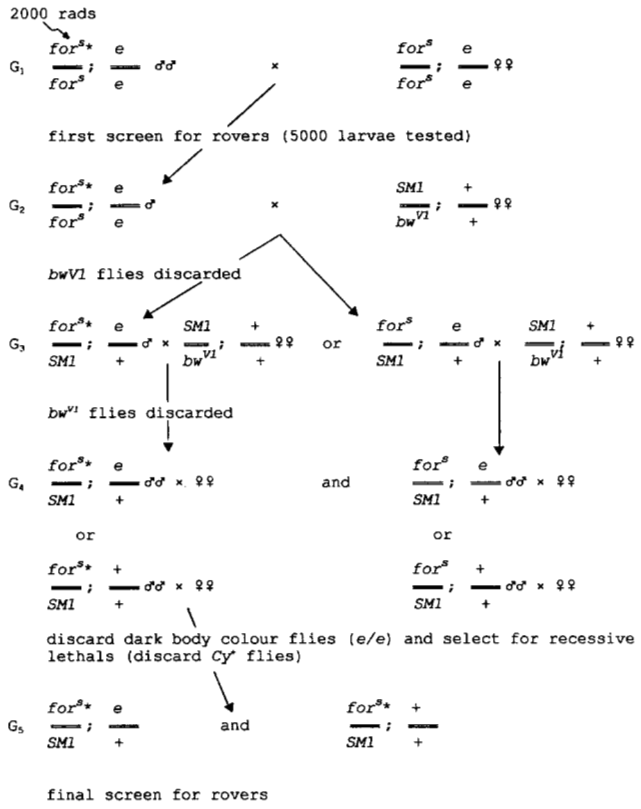

FIGURE 1.—Mutagenesis that led to uncovering *Chaser*. The standard sitter strain was used to attempt to lethal-tag for^s. The *ebony* marker on the third chromosome was selected out after mutagenesis. The second chromosome of these strains is designated for^s*, representing a chromosome *2* carrying a lethal allele, generated in a sitter background. The recessive lethal mutations on chromosome *2* were maintained using a second chromosome balancer (*SM1*), which has a dominant marker, *Curly* (*Cy*) to aid in identifying flies that carry the balancer chromosome. *SM1/SM1* and for^s*/for^s* are lethal. Chromosome *2* of the mutant strains is therefore balanced and maintained as $for^s*/SM1$. These flies were backcrossed to the standard sitter strain to screen for behavior. Strains with recessive lethal mutations were selected by discarding strains in which for^s*/for^s* (Cy^+, straight wings) survived. See text for further details.

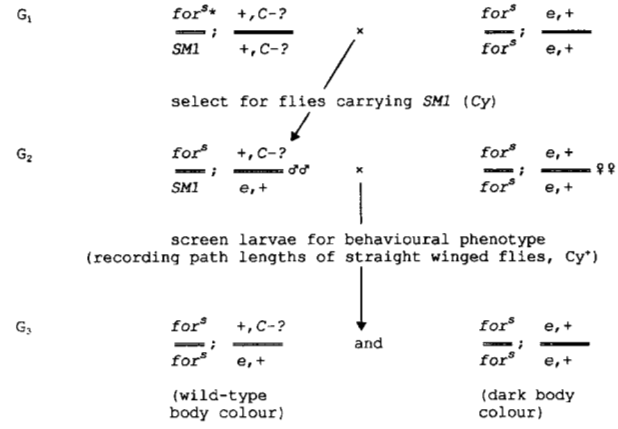

FIGURE 2.—Crossing scheme used to determine the chromosomal location of the mutation causing behavioural effect in C-1, C-2, and C-3. The second chromosome from the irradiated lines (for^s*) was substituted for a standard sitter chromosome in the first cross. The putative mutation is shown on chromosome *3* by C-?. We compared path lengths of larvae with one chromosome *3* from the three lines (C-1, C-2, C-3) to those without the irradiated chromosome *3* (*e/e, ebony* body color). We found that the presence of an irradiated chromosome *3* caused a significant increase in larval path length (see Table 2). This allowed us to map the mutations in all three lines to the third chromosome.

on the phenotype of the three lines, we classified them as *Chaser* strains and named them C-1, C-2 and C-3. Complementation analyses among the three irradiated lines and lethal alleles of the *for* gene showed that these newly induced lethals complemented with each other and were not allelic to *for*. The nature of the mutagenesis and screening procedures ensured that the resulting lethal lines had nonirradiated *X* chromosomes derived from sitters. However, it was possible that the mutations that resulted in a rover-like phenotype in a dominant fashion mapped to the other major autosome, chromosome *3*.

Chromosomal localization of *Chaser*: To determine whether the induced mutations that resulted in increased path lengths in the three lines mapped to the second or to the third chromosome, a crossing scheme and behavioral assay were followed as shown in Figure 2. The second chromosomes from the irradiated lines were substituted with standard sitter second chromosomes in the first cross. We then compared the path lengths of larvae with one copy of chromosome *3* from the irradiated strains to their concurrently tested sibs with standard sitter third chromosomes (e^{11}/e^{11}, *ebony*). This allowed us to determine if the behavioral effect was in fact due to a mutation on chromosome *2* or the other major autosome, chromosome *3*. We found the larval path lengths to be significantly longer in larvae with one copy of chromosome *3* from the irradiated strains compared with those of their genetically sitter sibs (Table 1). Therefore, the mutations that result in increased path length mapped to chromosome *3* in all three lines.

We derived iso-*3* chromosome lines from the three original *Chaser* strains. These iso-*3* lines exhibited a rover phenotype in a sitter (for^s/for^s) genetic background. Furthermore, there were no significant differences in the mean path lengths of for^s/for^s larvae carrying one or two copies of irradiated third chromosomes. One iso-*3* line from each of the three mutant strains was used for further genetic analyses. These three lines did not complement for behavior (see below) and are therefore likely allelic. We designate the three homozygous mutant strains as Csr-1, Csr-2 and Csr-3, derived from C-1, C-2 and C-3, respectively.

Behavioral effects of *Chaser* on rovers and sitters: To test the behavioral effects of the induced mutations on larval foraging behavior, we measured the path lengths of rovers and sitters carrying one copy of chromosome *3* from each of the *Chaser* iso-*3* lines. The path lengths

TABLE 1

Chromosomal location of the mutation responsible for the increase in larval path length in the original *Chaser* lines

Larval genotype	Path length (cm)	*P*
for^R/for^R; +/+	10.75 ± 0.60 (25)	0.0001
for^s/for^s; *e*, +/*e*, +	4.51 ± 0.32 (25)	
for^s/for^s; +, C-1/*e*, +	8.60 ± 0.52 (32)	0.0052
for^s/for^s; *e*, +/*e*, +	6.24 ± 0.61 (22)	
for^s/for^s; +, C-2/*e*, +	10.96 + 0.49 (25)	0.0001
for^s/for^s; *e*, +/*e*, +	7.41 ± 0.64 (15)	
for^s/for^s; +, C-3/*e*, +	8.56 ± 0.50 (34)	0.0004
for^s/for^s; *e*, +/*e*, +	5.36 ± 0.64 (15)	

Larval path lengths differ significantly (one way-ANOVA) in strains with standard sitter third chromosomes (*e/e, ebony*) compared with strains carrying third chromosomes from C-1, C-2, or C-3. The genotypes of the second and third chromosomes are shown in the first column. Path lengths are means ± SE, with number of larvae in parentheses.

of homozygous sitter and heterozygous rover larvae carrying one copy of the mutant third chromosome were measured with the concurrently tested standard rover and sitter strains. We have previously shown that for^R is completely dominant to for^s in larval foraging behavior (DE BELLE and SOKOLOWSKI 1987; DE BELLE *et al.* 1989). Larvae of the genotypes for^R/for^R and for^R/for^s behave as rovers to the same degree. We could therefore compare the foraging behavior of heterozygous rover larvae carrying one copy of the mutant third chromosome to standard rovers. The presence of one copy of a third chromosome from Csr-1 or Csr-2 significantly increased mean path length in rover larvae. The presence of one copy of chromosome *3* from any of the Csr-1, Csr-2 or Csr-3 lines significantly increased the path length of homozygous for^s/for^s larvae (Table 2).

To ensure that the observed increase in path length was attributable to foraging behavior and not a result of heightened general activity in the Csr lines, we analyzed the general locomotion of rover, sitter and *Chaser* larvae in the absence of food. The mean distance traveled (centimeters) ± standard error of standard rover (for^R; + = 11.99 ± 0.69) and sitter (for^s; *e*,+ = 13.66 ± 1.06) larvae did not differ from that of Csr-1 (for^s; *Csr-1* = 11.87 ± 0.91), Csr-2 (for^s; *Csr-2* = 14.41 ± 0.72) and Csr-3 (for^s; *Csr-3* = 13.07 ± 0.97) in the absence of food [one-way ANOVA, $F_{(4,120)} = 1.51$, $P = 0.2$]. The foraging behavioral effects of *Chaser* are therefore not due to heightened general activity.

Analyses of the possible allelism in Csr-1, Csr-2 and Csr-3: To determine whether the *Chaser* strains result from mutations on different genes on the third chromosome, we tested for recombination among the three dominant behavioral mutations on chromosome *3*. We did this by analyzing the behavior of progeny from crosses between heterozygous females carrying different combinations of each of the three mutations (for^s/for^s; +,*Csr-1*/+,*Csr-2* for^s/for^s; +,*Csr-1*/+,*Csr-3*, and for^s/for^s; +,*Csr-2*/+,*Csr-3*) and standard sitter (for^s/for^s; *e*,+/*e*,+) males (Figure 3). The frequency distributions of larval path lengths of the progeny of these crosses were compared with those of heterozygous for^s/for^s; +,*Csr*/*e*,+ and standard sitters for evidence of recombination. The frequency distributions of for^s/for^s; +,*Csr-1*/*e*,+, for^s/for^s; +,*Csr-2*/*e*,+ and for^s/for^s; +,*Csr-3*/*e*,+ were indistinguishable, and therefore pooled (Figure 3b). Recombination between the mutations would be evident if there was a decrease in mean larval path lengths, a greater variance in the frequency distributions of path lengths, and bimodality in path-length frequency distributions of the backcross progeny.

TABLE 2

Behavioral effects of *Csr* on for^R/for^s and for^s/for^s larvae

Larval genotype	Larval path length (cm)
for^R/for^s; *Csr-1*/+	12.33 ± 0.83 (24)
for^s/for^s; +, *Csr-1*/*e*, +	12.32 ± 1.26 (12)
for^s/for^R; *Csr-2*/+	11.91 ± 1.02 (25)
for^s/for^s; +, *Csr-2*/*e*, +	9.97 ± 0.57 (19)
for^s/for^R; *Csr-3*/+	9.23 ± 0.55 (32)
for^s/for^s; +, *Csr-3*/*e*, +	13.24 ± 0.37 (24)
for^R/for^R; +/+	8.00 ± 0.64 (25)
for^s/for^s; *e*, +/*e*, +	4.96 ± 0.47 (23)

Homozygous iso-3 lines were crossed to the standard rover and sitter strains. The genotypes of the second and third chromosomes are shown in the first column. In all cases, the presence of one copy of the third chromosome from *Csr-1*, *Csr-2* or *Csr-3* significantly increased for^s/for^s (sitter) larval path lengths. The presence of one copy of *Csr-1* or *Csr-2* significantly increased the path lengths of rover larvae (ANOVA, $F(7,176) = 14.24$; $P < 0.0001$). Path lengths are means ± SE, with number of larvae in parentheses.

The frequency distributions of larval path lengths in progeny from a cross between females heterozygous for *Csr-1*/*Csr-2*, *Csr-1*/*Csr-3*, or *Csr-2*/*Csr-3* and standard sitter males was not indicative of recombination (Figure 3, see figure caption for details). In all three cases, there was no decrease in the mean path lengths in the progeny of these crosses nor was there any evidence of bimodality in path-length frequency distributions. The variance in path-length frequency distributions did not differ from that of the *Csr*/+ control in all three test crosses [$F_{\max(4,303)} = 2.22$, $P > 0.1$]. However, the frequency distribution of *Csr-2*/*Csr-3* had a higher variance than the heterozygous *Csr*/+ control. We therefore progeny tested all larvae from this cross with path lengths of ≤7 cm. Nine larvae with sitter-like path lengths were backcrossed to standard sitters, and their progeny were tested. The sample sizes of the progeny tested varied between 10 and 45 larvae, and in all cases, the proportion of rovers to sitters was approximately equal. Therefore, the results of progeny testing support the notion that recombination did not occur between *Csr-2* and *Csr-3*. Taken together, our results strongly suggest that the three mutations in Csr-1, Csr-2 and Csr-3 either map to the same locus or are tightly linked.

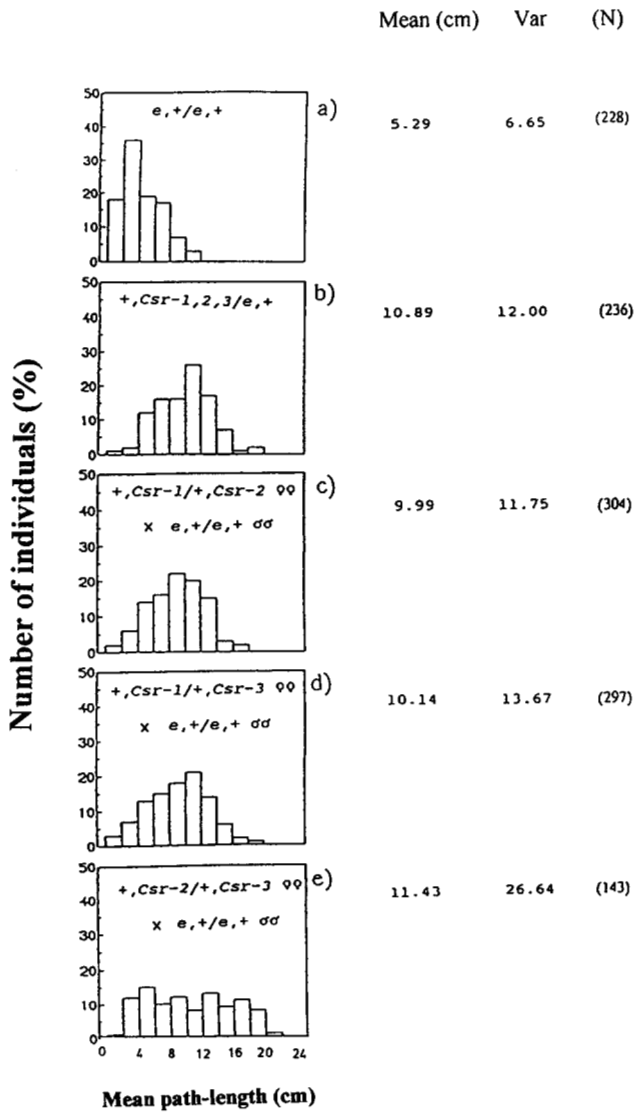

FIGURE 3.—Frequency distributions of sitter larvae (a) pooled iso-*3* lines heterozygous with standard sitter (*for*^{s}/*for*^{s}; +,*Csr-1,2,3*/*e*,+) (b) and the offspring of F_1 mutant females (*for*^{s}/*for*^{s}+,*Csr-1*/+,*Csr-2 for*^{s}/*for*^{s}; +,*Csr-1*/+,*Csr-3*, and *for*^{s}/*for*^{s}; +,*Csr-2*/*Csr-3*) backcrossed to sitter males (*for*^{s}/*for*^{s}; *e*,+/*e*,+) (c,d, and e, crosses shown on figure). All copies of chromosome *2* are *for*^{s}. If the induced mutations in the three lines (C-1, C-2 and C-3) map to different sites, we would expect to find third chromosome recombinants. This would result in a decrease in mean path length, a bimodal frequency distribution, and a greater variance in mean path length. The results do not show evidence of recombination. There is no significant difference in mean path lengths between b and c, d, and e. Furthermore, none of the frequency distributions showed bimodality. The distribution shown in e does have a significantly higher variance than the others. We therefore progeny tested all larvae from this cross with a path length of ≤7 cm. Progeny testing was done by individually back-crossing each of these larvae to standard sitters, and testing their larval behaviour. If *Csr-2* and *Csr-3* are not separable by recombination, then the proportion of sitter behaving larvae from this cross should be one-half. We found that the path lengths of larvae from the above cross were approximately half rover-like and half sitter-like, confirming the lack of recombination between *Csr-2* and *Csr-3*.

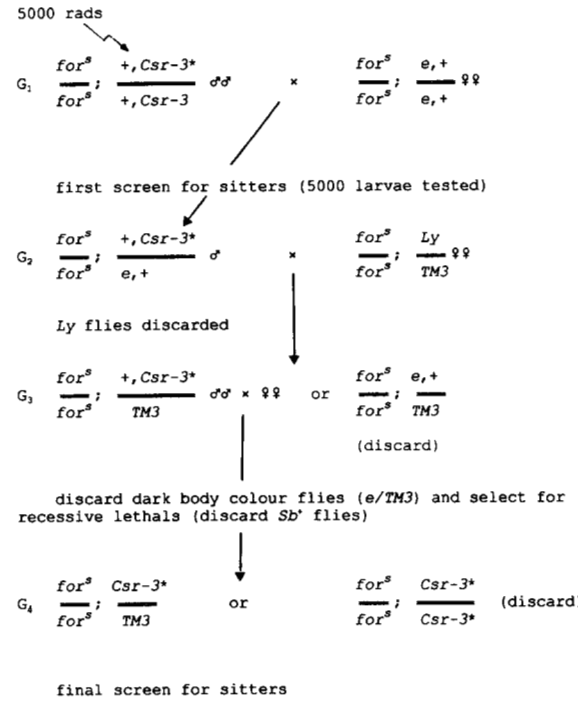

FIGURE 4.—Lethal tagging of *Csr-3*. We irradiated *Csr-3* to induce a lethal mutation in or tightly linked to Csr that was associated with a reversion in behavior from rover back to sitter. Male flies with the genotype *for*^{s}/*for*^{s}; +,*Csr-3*/+,*Csr-3* were irradiated and subsequently crossed to standard sitter virgin females. The male progeny of this cross were screened for a change in behavior from rover to sitter, and a recessive lethal mutation on chromosome *3*. See text for further details.

Lethal tagging of the *Chaser* gene: To localize *Csr*, we reverted the *Chaser* mutation associated with Csr-3 by the lethal-tagging method (DE BELLE *et al.* 1989). Briefly, *for*^{s}/*for*^{s}; +,*Csr-3*/+,*Csr-3* males were irradiated with 5000 rads of gamma radiation and crossed to *for*^{s}/*for*^{s}; *e*,+/*e*,+ virgin females (Figure 4). From this cross, 5000 progeny were screened for a change in larval behavior from rover to sitter. Individual male progeny from this cross with sitter-like path lengths were used to establish separate lines with balanced third chromosomes (see Figure 4). Each line was then screened for recessive lethal mutations on chromosome *3* by selecting lines in which only balanced third chromosome adults (*Stubble, Sb*) eclosed. Lines without recessive lethals were discarded.

Gamma induced Csr-3 derivatives with behavioral alterations from rovers to sitters and recessive lethal mutations on chromosome *3* could be due to random lethal mutations on the third chromosome that occurred as second-site events along with nonlethal mutations in *Csr-3* that alter the larval phenotype from rover to sitter or lethal mutations (such as deletions) in or close to the *Csr-3* gene that cause a lethality along with a reversion of the dominant *Csr-3* allele (rover-like) to a sitter-like larval foraging behavior. To separate these two events, and to determine if any of the resulting lethals share a common lethal locus, we conducted pairwise comple-

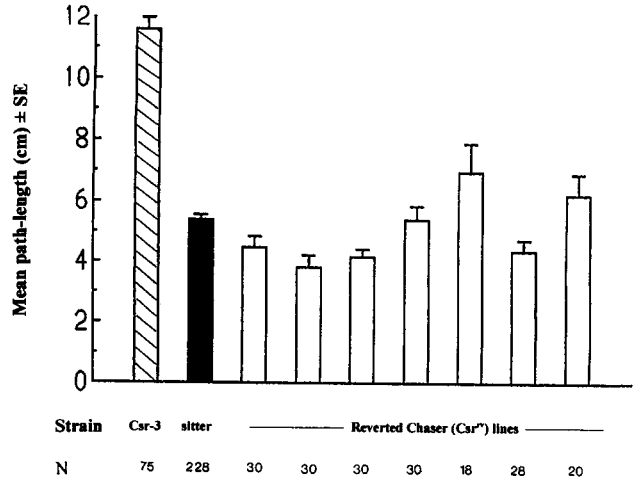

FIGURE 5.—Mean larval path lengths (centimeters) ± standard error of the seven Csr^{rv} lines compared with *Csr-3* and standard sitters. All copies of the second chromosome are *for*^{s}. Chromosome *3* genotypes are +,*Csr-3*/*e*,+ and *e*,+/*e*,+ for Csr-3 and standard sitter, respectively. The seven Csr^{rv} lines are shown in order, from Csr^{rv1} to Csr^{rv7}. Sample sizes are shown on the figure.

mentation tests for lethality at 25 and 29° with each of the lines carrying recessive lethals. Independently derived lines that share a lethal locus and a behavioral alteration from rover to sitter should define the *Csr-3* gene.

We identified 23 lines with an alteration in foraging behavior from rover to sitter and a recessive lethal mutation on chromosome *3* using the scheme outlined in Figure 4. All 23 recessive lethals were homozygous lethal at 25°. Seven of these 23 lines failed to complement for viability with one another in all combinations at 29°, but complemented fully at 25°. The path lengths of the seven lines that did not complement for lethality at 29° are presented in Figure 5.

Cytological analysis of the reverted *Chaser-3* lines: Gamma radiation often results in chromosomal rearragements that are detectable in the salivary chromosomes. We therefore cytologically characterized the salivary chromosomes of the reverted Csr-3 (Csr^{rv}) lines in *Csr*^{rv}/+ heterozygotes. Lines with putative translocations between the second and third chromosomes were further characterized by crossing males with one copy of the irradiated second and third chromosomes heterozygous with a dominant marker on chromosomes *2* and *3* to wild-type females (*i.e.*, *Pu*/*Csr*^{rv}; *Ly*/*Csr*^{rv} ♂♂ x +/+; +/+ ♀♀). Deviations from the expected 1:1:1:1 phenotypic ratio in the progeny of this cross indicate the presence of a translocation between the second and third chromosomes.

Salivary chromosome squashes showed rearrangements in three of the seven Csr^{rv} lines. One line, Csr^{rv2}, had three breakpoints at cytological positions 73B2–5, 79D2-E1, and 96A2–6, as well as a heterochromatic break. The new order is 100–96A|79D-96A|73B-61 plus an insertion of 73B2–5-79D2-E1 into heterochromatin. We showed that this insertion was in chromosome *2* genetically. Csr^{rv2} is therefore a complex translocation (data not shown). The second line associated with a rearrangement, Csr^{rv4}, had one heterochromatic breakpoint and one breakpoint at 50A4–10. It was shown genetically that the heterochromatic break was in chromosome *3*. Thus Csr^{rv4} is a simple reciprocal translocation. The third line associated with a rearragement, Csr^{rv7} , had two breakpoints, one heterochromatic break in chromosome *3* and one euchromatic break at cytological position 93F2–4. Csr^{rv7} is therefore either a paracentric inversion or a T(3;4).

Deficiency mapping of the lethal-tagged Csr-3 revertants: Lethal complementation analyses were performed utilizing the seven members of the complementation group identified among the Csr^{rv} lines and deficiencies that uncovered chromosomal breaks detected in the cytological analysis of these. However, we determined the left breakpoint *Df(3R)*crb87–4 to be 95D1–2, and not 95F15 as reported by LINDSLEY and ZIMM (1992). All the deficiencies used were first made heterozygous with the same balancer chromosome (*TM3, Sb*) that was used to balance the lethals. Deficiencies were tested for complementation with the Csr^{rv} lethals at both 25 and 29°. Complementation was detected by the presence of *Sb*$^{+}$ progeny at the expected frequencies.

The complementation map that resulted from the deficiency analyses of the Csr^{rv} lines is shown in Figure 6. The overlapping deletions *Df(3R)*crb87–4 and *Df(3R)*crb87–5 did not complement for viability with all seven Csr^{rv} lines at 29°, although there was full complementation for viability at 25°. Interestingly, although Csr^{rv2} is fully viable when heterozygous with both deficiencies at 25°, flies heterozygous for *Csr*^{rv2}/*Df(3R)*crb87–4 and *Csr*^{rv2}/*Df(3R)*crb87–5 are sterile. Because of the lack of complementation of the Csr^{rv} lines with deletions that uncover the *crumbs* (*crb*) gene, we tested the complementation pattern of a lethal allele of *crb* (*crb*^{11A}) and the seven revertant lines. *crb*^{11A} complemented fully for viability with all seven Csr^{rv} lines at both 25 and 29°. The temperature-sensitive recessive lethals associated with Csr-3 revertants may therefore define a new gene that lies between 95F7–96A1 on the right arm of chromosome *3*.

We also performed pairwise complementations with the three iso-*3* Csr lines and the deletions that uncover the recessive lethal mutations associated with the revertants. These three lines are fully viable at both 25 and 29°. Two of the three lines, Csr-2 and Csr-3 complement fully for viability with the two deletions. However, at 29°, Csr-1 shows a complementation pattern with deletions *Df(3R)*crb87–4 and *Df(3R)*crb87–5, which indicates semilethality (7% *Sb*$^{+}$ progeny). Thus we conclude that *Csr* is located at cytological position 95F7–96A1.

DISCUSSION

In this study, we identified and genetically localized *Csr*, a novel gene that has a major effect on the foraging

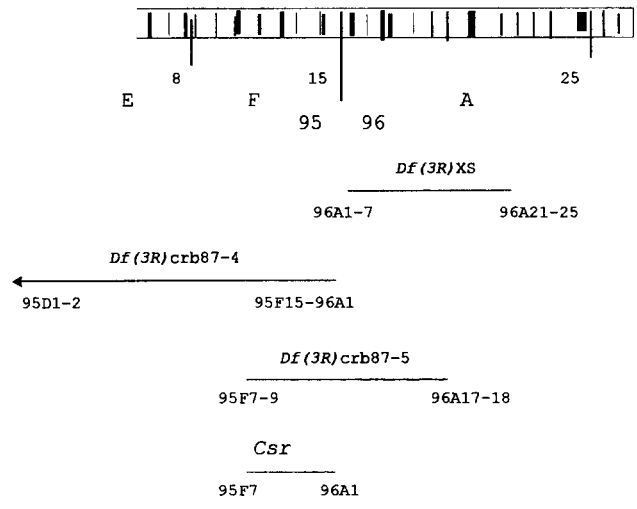

FIGURE 6.—Deficiency map of Csr^{rv} lines. Pairwise complementation crosses were done with the seven revertant lines and the three deletion lines that have chromosomal deficiencies in the 96A cytological region. The overlapping deficiencies *Df(3R)*crb87–4 and *Df(3R)*crb87–5 did not complement for viability with all seven Csr^{rv} lines at 29°C, although there was full complementation for viability at 25°C. These results map the recessive lethal mutations associated with the revertant lines to cytological region 95F7–96A1. We also performed pairwise complementation crosses between the three deletion lines. *Df(3R)*XS complemented fully for lethality with *Df(3R)*crb87–4, but not with *Df(3R)*crb87–5. This was true at both 25 and 29°C. *Df(3R)*crb87–4 and *Df(3R)*crb87–5 did not complement for viability at either temperature.

behavior of *D. melanogaster* larvae. In a mutagenesis screen to identify mutations in the *for*^{s} allele as well as modifiers of *for*^{s}, we recovered three lines with mutations that increased larval path length in a dominant fashion. In all three lines, the mutations mapped to chromosome *3*, indicating that they were not in the *for* gene, but likely modifiers of *for*. The behavioral effects of the three lines were not separable by recombination. This led us to believe that the increased path length in the three lines mapped to the same gene. We have called this gene *Csr*. To further localize *Csr*, we used the lethal-tagging technique [as in (DE BELLE *et al.* 1989)] to revert *Csr-3*.

Csr is a dominant behavior mutation that results in "rover-like" foraging behavior in larvae with a sitter genetic background. We hypothesized that if *Csr* was a gain of function mutation, it should be revertable. The reversion of *Csr* would result in the reversion of its behavioral effect on larval path length, from rover back to sitter. We readily obtained seven revertants of *Csr-3* (*Csr*^{rv}), with "sitter-like" path lengths. All seven lines were associated with lethal mutations on chromosome *3*. The probability of obtaining seven independently generated noncomplementing third chromosome lethals with sitter-like foraging phenotypes is vanishingly small. We therefore attribute the high coicidence of lethal alleles to the screening of "sitter-like" progeny following mutagenesis of *Csr-3*.

The seven Csr^{rv} lines did not complement for viability with deletions *Df(3R)*crb87–4 and *Df(3R)*crb87–5 at 29°. Because recessive lethal mutations are either in or tightly linked to *Csr-3*, this complementation pattern maps *Csr* to cytological region 95F7–96A1. Interestingly, the complementation pattern of one of the viable *Csr* alleles with the deletions *Df(3R)*crb87–4 and *Df(3R)*crb87–5 mimmicks that of the revertants. Although *Csr-1* is viable at 29°, it does not complement fully for viability with deletions *Df(3R)*crb87–4 and *Df(3R)*crb87–5 at 29°. This not only supports the notion that we have in fact lethal tagged the *Csr* gene in our mutagenesis of *Csr-3*, but also that *Csr-1* and *Csr-3* are allelic.

The lethal-tagging technique has allowed us to localize *Csr* to a small region on the third chromosome. This supports the power of this strategy to genetically localize major genes that influence quantitative traits, such as behavioral traits. The genetical localization of *Csr* is the first step to its molecular characterization. This characterization will be greatly facilitated by the large number of mutants that we have generated in the process of lethal-tagging *Csr*. The further characterization of *Csr* will undoubtably help to clarify the biochemical pathways involved in foraging behavior.

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