Genetic Localization of *foraging* (*for*): A Major Gene for Larval Behavior in *Drosophila melanogaster*

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ABSTRACT

Localizing genes for quantitative traits by conventional recombination mapping is a formidable challenge because environmental variation, minor genes, and genetic markers have modifying effects on continuously varying phenotypes. We describe "lethal tagging," a method used in conjunction with deficiency mapping for localizing major genes associated with quantitative traits. Rover/sitter is a naturally occurring larval foraging polymorphism in *Drosophila melanogaster* which has a polygenic pattern of inheritance comprised of a single major gene (*foraging*) and minor modifier genes. We have successfully localized the lethal tagged *foraging* (*for*, 2-10) gene by deficiency mapping to 24A3-C5 on the polytene chromosome map.

A variety of induced behavioral mutants have been isolated and characterized in *Drosophila melanogaster* (review: HALL 1985). These mutants define genes essential to the expression of normal (wild type) behavioral phenotypes. In contrast, we are interested in behavioral variants found in natural populations of *D. melanogaster*. Our goal is to localize major genes associated with quantitative or continuous patterns of variation.

Localizing genes involved in the expression of quantitative traits is essential for understanding their character and function (THODAY 1961). Most quantitative traits are influenced by many genes (polygenic inheritance); however, the actual number of genes involved and the magnitude of their individual effects is a subject of controversy (FALCONER 1981). The classical view is that hundreds of genes each with small equal and additive effects are involved (MATHER and JINKS 1982), while more recently it has been proposed that quantitative traits are controlled by relatively few major genes modified by minor genes (THODAY and THOMPSON 1976).

Conventional recombination mapping techniques are difficult to apply to genes controlling quantitative traits since these traits are strongly influenced by environmental variation and are usually controlled by many genes. In addition, quantitative phenotypes can be modified by the pleiotropic effects of genetic markers introduced through crosses made to marked strains (*e.g.* WELLER, SOLLER and BRODY 1988). Taken together, these genetic and environmental factors result in phenotypic overlap among genotypically distinct classes for the quantitative trait of interest. Consequently, in mapping experiments, it is difficult to accurately score marker classes for genes controlling quantitative traits. Indeed, methods for genetic localization of quantitative traits (review: THOMPSON and THODAY 1979) can be laborious and costly (*e.g.* EDWARDS, STUBER and WENDEL 1987; PATERSON *et al.* 1988; SHRIMPTON and ROBERTSON 1988a,b; LANDER and BOTSTEIN 1989), even when a large proportion of phenotypic variation can be attributed to one or a few segregating genes with large effects (STEWART 1969a,b; THOMPSON 1975; THODAY and THOMPSON 1976). Here we describe and demonstrate "lethal tagging": a procedure used in conjunction with deficiency mapping for localizing major genes controlling quantitative traits, a strategy which is not limited by the difficulties encountered when using the methods cited above.

Rover/sitter is a naturally occurring behavioral polymorphism in *D. melanogaster* larvae (SOKOLOWSKI 1980, 1982, 1985). The phenotype is measured as the distance ("path length") a larva travels while foraging in a yeast coated Petri dish. Rovers have significantly longer paths than sitters. Phenotypes are best represented by frequency distributions (Figure 1). A carefully controlled environment is required to minimize the phenotypic overlap between distinct genotypes (GRAF and SOKOLOWSKI 1989).

Expression of behavioral differences between rovers and sitters is conditional on the distribution of food and moisture in the environment (SOKOLOWSKI, KENT and WONG 1983). Both rover and sitter larvae have high locomotor scores (long paths) when placed in dry, non-nutritive environments (SOKOLOWSKI,

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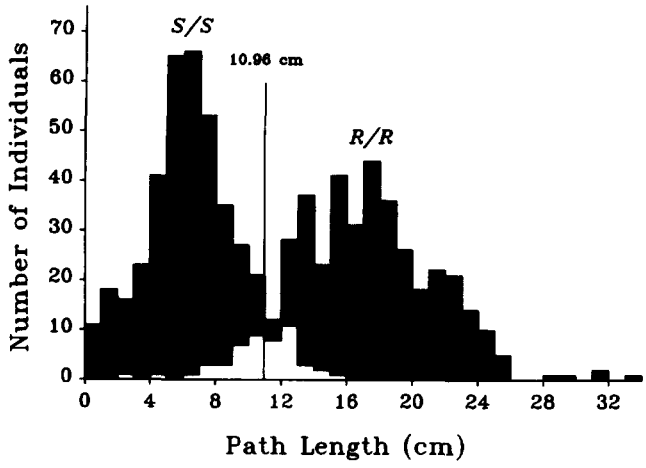

FIGURE 1.—Frequency distributions of larval path lengths for tests of standard sitter ("*S*"/"*S*"; $N = 401$, $\bar{X} \pm SE = 6.47 \pm 0.14$ cm) and rover ("*R*"/"*R*"; $N = 398$, $\bar{X} \pm SE = 17.11 \pm 0.22$ cm) strains reported herein. Overlap between the two distributions (unshaded) is 6% and represents the probability of genotype misclassification given a discrimination point of approximately 11 cm between sitters and rovers.

KENT and WONG 1984). The sitter morph does not have a low general activity or "sick" phenotype. Further support for this statement comes from the finding that rovers and sitters do not differ in developmental rate (SOKOLOWSKI, KENT and WONG 1984) or body size [measured as larval length and width reached by the third instar (GRAF and SOKOLOWSKI 1989)]. In the laboratory, rover and sitter strains show no obvious differences in fertility, fecundity or survivorship. In addition, the abundance of both rover and sitter larvae in natural populations (SOKOLOWSKI 1980, 1982) is not indicative of a general difference in vigor, but rather, may reflect disruptive selection acting on the different forager types in heterogeneous environments (SOKOLOWSKI 1985, 1987; SOKOLOWSKI and TURLINGS 1987).

Rover/sitter is a quantitative trait influenced by one major gene with rover dominant to sitter, and modified by minor genes (DE BELLE and SOKOLOWSKI 1987). Whole chromosome analyses of both established laboratory (SOKOLOWSKI 1980) and recently field-derived strains (BAUER and SOKOLOWSKI 1985) revealed a predominantly second chromosome genetic basis for rover/sitter. Compound autosome analysis localized the major gene to the left arm of chromosome-2 (DE BELLE and SOKOLOWSKI 1989). Attempts to further localize rover/sitter using standard recombination mapping did not succeed.

In the present study, we used a high dosage of gamma radiation (5000 rad) to induce mutations in gametes carrying the dominant rover allele, and recovered larvae expressing the sitter phenotype. Lethal alleles or deletions of the major gene and/or adjacent vital gene(s) were coincidentally generated. These recessive lethal mutations are completely penetrant, and thus have discontinuous phenotypes in contrast to the continuously varying behavioral phenotypes of rovers and sitters. Consequently, the "lethal tagged" major gene for rover/sitter, a quantitative trait, could then be cytogenetically localized by deficiency mapping.

TABLE 1

Deficiencies used to localize lethal mutations on "*S(R)*" chromosomes

Deficiency	Cytology	References[a]	Source
Df(2L)al	21B8-C1; 21C8-D1	*d, f, g, k*	*m*
Df(2L)S2	21C6-D1; 22A6-B1	*f, g, i, k*	*m*
Df(2L)ast1	21C7-8; 23A1-2	*i, k*	*m*
Df(2L)ast2	21D1-2; 22B2-3	*i, k*	*m*
Df(2L)S3	21D2-3; 21F2-22A1	*f, g, i*	*c*
Df(2L)ed^{Sz}	24A3-4; 24D3-4	*h, k, l*	*m*
Df(2L)ed dp^{h1}	24C3-5; 25A1-4	*j, k, l*	*m*
Df(2L)M-z^{B}	24E1-2; 24F6-7	*g, h, j, k, l*	*m*
Df(2L)c11	25D7-E1; 25E6-F3	*a, e, g, k, l*	*m*
Df(2L)GdhA	25D7-E1; 26A8-9	*b, e, g, k, l*	*c*
Df(2L)c17	25E1-2; 26A7-8	*a, e, g, k, l*	*m*

[a] *a*, ASHBURNER *et al.* (1980); *b*, GRELL (1967); *c*, Indiana stock center; *d*, KOROCHKINA and GOLUBOVSKY (1978); *e*, KOTARSKI, PICKERT and MACINTYRE (1983); *f*, LEWIS (1945); *g*, NUSSLEIN-VOLHARD, WIESCHAUS and KLUDING (1984); *h*, REUTER and SZIDONYA (1983); *i*, ROBERTS *et al.* (1985); *j*, SEMESHIN and SZIDONYA (1985); *k*, SCHUPBACH and WIESCHAUS (1989); *l*, SZIDONYA and REUTER (1988a,b); *m*, E. WIESCHAUS.

MATERIALS AND METHODS

Strains and chromosomes: We used two strains of *D. melanogaster* isogenic for chromosomes-*2* and -*3* (standard strains). These were E_2E_3, expressing a sitter phenotype (SOKOLOWSKI 1980; DE BELLE and SOKOLOWSKI 1987) and B15B15, expressing a rover phenotype (BAUER and SOKOLOWSKI 1985; DE BELLE and SOKOLOWSKI 1987). We designate second chromosomes from sitter as "*S*", rover as "*R*", and those from newly generated sitters derived from rover as "*S(R)*". *In(2LR)SM1, al*^{2} *Cy cn*^{2} *sp*^{2}*/In(2LR)bw*^{V1}, *ds*^{33k} *bw*^{v1} was employed as a second chromosome balancer strain and is hereafter referred to as *SM1/bw*^{V1}. *In(2LR)SM5, al*^{2} *ds*^{33k} *Cy lt*^{v} *cn*^{2} *sp*^{2}*/Sp Bl L*^{2} is a balanced dominantly marked second chromosome strain which we used for recombination mapping. The above chromosomes and mutations are described by LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1985, 1987). Chromosome-*2* deficiencies used in this study are listed in Table 1. 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 a L:D 12:12 photocycle with lights on at 0800 hr.

Behavioral assay: The locomotor component of foraging behavior in third instar larvae was quantified using a procedure described by DE BELLE and SOKOLOWSKI (1987) which we briefly outline here. Larvae were harvested from matings between 250 females and 125 males aged 2 to 5 days (posteclosion). One-hundred first instar larvae were collected over a 3 hr period and placed in Petri dishes containing 35 ml of culture medium where they developed to third instar larvae under standard conditions (SOKOLOWSKI, KENT and WONG 1984). The maximum expression of genetically based differences between rovers and sitters occurs during this stage of larval development (GRAF and SOKOLOWSKI 1989). Foraging third instar larvae (96 ± 1.5

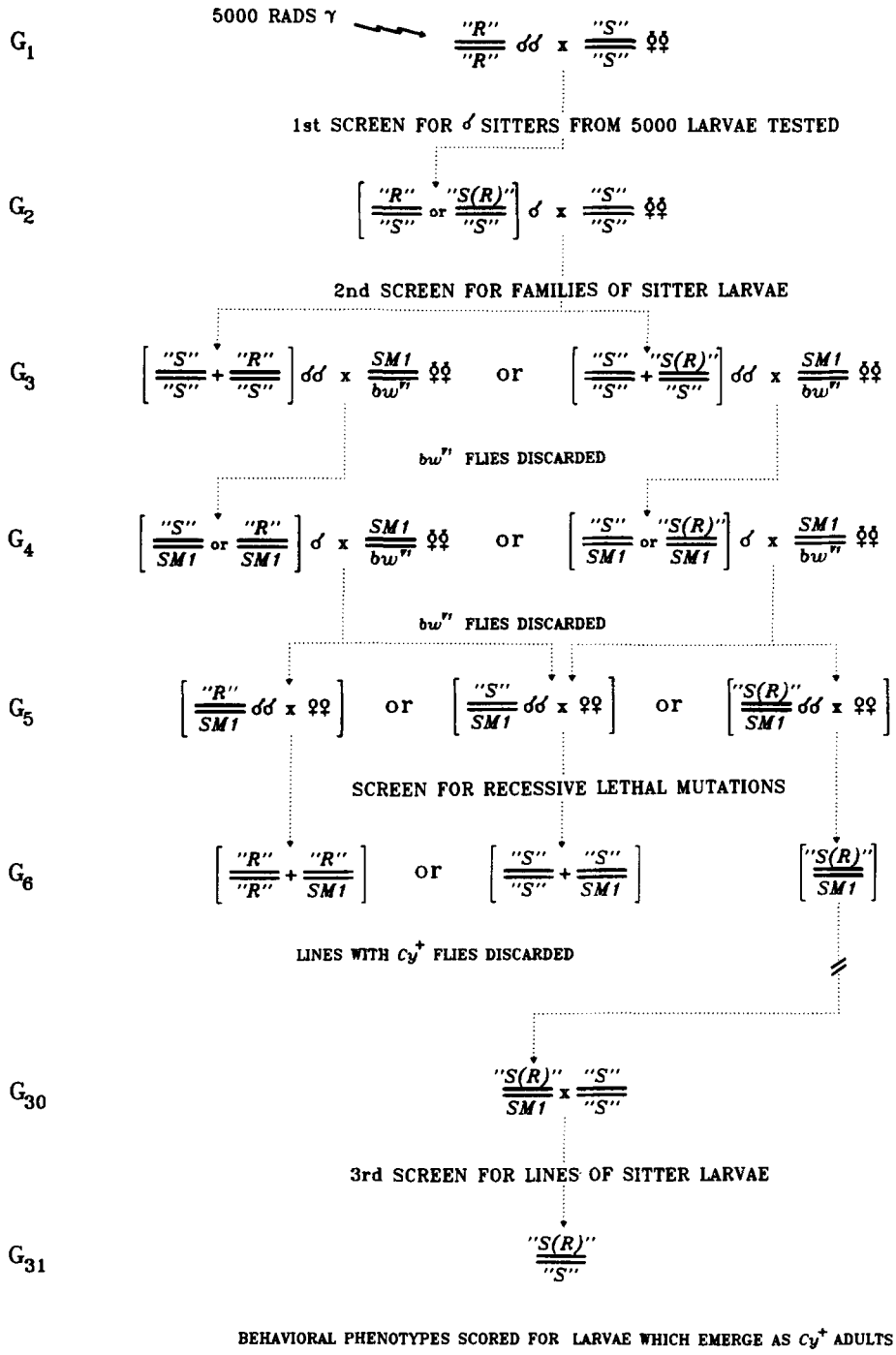

FIGURE 2.—Lethal tagging protocol. Second chromosome designations: "*S*", standard sitter strain; "*R*", standard rover strain; "*S(R)*", recessive lethal conferring a sitter phenotype derived from the standard rover strain; *SM1*, balancer marked with *Curly* (*Cy*); bw^{V1}, balancer marked with *brown variegated*. "*R*"/"*R*" adult males were treated with 5000 rad of gamma radiation from a ^{60}Co source and mated to "*S*"/"*S*" virgin females. Five-thousand individual G_2 (generation two) third instar larvae ("*R*"/"*S*" and "*S(R)*"/"*S*") were tested in the first behavioral screen. Of these, 546 sitter larvae were selected and maintained in separate vials, from which 220 sitter males eclosed. Second chromosomes were always kept in males to maintain complete linkage (MORGAN 1912). Individual G_2 sitter males were mated to 25 "*S*"/"*S*" tester virgin females to replicate each irradiated second chromosome. All G_3 third instar larvae (either "*S*"/"*S*" and "*R*"/"*S*" or "*S*"/"*S*" and "*S(R)*"/"*S*") from each mating were tested in a second behavioral screen. G_3 males from 58 sitter families were mated to $SM1/bw^{V1}$ females to balance intact second chromosomes. Six G_4 *Cy* progeny *per* family were sampled and individually backcrossed to $SM1/bw^{V1}$ giving a 98.4% probability *per* family of replicating the irradiated "*R*" second chromosome. In G_5 only *Cy* progeny within each vial were bred. G_6 families consisting of only *Cy* progeny had new second chromosome lethal mutations linked with alteration of behavior from rover to sitter. Lethal alleles serve as genetic tags identifying each independently generated "*S(R)*" chromosome. Confirmed lethal families were maintained as true breeding heterozygous lines ("*S(R)*"/*SM1*) owing to the lethality of both homozygotes in each culture. Twenty-five generations following completion of this protocol a third behavioral screen determined the stability of behavioral alteration in each line. "*S(R)*"/*SM1* males were mated with "*S*"/"*S*" sitter females. Path lengths of "*S(R)*"/"*S*" individuals were compared with those of standard rover and sitter strains.

hr posthatching) were individually placed 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). Path lengths made by foraging larvae during 5-min tests were measured. These were classified as rovers or sitters by comparison with those of concurrently tested standard strains. Behavioral testing occurred on each day within a 6-hr interval beginning at 1200 hr at room temperature under homogeneous overhead illumination.

Lethal tagging: The strategy for lethal tagging is briefly outlined below (Figure 2). Gamma-irradiated homozygous rover males were mated to homozygous sitter females. In the absence of mutagenesis, this mating produces heterozygotes having well characterized rover phenotype indistinguishable from the homozygous rover strain (DE BELLE and SOKOLOWSKI 1987). With this in mind, we screened progeny larvae for mutations from rover to sitter behavior in the well defined genetic background of the heterozygote. We anticipated that in some cases, coincident with behavioral alteration would be the induction of lethal "sitter derived from rover" alleles of the behavioral gene (if it is a vital gene). Alternatively, we might generate deletions of the dominant rover allele and adjacent vital genes. We used a high dosage of gamma radiation (5000 rad) in order to "tag" chromosomes with lethals, so that they were distinguishable from isogenic tester chromosomes in subsequent breeding. Noncomplementing, independently induced lethal chro-

TABLE 2

Behavioral phenotypes of heterozygous "*S(R)*"/"*S*" larvae

	N	Path length (cm)[a] $\bar{X}$	SE	Phenotype[b]
"*S(R)*"/"*S*" heterozygotes				
"*S(R)*"*92*/"*S*"	20	7.89	0.64	sitter
"*S(R)*"*136*/"*S*"	25	7.91	0.81	sitter
"*S(R)*"*164*/"*S*"	17	8.33	0.57	sitter
"*S(R)*"*172*/"*S*"	16	7.64	0.87	sitter
"*S(R)*"*184*/"*S*"	22	4.96	0.48	sitter
Standard strains				
"*S*"/"*S*"	151	6.33	0.24	sitter
"*R*"/"*R*"	148	17.80	0.35	rover

[a] Path lengths were significantly different [analysis of variance (ANOVA), $F(6,392) = 153.09$, $P < 0.0001$].

[b] A Student-Newman-Keuls multiple range test [SNK, $P \leq 0.05$ (ZAR 1984)] resulted in two path length groupings. All "*S(R)*"/"*S*" heterozygotes differed from the "*R*"/"*R*" rover strain but not from the "*S*"/"*S*" sitter strain.

TABLE 3

Behavioral phenotypes of heterozygous "*S(R)*"/"*R*" larvae

	N	Path length (cm)[a] $\bar{X}$	SE	Phenotype[b]
"*S(R)*"/"*R*" heterozygotes				
"*S(R)*"*92*/"*R*"	40	17.09	0.57	rover
"*S(R)*"*136*/"*R*"	44	14.06	0.50	rover
"*S(R)*"*164*/"*R*"	38	13.37	0.48	rover
"*S(R)*"*172*/"*R*"	40	12.94	0.48	rover
"*S(R)*"*184*/"*R*"	40	12.92	0.54	rover
Standard strains				
"*S*"/"*S*"	50	5.65	0.47	sitter
"*R*"/"*R*"	50	15.15	0.38	rover

[a] Path lengths differed significantly (ANOVA, $F(6,295) = 59.31$, $P < 0.0001$).

[b] SNK test ($P \leq 0.05$) results showed two path length groupings. All "*S(R)*"/"*R*" heterozygotes differed from the "*S*"/"*S*" sitter strain but not from "*R*"/"*R*" rovers.

TABLE 4

Behavioral phenotypes of homozygous "*S(R)*"/"*S(R)*" larvae

	N	Path length (cm)[a] $\bar{X}$	SE	Phenotype[b]
"*S(R)*"/"*S(R)*" homozygotes				
"*S(R)*"*92*/"*S(R)*"*92*	7	8.38	1.47	sitter
"*S(R)*"*172*/"*S(R)*"*172*	9	7.79	1.06	sitter
"*S(R)*"*184*/"*S(R)*"*184*	15	9.47	0.91	sitter
Standard strains				
"*S*"/"*S*"	100	7.47	0.22	sitter
"*R*"/"*R*"	100	18.09	0.46	rover

[a] Path lengths differed significantly (ANOVA, $F(4,226) = 119.49$, $P < 0.0001$).

[b] SNK test ($P \leq 0.05$) results showed two path length groupings. All "*S(R)*"/"*S(R)*" homozygotes differed from the "*R*"/"*R*" rover strain but not from "*S*"/"*S*" sitters.

mosomes which also exhibited loss of rover allele function in behavioral tests served to localize the rover/sitter gene.

We determined the dominance relationships between sitter ("*S*"), rover ("*R*") and sitter derived from rover ("*S(R)*") alleles. "*S(R)*"/"*S*" behavioral phenotypes were measured for each lethal chromosome (Figure 2). "*S(R)*"/"*R*" phenotypes were similarly scored in Cy^+ progeny from matings between "*S(R)*"/*SM1* males and "*R*"/"*R*" females. Lethality was expressed during a specific stage of pupal development in three of the behaviorally mutant lines. Larval phenotypes of "*S(R)*"/"*S(R)*" homozygotes could therefore be assayed by testing the path lengths of progeny of "*S(R)*"/*SM1* heterozygotes followed by scoring individuals which failed to eclose.

Complementation analysis: We anticipated that two types of lethal-bearing "*S(R)*" chromosomes had been constructed. Lethals might have occurred at random locations on chromosome-*2* as second-site events in conjunction with mutations of the rover allele to sitter. Alternatively, lethals which directly resulted in the loss of rover allele expression would be lethal alleles of the behavioral gene and/or deletions of rover and adjacent vital gene(s). Accordingly, pairwise tests of complementation were performed between all lethal "*S(R)*" chromosome-*2* lines to determine if any share a common lethal locus. Second-site lethals should complement since random mutations would not likely occur at the same locus. In contrast, independent chromosomes bearing lethal sitter derived from rover alleles or deletions should fail to complement and would thus serve to identify the major rover/sitter gene.

Recombination mapping: We determined approximate map positions of each lethal complementation group with respect to the genetic markers *Sternopleural* (*Sp*, 2-22.0), *Bristle* (*Bl*, 2-54.8) and *Lobe-2* (L^2, 2-72.0). "*S(R)*"/*SM1* females were mated to *Sp Bl* L^2 males. *Sp Bl* L^2/Cy^+ female progeny were then backcrossed to "*S(R)*"/*SM1* males. Viable Cy^+ progeny from this cross were scored for *Sp*, *Bl* and L^2. The positions of lethals can be inferred from the underrepresentation of specific classes of recombinants. When possible, the backcross was performed with an independent allele in order to complement second-site lethals which might have been coincidentally generated in the mutagenized "*S(R)*" second chromosome. We accounted for differences in viability attributable to the genetic markers by making pairwise comparisons between segregating *Cy* (control) marker classes. A correction coefficient was determined from the observed ratio of each reciprocal marker class pair. The actual scores of Cy^+ (test) marker classes were multiplied by these coefficients to compensate for the influence of markers on viability, providing corrected scores for recombination mapping of lethal complementation groups.

deficiency mapping: Lethal-bearing chromosomes were tested for complementation with the deficiencies listed in Table 1. Linkage of each deficiency is maintained over a balancer chromosome marked with *Cy*. Matings involving each lethal "*S(R)*" chromosome and deficiency were performed. On the basis of pseudodominance, recessive lethal mutations were localized between deficiency breakpoints in matings from which Cy^+ progeny were not observed.

Cytological analysis: All lethal "*S(R)*" chromosomes were cytologically characterized. Polytene chromosome preparations were made from larval salivary glands obtained from "*S(R)*"/"*R*" heterozygotes.

RESULTS

Lethal tagging: We generated five lethal lines expressing altered behavioral phenotypes which do not differ significantly from the standard "*S*"/"*S*" sitter strain (Table 2). Dominance relationships between

TABLE 5

Recombination analysis of "*S(R)*" lethal complementation groups

	Marker classes								
	+	*Sp*	*Bl*	L^2	*Sp Bl=1*	*Sp* L^2	*Bl* L^2	*Sp Bl* L^2	Σ
Lethal complementation groups									
"*S(R)*"*92*/"*S(R)*"*172*	175	206	15	15	67	20	52	383	933
"*S(R)*"*136*	1	2	0	2	0	0	151	377	533
"*S(R)*"*164*	22	3	13	2	40	1	73	229	383
Viability control[a]									
Cy	1172	259	42	81	152	13	145	361	2225
Correction	1	1	1	1.88	1	3.23	1.79	3.25	
Lethal complementation groups corrected for viability[b]									
"*S(R)*"*92*/"*S(R)*"*172*[c]	175	206	15	28	67	65	93	1245	1894
"*S(R)*"*136*[d]	1	2	0	4	0	0	270	1225	1502
"*S(R)*"*164*[e]	22	3	13	4	40	3	131	744	960

[a] Marker classes are corrected for viability differences according to those observed in the *Cy* control groups. Correction coefficients were determined by comparing control groups produced by reciprocal recombination events.

[b] Corrected scores were calculated by multiplying actual scores by correction coefficients.

[c] Noncomplementing lethals ("*S(R)*"*92*, "*S(R)*"*172* and "*S(R)*"*184*) map at 2-10, 12 cM distal to *Sp*.

[d] "*S(R)*"*136* has two lethals; one distal to *Bl*, the other proximal to L^2.

[e] The "*S(R)*"*164* lethal maps at 2-19, three cM distal to *Sp*.

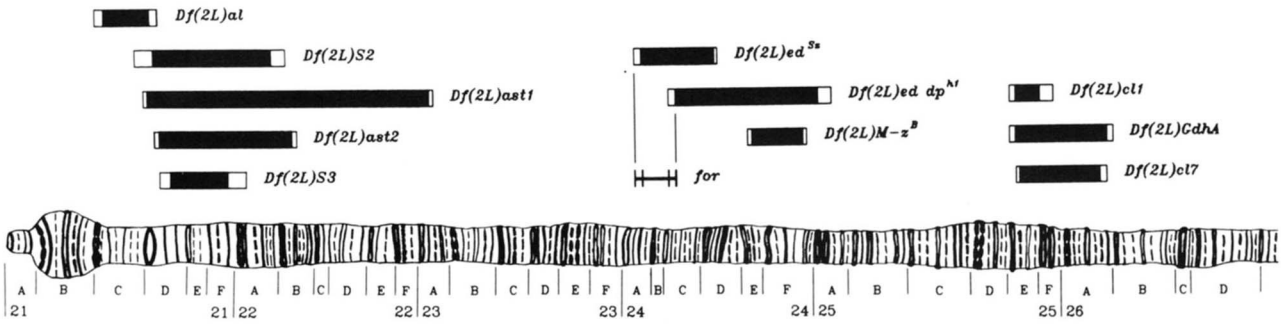

FIGURE 3.—Cytogenetic mapping of *for* on the revised polytene chromosome-2 map of the left arm distal to *Sp* (drawn from BRIDGES 1942). Deficiencies used to locate *for* on this map are indicated by shaded bars, and listed in Table 1. Uncertainty about the exact location of breakpoints is indicated by unshaded regions on each bar. The lethal tagged *for* gene fails to complement with $Df(2L)ed^{Sz}$ but complements with all other deficiencies including $Df(2L)ed\ dp^{h1}$. Thus, the cytological location of *for* is 24A3-C5.

rover ("*R*") and sitter derived from rover ("*S(R)*") alleles are compared in Table 3. Path lengths of all five "*S(R)*"/"*R*" heterozygotes are not significantly different from "*R*"/"*R*" rover homozygotes. This observation suggests that behavioral alteration has not arisen through mutation of a dominant *trans*-acting suppressor of the rover allele. Behavioral phenotypes of "*S(R)*"*92*/"*S(R)*"*92*, "*S(R)*"*172*/"*S(R)*"*172* and "*S(R)*"*184*/"*S(R)*"*184* larvae could be assayed since lethal expression occurs after the grey wing stage of pupal development (BAINBRIDGE and BOWNES 1981). (Lethality is expressed during larval development in "*S(R)*"*136*/"*S(R)*"*136* and could not be associated with a specific developmental stage in "*S(R)*"*164*/"*S(R)*"*164*.) The behavior of homozygous "*S(R)*"/"*S(R)*" larvae does not differ significantly from that of "*S*"/"*S*" sitters (Table 4). Indeed, behavioral tests of "*S(R)*"/"*S*", "*S(R)*"/"*R*" and "*S(R)*"/"*S(R)*" showed that the effect of "*S(R)*" second chromosomes on larval path length is not distinguishable from that of the "*S*" sitter second chromosome.

Complementation analysis: We performed pairwise tests of complementation between all five lethal "*S(R)*" chromosomes. Three fail to complement ("*S(R)*"*92*, "*S(R)*"*172* and "*S(R)*"*184*) and are therefore concluded to be allelic. These lethals "tag" the rover/sitter major gene which we call *foraging* (*for*; DE BELLE, HILLIKER and SOKOLOWSKI 1987). "*S(R)*"*136* and "*S(R)*"*164* lethal alleles complement with all other lethals. They likely resulted from nonlethal mutations of the rover allele to sitter and second-site lethal mutations concurrently generated elsewhere on the second chromosome.

Recombination mapping of *for*: Noncomplementing lethals (and thus *for*) map at approximately 2-10, 12 centimorgans (cM) to the left of *Sp* (Table 5). "*S(R)*"*136* has two lethals, one distal to *Bl*, one proximal to L^2. "*S(R)*"*164* has a single lethal which maps to 2-19, three cM distal to *Sp*.

deficiency mapping of *for*: The lethals tagging *for* fail to complement with $Df(2L)ed^{Sz}$ but complement with all other deficiencies including $Df(2L)ed\ dp^{h1}$

(Figure 3). This result places the noncomplementing lethals (*for*) at 24A3-C5, a seven to eleven band interval on the salivary gland polytene chromosome map (LEFEVRE 1976; SORSA 1988). Rearrangements were not observed in this region of *"S(R)"92*, *"S(R)"172* and *"S(R)"184* chromosomes indicating that these lethals are associated with very small deficiencies or are lethal alleles of *for*.

DISCUSSION

In this study, we generated five lethal tagged *"S(R)"* chromosomes. Three of these have identical patterns of lethal expression, do not complement, and identify the major gene (*foraging*) associated with the rover/sitter foraging polymorphism. The probability of obtaining three independently generated noncomplementing second chromosome lethals from a sample of five by chance alone is vanishingly small. We therefore attribute the high coincidence of lethal alleles to selection of alternative behavioral phenotypes following mutagenesis at the *foraging* locus. Results of deficiency and recombination mapping are in close agreement since both $Df(2L)ed^{Sz}$ and $Df(2L)ed\ dp^{h1}$ uncover *echinoid* (*ed*) which maps at 2-11.0 (REUTER and SZIDONYA 1983; SZIDONYA and REUTER 1988a,b). The *foraging* locus is included in $Df(2L)ed^{Sz}$ but not $Df(2L)ed\ dp^{h1}$ and maps at 2-10. Our success with the lethal tagging strategy indicates a potential for analyses of major genes controlling other quantitative behavioral traits and indeed, quantitative traits in general.

Dominant rover and recessive sitter alleles are designated as for^R and for^s respectively. We have concurrently generated $for^{s(R)}$ alleles and pupal-lethal mutations. Noncomplementing lethals are either tightly lined to the *foraging* locus or lethal $for^{s(R)}$ alleles. The latter possibility would suggest that the *for* gene product is essential for complete metamorphosis. Alternatively, *for* may control the expression of two functionally distinct phenotypes; larval behavior and a physiological function vital for metamorphosis.

The fact that appreciable frequencies of both for^R and for^s alleles can be found in natural populations of *D. melanogaster* (SOKOLOWSKI 1980, 1982) is thus far a unique aspect of this behavioral gene and favors continued investigation of both proximate and ultimate questions concerning the differences between rovers and sitters. Moreover, localization of *for* permits further analysis of the locus and its immediate genetic environment: an important milestone toward its molecular characterization (HALL 1985).

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