Genetic analysis of the *foraging* microregion of *Drosophila melanogaster*

J. STEVEN DE BELLE[1] AND MARLA B. SOKOLOWSKI
Department of Biology, York University, 4700 Keele Street, North York, Ont., Canada M3J 1P3
AND
ARTHUR J. HILLIKER
Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ont., Canada N1G 2W1

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The rover/sitter polymorphism in *Drosophila melanogaster* larval behaviour is a unique example of a genetically determined, naturally occurring behavioural polymorphism. Allelic variation at the *foraging* locus (*for*) accounts for the rover (long foraging paths) and sitter (short foraging paths) phenotypes. We previously developed lethal tagging and used deficiency mapping to place *for* in the 24A3-C5 interval on the polytene chromosome map, thereby defining the *for* microregion. Here, we subjected this microregion to mutational analysis to (*i*) isolate putative lethal *foraging* mutations and characterize their behavioural phenotypes to assess whether or not *for* is a vital locus, (*ii*) generate cytologically detectable chromosome rearrangements with breakpoints in or near *for* for more precise localization and for future molecular analysis of the *for* gene, and (*iii*) identify other gene loci in the immediate vicinity of the *for* locus. We recovered 10 gamma-induced and 33 ethyl methanesulfonate (EMS) induced new mutations that define seven complementation groups in 24A3-D4. Two new EMS-induced lethal *for* alleles and four gamma-induced rearrangements with breakpoints in *for* were identified, which allowed us to further localize *for* to 24A3-5. All lethal mutations in *for* resulted in an altered behavioural phenotype providing evidence that both vital and behavioural functions are encoded by *for*.

Key words: behaviour, genetics, *foraging* microregion, *Drosophila melanogaster*, larvae.

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Le polymorphisme nomade/sédentaire du comportement larvaire chez le *Drosophila melanogaster* est un exemple unique de polymorphisme behavioriste, lequel survient naturellement et est déterminé génétiquement. Une variation allélique au locus *for* (lié à l'affourragement) est à l'origine des phénotypes nomade (longs sentiers d'affourragement) et sédentaire (courts sentiers d'affourragement). Ayant déjà procédé au développement d'un marquage létal sélectif, nous avons utilisé la cartographie déficiente pour placer le locus *for* dans l'intervalle 24A3-C5 sur la carte du chromosome polytène, définissant ainsi la microrégion *for*. Cette microrégion a été soumise à une analyse mutationnelle pour : (*i*) isoler les mutations létales présumées liées à l'affourragement et caractériser leurs phénotypes behavioristes en vue d'établir si, oui ou non, le locus *for* est vital; (*ii*) favoriser la production de réarrangements chromosomiques décelables cytologiquement, avec fractures dans ou près de *for*, en vue d'une localisation plus précise et d'analyses moléculaires futures du gène *for* et (*iii*) identifier d'autres locus géniques dans le voisinage immédiat du locus *for*. Dix nouvelles mutations induites par rayons gamma et 33 autres induites par EMS ont été recouvrées, lesquelles ont permis de définir sept groupes de complémentation dans la microrégion 24A3-D4. Deux nouveaux allèles *for* létaux, induits par EMS, et quatre réarrangements avec fractures dans le *for*, induits par rayons gamma, ont été identifiés, permettant de localiser le *for* plus précisément dans la microrégion 24A3-5. Toutes les mutations létales dans le *for* se sont traduites par un phénotype behavioriste altéré, ce qui démontre que les fonctions vitales et behavioristes sont encodées par le *for*.

Mots clés : comportement, génétique, *foraging* microrégion, *Drosophila melanogaster*, larves.

[Traduit par la rédaction]

Introduction

Behaviour is perhaps the most complex of phenotypes because it reflects the development and function of whole organisms and is remarkably plastic in response to environmental variation (Sokolowski 1992). Consequently, the genetic and molecular analyses of behaviour are challenging endeavours. Genetic dissection of the mechanisms underlying behavioural phenotypes requires the generation of mutations that disrupt some aspect of normal behaviour (Benzer 1973; Hall 1985; Kyriacou 1990). However, studies of induced mutants do not necessarily aid in understanding the genetic basis of variation found in natural populations. From an evolutionary perspective, genetically based behavioural variations found in nature are of particular interest because they may represent the types of variants on which natural selection acts.

The rover/sitter larval foraging polymorphism in *Drosophila melanogaster* is a well-characterized, naturally occurring behavioural phenotype for which a hereditary basis has been established, namely allelic variation at a major gene on the second chromosome (Sokolowski 1980, 1982, 1985; de Belle and Sokolowski 1987, 1989). Larvae of *D. melanogaster* in both wild and laboratory populations exhibit one of two alternative phenotypes. "Rover" larvae have long foraging paths while feeding, whereas "sitter" larvae have shorter paths. This "path length" phenotype is defined by the distance a larva travels while foraging in

[1]Present address: Theodor-Boveri-Institut (Biozentrum), Lehrstuhl für Genetik, Universität Würzburg, Germany.

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a yeast-coated Petri dish during a specific time period and is therefore a quantitative trait.

Classical genetic mapping techniques proved unsuitable for precise localization of the major gene for the rover/sitter trait; however, we refined its genetic localization using the "lethal tagging" approach in conjunction with deficiency mapping (de Belle *et al.* 1989). Briefly, we screened gamma-irradiated "rover" second chromosomes (heterozygous over recessive "sitter" chromosomes) for mutations giving rise to both recessive "sitter derived from rover" behavioural phenotypes and coincident recessive lethality. Three non-complementing lethal mutations ($for^{s(R)92}$, $for^{s(R)172}$, $for^{s(R)184}$) associated with altered foraging behaviour were recovered. These greatly facilitate the localization of the *foraging* (*for*) locus as they "tag" *for* with an easily localized genetic marker (lethality). We concluded that the lethals are either recessive lethal alleles of *for*, or small deletions uncovering both *for*, and one or more adjacent vital functions. The lethal tagged *for* gene was localized by recombination to map position 2-10 and by deficiency mapping to polytene chromosome interval 24A3-C5 (de Belle *et al.* 1989). Two lines with viable sitter derived from rover alleles, $for^{s(R)136}$ and $for^{s(R)164}$, were also recovered from the lethal tagging experiments. These lines carried second site lethal mutations that have since been crossed off by recombination with a lethal *for* mutation.

At this stage in our analysis of foraging, we wish to exploit the sophisticated genetic, cytological, and molecular techniques used to characterize specific genes in *Drosophila*. Therefore, we have undertaken a detailed genetic analysis of the *for* microregion as defined by $Df(2L)ed^{Sz}$. We screened for lethal mutations mapping within interval 24A3-D4 to (*i*) isolate putative lethal *foraging* mutations and characterize their behavioural phenotypes, (*ii*) generate cytologically detectable chromosome rearrangements with breakpoints in or near *for* for more precise localization and for future molecular analysis of the *for* gene, and (*iii*) identify and characterize lethal complementation groups within this chromosome interval.

Materials and methods

D. melanogaster *strains and chromosomes*

Strains used in the mutagenesis scheme were BB, an isogenic for^R "rover" strain; EE, an isogenic for^s "sitter" strain (de Belle and Sokolowski 1987); and balancer chromosomes used to maintain lethal chromosome-2 mutations, $In(2LR)SM1$, al^2 Cy cn^2 $sp^2/In(2LR)bw^{V1}$, ds^{33k} bw^{V1} (referred to as $SM1/bw^{V1}$), and $In(2LR)SM5$, al^2 ds^{33k} Cy lt^v cn^2 sp^2 (referred to as *SM5*). Previously reported mutants from the region were three lethal alleles of *for* ($for^{ls(R)92}$, $for^{ls(R)172}$, and $for^{ls(R)184}$, described in de Belle *et al.* 1989), two *slp* alleles (slp^{7L48}, and slp^{11M105} (Coulter and Wieschaus 1988), provided by D. E. Coulter), and one *ed* allele. Chromosome rearrangements employed in this study are listed in Table 1. $Df(2L)ed^{Sz}$, *al b* L^2 (referred to as $Df(2L)ed^{Sz}$, L^2) was made from $Df(2L)ed^{Sz}$, *al b*, and *Sp Bl* L^2 (provided by D. Morton) chromosomes. We also used (*i*) *bw; ve st e* and (*ii*) *cn bw* (Lindsley and Zimm 1992), which were obtained from the Bowling Green stock center.

Strains were grown in 6-oz (1 oz = 28.413 cm^3) plastic bottles on a dead yeast, sucrose, and agar medium under conditions of 25 ± 1°C, 15 ± 1 mbar (1 mbar = 100 Pa) vapor pressure deficit, and a 12-h light - 12-hr dark regime with lights on at 08:00 (standard conditions).

TABLE 1. Chromosome rearrangements used to localize mutations within 24A3-D4

Rearrangements	Cytology	References*
$Df(2L)ed^{Sz}$, *al b*	24A3-4; 24D3-4	1, 7, 9
$Df(2L)sc^{19-8}$	24C2-8; 25C8-9	5, 9
$Df(2L)ed$ dp^{h1}	24C3-5; 25A2-3	1, 7, 8, 9
$Df(2L)M11$	24D3-4; 25A2-3	5, 7, 8, 9
$Dp(2;1)B19$, ed $dp^{o2\ cl}$	9B-C; 24D2-5; 25F1-2	5, 7, 8, 9
Order: 1A to 9B-C:25F2-1 to 24D5-2:9B-C to 20F		
$In(2L)$ for^{A9}	24A2-B1; 27C5-D2	10
$In(2L)for^{B29}$	24A2-B1; 27C7-D1	10
$T(Y;2)L126$	h25D; 24C2-D1	4, 6
$T(Y;2)D6$	Y^SXh; 24C2-D1; 24D2-5; 25D1-2	4, 6, 11†
Order: 21A to 24C2-D1:25D2-1 to 24D5-2:Y^SXh; Y^SXh:25D1-2 to 60F		
$T(2;3)8r4$, cn bw	22A2-B1; 24A2-5; 60A2-B1; 60E; 3het	2, 6
Order: 21A to 22A2-B1:24A2-5 to 60A2-B1:60E to F; 61A to 3het:(60A2-B1 to 60E:22A2-B1 to 24A2-5):3het to 100F		
$T(2;3)for^{B4}$ =		10
$T(2;3)B4a$ +	21D1-2; 93F7-9 +	
$T(2;3)B4b$ +	2Rhet; 87F +	
$Tp(2)for^{B4}$	24A2-B1; 26D5-E1; 46E	
Order: 21A to 21D1-2:93F9-7 to 87F:2Rhet to 26E1-D5:46E to 60F; 61A to 87F:2Rhet to 46E:24A2-B1 to 26D5-E1:24B1-A2 to 21D2-1:93F7-9 to 100F		
$T(2;3)slp^{A10}$	24C2-D1; 3het	10
$T(2;3)9$	24D1-2; 3het	3
$T(2;3)7$	24D2-E1; 78C	3
$T(2;3)ed^{A16}$ =		10
$T(2;3)ed^{A16}$ +	24D2-5; 87D5-11 +	
$In(3LR)A16$	64E1-2; 95A2-4	
Order: 21A to 24D2-5:87D11-5 to 64E2-1:95A2-4 to 100F; 61A to 64E1-2:95A4-2 to 87D11-5:24D2-5 to 60F		

*1, de Belle *et al.* 1989; Schupbach and Wieschaus 1989; provided by E. Wieschaus; 2, Eberl *et al.* 1989; 3, Hilliker and Trusis-Coulter 1987; 4, Lindsley *et al.* 1972; Gatti and Pimpinelli 1983; provided by D. L. Lindsley; 5, provided by the Bowling Green stock center; 6, reanalyzed, this laboratory; 7, Reuter and Szidonya 1983; 8, Semeshin and Szidonya 1985; 9, Szidonya and Reuter 1988*a*, 1988*b*; 10, this report; 11, Velissariou and Ashburner 1980.

†The most recent previous cytological analysis of *T(Y;2)D6*: T(Y;2)Y^SXh;25D2-3 + In(2L)24C4-6;25D2-3 + Df(2L)24C3;24E1.2. This is likely incorrect if 24C4-6 is deleted.

Isolation of mutations in region 24A3-D4

Two-day-old BB (for^R/for^R) males were treated with either 5000 rad of gamma radiation from a ^{60}Co source or fed a solution of 12 mM ethyl methanesulfonate (EMS) in 1% sucrose for 24 h (modified from Lewis and Bacher 1968). They were crossed to $SM1/bw^{V1}$ virgin females to balance mutagenized second chromosomes (indicated by * below). EMS-fed males were allowed to recover for 24 h prior to mating. Mutagenized males were discarded after 4 days. Individual $*/SM1$ F_1 males were each mated to three $Df(2L)ed^{Sz}$, $L^2/SM5$ virgin females in separate vials. Putative new recessive lethal mutations in region 24A3-D4 were scored when progeny of these crosses (F_2) consisted of only *Cy* flies. $*L^+/SM5$ adults from these vials were used to initiate individual balanced lethal lines (referred to as $*/SM5$ below).

Complementation

Complementation tests were done at 25 and 29°C to isolate temperature sensitive (ts) mutations (Suzuki *et al.* 1967). All progeny,

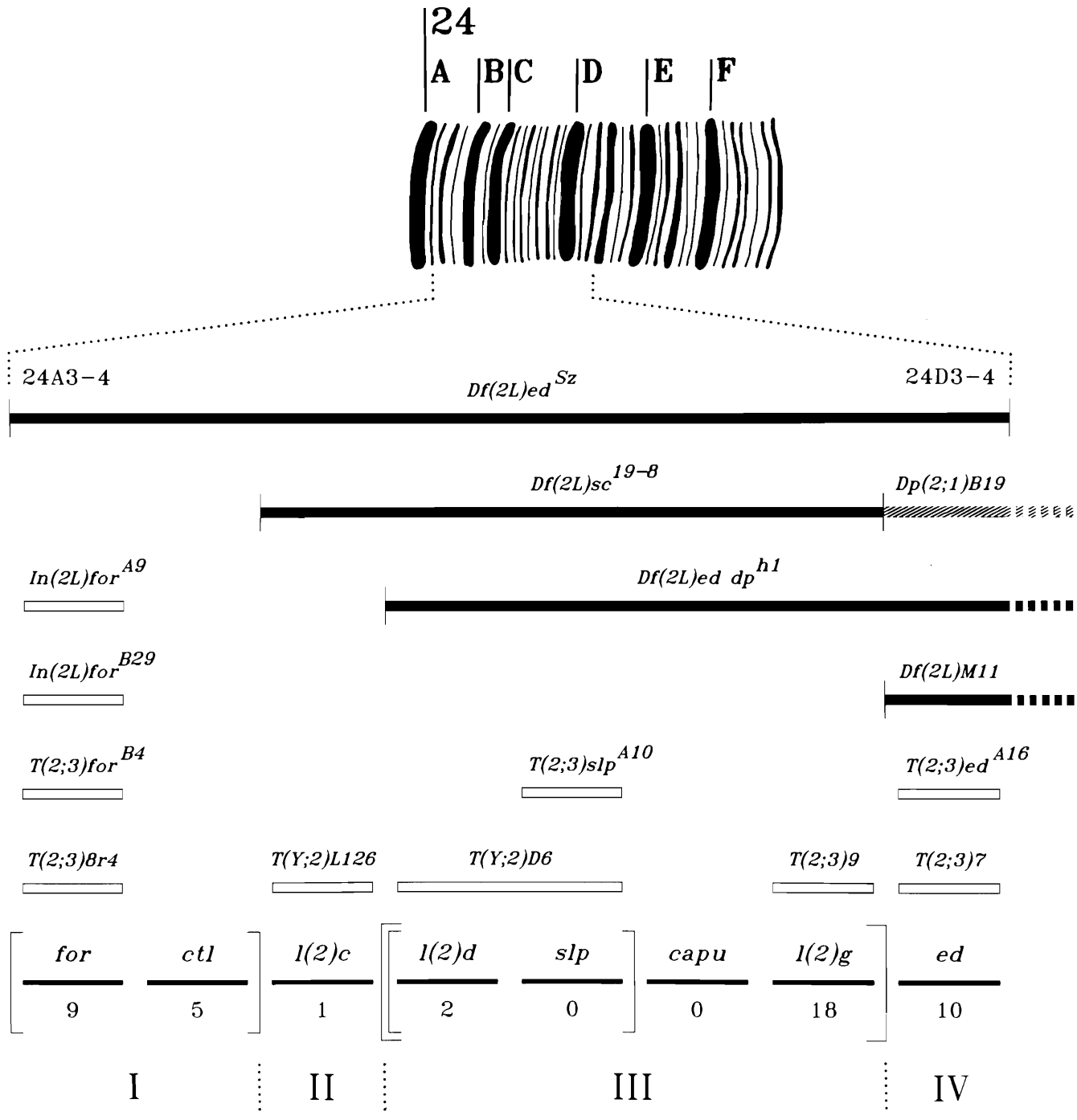

FIG. 1. Complementation map of polytene chromosome interval 24A3-D4 (chromosome redrawn from Sorsa 1988). The extent of chromosome deletions are represented by solid bars. Empty bars represent rearrangement breakpoints. The duplication *Dp(2;1)B19* (shaded bar) is maintained over the deficiency $Df(2L)sc^{19\text{-}8}$; these chromosomes are shown superimposed. Subintervals defined by the breakpoints of adjacent chromosome deletions are indicated by Roman numerals. Complementation groups are shown, along with the number of identified alleles. The relative positions of complementation groups not separated by deficiency breakpoints are tentative and placed within brackets. Alleles of *for* include seven described in previous studies (see Results). Five mutant alleles of *capu* are known to map between 24C3 and 24D4 (Manseau and Schupbach 1989). We did not isolate new *capu* alleles in this study, since our screens were not designed to recover mutations associated with male or female sterility. Mutant alleles of *Shaw* (Butler *et al.* 1989) are not currently known to exist.

no fewer than 50, were scored from each cross. Most complementation tests were between mutations maintained in heterozygotes with *Cy*-marked chromosome-*2* balancers. Thus we expected that fully complementing lethals would give rise to $Cy{:}Cy^+$ progeny in a 2:1 ratio. A cross was scored as "viable" when the proportion of Cy^+ progeny was greater than 10%. Crosses yielding up to 10% Cy^+ progeny were scored as "semilethal," while those with no Cy^+ flies were scored as "lethal." Critical classes recovered from semilethal crosses were progeny tested to confirm Cy^+/Cy^+ genotypes. Results from crosses made at 25 and 29°C were pooled when they did not differ significantly (χ^2 analysis of 2×2 contingency tables; Zar 1984).

All mutations were tested for complementation with four deficiencies found in the 24A3-D4 interval (Table 1). Within each

subinterval, all combinations of mutations were assayed for complementation. Additional complementation tests were made between representative alleles of complementation groups within different subintervals.

Recombination

A class of lethal mutations was recovered that was lethal in combination with *Df(2L)ed*^{Sz}, L^2 and found to complement all other *for* microregion deletions employed. To establish whether these mutations map to subinterval I (24A3-C5) or are secondary mutations mapping elsewhere, frequencies of recombination between each mutation and *for*$^{ls(R)172}$ were measured. Males from each */*SM5* line were mated to *for*$^{ls(R)172}$*SM1* virgin females. At least 25 F_1 */for*$^{ls(R)172}$ female progeny were collected from each cross and mated to 25 *Df(2L)ed*^{Sz}, L^2/*SM5* males in bottles. These were rebrooded once. No fewer than 500 F_2 progeny were scored per mutant line for the presence of $*^+$ *for*^{R}/*Df(2L)ed*^{Sz}, L^2 recombinants. Lethals mapping more than 2 cM from *for* were judged to be outside the deleted region defined by *Df(2L)ed*^{Sz}. Of 32 new recessive lethals, initially localized to subinterval I, 22 were mapped by recombination to points greater than 2 cM from *for* and therefore judged to be outside the region defined by *Df(2L)ed*^{Sz}. These undoubtedly represent alleles of secondary lethals that were linked to the deletion (see Mukai 1964; Hilliker 1976).

A similar strategy was used to determine whether complex patterns of complementation involving two alleles of different groups were attributable to second-site lethals common to both chromosomes, rather than to either small deficiencies or interallelic complementation. Males bearing mutation(s) that failed to complement with alleles of two complementing loci ($*^{ab}$/*SM5*) were mated to females *trans*-heterozygous for lethal alleles of both loci ($*^a/*^bF_1$ from matings between $*^a$/*SM5* and $*^b$/*SM5*). From each cross, F_2 progeny were scored for a Cy^+ recombinant class.

Nomenclature

We have named new lethal complementation groups in alphabetical order (distal to proximal). Alleles were designated alphanumerically according to the mutagenesis experiment (gamma, A–B; EMS, C–I) and the order of each isolate. For example, the most distal lethal that is the third isolate from the second gamma screen would be designated *l(2)a*^{B3}. In addition, *ts, cl,* and *sl* were included in the nomenclature of temperature sensitive, conditional lethal, and semilethal alleles, respectively. The names of previously identified complementation groups were retained. New visible loci were named according to their phenotype.

When chromosome rearrangements involving heterochromatic breakpoints could not be fully characterized by polytene chromosome analysis we distinguished 2-3 translocations from 2-2 transpositions on the basis of segregation data from test crosses to *bw; ve st e* females.

Behaviour

The locomotory component of foraging behaviour in third instar larvae was quantified using a procedure described in de Belle and Sokolowski (1987), which we briefly outline here. Foraging third instar larvae (96 ± 2 h post-hatching) were collected and washed in distilled water. Larvae were individually placed in Petri dishes (8.5 cm diameter × 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). The path length made by a foraging larva during a 5-min test was measured and classified as rover or sitter by comparison with paths of concurrently tested *for*^{R}/*for*^{R} and *for*^{s}/*for*^{s} standard strains, or in one case by comparison to the strain from which the mutant was derived (see Results). Behavioural testing occurred on each day within an 8-h interval beginning at 11:00 at room temperature under homogeneous overhead illumination.

Larval behaviour was tested for mutations in subinterval I and lethals from other subintervals were included as controls. Behavioural phenotypes were always measured in progeny from */*SM5* by *for*^{s}/*for*^{s} matings. Path lengths were scored for larvae that eclosed as Cy^+ adults. Strains were tested "blind" and in random order.

TABLE 2. Behavioural phenotypes expressed by mutations in 24A3-D4

	N	Path length (cm)* ($\bar{X}$ ± SE)	Phenotype†
for/for^s heterozygotes			
In(2L)for^{A9}/*for*^{s}	61	11.85 ± 0.53	Intermediate
In(2L)for^{B29}/*for*^{s}	55	12.62 ± 0.56	Intermediate
T(2;3)for^{B4}/*for*^{s}	66	11.91 ± 0.50	Intermediate
for^{lH6}/*for*^{s}	85	12.59 ± 0.44	Intermediate
for^{l113}/*for*^{s}	65	12.91 ± 0.49	Intermediate
ctl/for^s heterozygotes			
ctl^{clB13}/*for*^{s}	24	15.17 ± 0.77	Rover
ctl^{cl11}/*for*^{s}	23	15.97 ± 1.24	Rover
ctl^{cl17}/*for*^{s}	41	12.83 ± 0.64	Intermediate
ctl^{cl110}/*for*^{s}	69	12.56 ± 0.48	Intermediate
ctl^{cl114}/*for*^{s}	29	13.55 ± 0.65	Intermediate
l(2)c/for^s heterozygotes			
l(2)c^{E17}/*for*^{s}	24	16.30 ± 0.92	Rover
ed/for^s heterozygotes			
ed^{lC2}/*for*^{s}	15	16.31 ± 1.11	Rover
Standard strains			
for^s	250	7.99 ± 0.22	Sitter
for^R	249	16.59 ± 0.26	Rover

*Path lengths were significantly different (ANOVA, $F_{(13, 1040)} = 50.11$ $P < 0.0001$).

†Behavioural phenotypes classified as rover were not significantly different from the *for*^{R} rover strain. Intermediate phenotypes were significantly different from both standard strains (Student-Newman-Keuls multiple range test, $P \leq 0.05$).

Results

The results of our cytogenetic and complementation analysis of the 24A3-D4 region is summarized in Fig. 1. The EMS and gamma irradiation screems of 5178 and 5053 second chromosomes produced 33 and 10 new mutations, respectively, which mapped within *Df(2L)ed*^{Sz}.

Complementation groups of subinterval I (24A3-4 to 24C2-5)

Included in this subinterval were mutants complementing with all deficiencies except *Df(2L)ed*^{Sz} (Fig. 1). Two lethal complementation groups define this subinterval; their relative positions could not be determined.

One of these lethal complementation groups corresponds to the *for* locus. Previously identified *for* mutations include three lethal and two nonlethal alleles of *for* (de Belle *et al.* 1989) and a lethal breakpoint associated with *T(2;3)8r4* (Table 1). The four nonlethal alleles include two naturally occurring alleles (*for*^{R} and *for*^{s}) and two gamma-induced alleles (*for*$^{s(R)136}$ and *for*$^{s(R)164}$).

T(2;3)8r4 was generated from a standard sequence strain *cn bw* in an unrelated study (Eberl *et al.* 1989). We found that the lethal phenotype of *T(2;3)8r4* failed to complement with all lethal alleles of *for*. The revised cytology of *T(2);3)8r4* (Table 1) allowed us to narrow down the *for* lethal breakpoint to 24A3-5. Since *T(2;3)8r4* has a lethal breakpoint in *for*, we tested its larval behaviour along with *cn bw*, the control strain. Indeed *T(2;3)8r4/for*^{s} larvae

TABLE 3. Complementation matrix for *cutlet* phenotypes*

	ctl^{clB13}	ctl^{cl11}	ctl^{cl17}	ctl^{cl110}	ctl^{cl114}
$Df(2L)ed^{Sz}$, L^2					
29°C	–	–	sl	–	–
25°C	–	sl	+	–	sl
$Df(2L)ed^{Sz}$					
29°C	+	ctl	+	ctl	ctl
25°C	+	ctl	+	ctl	ctl
ctl^{cl114}					
29°C	+	ctl, sl	+	ctl, sl	
25°C	+	ctl	+	ctl	
ctl^{cl110}					
29°C	+	ctl	–		
25°C	+	ctl	–		
ctl^{cl17}					
29°C	ctl	+			
25°C	ctl	+			
ctl^{cl11}					
29°C	+				
25°C	+				

*Critical class viabilities: +, viable (>10% of progeny); sl, semilethal (<10% of progeny); –, lethal; ctl, *cutlet* visible phenotype.

expressed strong sitter phenotypes (mean ± SE, sample size was 6.97 ± 0.39 cm, 57) compared with the *cn bw/for*^{s} control larvae, which had significantly longer path lengths (12.71 ± 0.41, 90) (Student's *t*-test, $t_{(56, 89)} = 9.54$, $P < 0.0001$). This provided evidence that the lethal and behavioural function are attributable to the same locus. However, two other possibilities exist. One is that there is a small deletion that includes the *for* breakpoint and an adjacent vital function. The other possibility is that the behavioural alteration we found results from position affect variegation, since *T(2;3)8r4* includes the transposition of a chromosome-*2* segment (22A2-B1 to 24A2-5) into the centric heterochromatin of chromosome-*3* (see Discussion).

We isolated five new lethal mutations of *foraging*. Three mutations induced by gamma radiation are rearrangements, each with breakpoints in 24A2-B1 (*In(2L)for*^{A9}, *In(2L)for*^{B29}, and *T(2;3)for*^{B4}; Table 1 and Fig. 1). Two EMS-induced mutations were found to be cytologically normal lethal alleles, *for*^{lH6} and *for*^{l113}. All 36 *for* heteroallelic combinations were fully lethal except *In(2L)for*^{B29}/*T(2;3)8r4*, which was semilethal at 25°C and lethal at 29°C. All new lethal *for*^{l} mutations expressed altered (intermediate) behavioural phenotypes that were significantly different from *for*^{R} rovers (Table 2), once again providing evidence that the lethal and behavioural functions are encoded by the same gene (see Discussion).

The cutlet (*ctl*) complementation group mapped adjacent to *for* (Fig. 1) and did not have any previously identified mutations. We recovered four EMS- and one radiation-induced allele as lethals or semilethals when heterozygous with $Df(2L)ed^{Sz}$, L^2 at 29°C. They were viable over all other chromosome rearrangements used in this study, including $Df(2L)ed^{Sz}$, L^+. Hemizygous lethality of all *ctl* alleles appears to be conditional on an interaction with L^2. Chromosomes bearing *ctl* alleles were all homozygous lethal and cytologically normal. There is a complex pattern of allelic complementation in the *ctl* locus (Table 3). All *ctl* mutations were allelic since *trans*-heterozygotes were either lethal or expressed a characteristic visible phenotype. However, no single putative *ctl* allele failed to complement with all other alleles (Table 3). In certain heteroallelic combinations, we observed roughened eyes, scalloped trailing, and (or) notched end wing margins resembling certain mutant alleles of cut, Notch, and scalloped (Lindsley and Zimm 1992). The *ctl* visible phenotypes are recessive, aside from the semidominance of the roughened eye conferred by ctl^{clB13}.

All combinations of *foraging* and *cutlet* alleles except one were fully viable and wild type in visible phenotype. *for*^{l113} and ctl^{cl110} chromosomes were found to share a common second-site lethal mutation. We mapped this secondary lethal to a point greater than 15 cM from the two genes based on frequencies of Cy^+ ctl^+ recombinant progeny recovered from each of the following crosses (data not shown): (*i*) *for*^{lH6}/ctl^{cl110} females mated to *for*^{l113}/*SM5* males and (*ii*) *for*^{l113}/ctl^{cl17} females mated to ctl^{cl110}/*SM5* males.

Larval path length was measured for all five *ctl* alleles heterozygous with *for*^{s} (Table 2). Both ctl^{clB13} and ctl^{cl11} expressed rover phenotypes. The alleles ctl^{cl17}, ctl^{cl110}, and ctl^{cl114} when heterozygous with *for*^{s} gave rise to intermediate behavioural phenotypes, which were not significantly different from those associated with the *for* lethal mutations recovered in this study. Two mutations mapping to different subintervals, *l(2)c*^{E17} and *ed*^{lC2}, were included as controls in our behavioural experiments (Table 2). As expected, both controls expressed strong rover phenotypes that were not significantly different from the *for*^{R} standard strain. The observation that three of five *ctl* alleles have a coincident altered larval foraging phenotype leads us to speculate that *for* and *ctl* may constitute a complex locus (see Discussion).

The altered intermediate behavioural phenotypes associated with the *for*^{l} mutations recovered in the present study were less extreme than the sitter phenotypes expressed by the *for*$^{ls(R)}$ alleles (described in de Belle *et al.* 1989). We attribute this difference to genetic background effects. Of necessity, the crossing scheme used in the present study resulted in genetic backgrounds that differ from those produced in de Belle *et al.* (1989). Both schemes employed chromosome-*2* balancers to maintain intact linkage of second chromosomes. However, the *for*$^{ls(R)}$ alleles from our previous study are balanced over *SM1*, whereas the *for*^{l} mutations described in this report are balanced over *SM5* (from the $Df(2L)ed^{Sz}$/*SM5* strain). Larval path lengths of *for*^{R}/*SM5* were significantly longer than those of both *for*^{R} homozygotes and *for*^{R}/*SM1* (data not shown). We therefore conclude that the *for*^{l} mutations described in this report gave rise to intermediate rather than sitter phenotypes because the genetic background of $Df(2L)ed^{Sz}$/*SM5* enhances larval path length. Thus the observation of significant reductions in path lengths associated with all *for*^{l} mutations recovered in our lethal screen supports the hypothesis that *for* is a vital locus.

Complementation groups of subinterval II (24C2 to 24C3-5)

Mutations failing to complement with $Df(2L)ed^{Sz}$ and $Df(2L)sc^{19-8}$ but complementing with *Df(2L)ed dp*^{hl} and *Df(2L)M11* define subinterval II (Fig. 1). *T(Y;2)L126* was previously identified and has a lethal breakpoint in 24C2-D1 (Table 1).

The single complementation group in subinterval II is *l(2)c* (Fig. 1), represented by *T(Y;2)L126* and one new EMS-induced allele designated *l(2)c*^{E17}, which failed to comple-

ment. We initially considered that subinterval II might include a second vital genetic function. *T(Y;2)L126* and *T(Y;2)D6* (Table 1; see below) were lethal in combination at 29°C and semilethal at 25°C, while $l(2)c^{E17}$ was fully viable with *T(Y;2)D6*. However, restriction fragment analysis of subinterval II failed to detect a *T(Y;2)D6* breakpoint (D. E. Coulter, personal communication). We therefore suggest that the semilethality of *T(Y;2)L126/T(Y;2)D6* heterozygotes may result from a common second-site mutation.

Complementation groups of subinterval III (24C3-5 to 24D3-4)

Mutations failing to complement with $Df(2L)ed^{Sz}$, $Df(2L)sc^{19-8}$, and $Df(2L)ed\ dp^{h1}$ but complementing with *Df(2L)M11* were placed in subinterval III (Fig. 1). Previously identified mutations in this subinterval are the lethal breakpoints of *T(Y;2)D6* and *T(2;3)9* (Table 1) and *slp* (Coulter and Wieschaus 1988) and *capu* (Manseau and Schupbach 1989). *T(Y;2)D6* is a complex rearrangement and may be deficient for bands in 24C (Velissariou and Ashburner 1980). Indeed, we observed a deletion with breakpoints 24C2-D1; 24D2-5 associated with *T(Y;2)D6*. We recovered 21 new lethal mutations mapping to this subinterval (two induced with gamma radiation and 19 with EMS). Taken together they define four complementation groups in subinterval III.

l(2)d is represented by two new EMS-induced lethal alleles designated $l(2)d^{F14}$ and $l(2)d^{F16}$ (Fig. 1), which were lethal as *trans*-heterozygotes and both were lethal with *T(Y;2)D6*. Combinations of these alleles with all other mutations in subinterval III (including recessive lethal slp^{7L48} and slp^{11M105} alleles) were fully viable and did not show obvious visible mutant phenotypes. Furthermore, *l(2)d* alleles did not produce female sterility or embryonic lethality in combination with alleles of *capu* (L. J. Manseau, personal communication).

We isolated one mutation of the *sloppy paired* complementation group (Fig. 1), a radiation-induced rearrangement designated $T(2;3)slp^{A10}$ with a breakpoint in 24C2-D1 (Table 1). It failed to complement with *T(Y;2)D6*. Both of these rearrangements were completely lethal with slp^{7L48} and slp^{11M105}. $T(2;3)slp^{A10}$ was fully viable with mutations from all other complementation groups, with the following exceptions. Three of the 19 *l(2)g* alleles ($l(2)g^{G7}$, $l(2)g^{H21}$, and $l(2)g^{13}$; see below) appeared cytologically normal but were semilethal in combination with $T(2;3)slp^{A10}$. We suggest that this result may be attributable to position-effect variegation associated with the $T(2;3)slp^{A10}$ heterochromatic breakpoint, which might be detectable in heterozygotes for certain extreme alleles of *l(2)g*.

l(2)g is defined by *T(2;3)9* (Fig. 1), a previously described chromosome rearrangement that has a breakpoint in 24D1-2 (Table 1). An additional 18 new lethal alleles were recovered, all but 1 of which were EMS induced. Among *l(2)g* alleles, 8 of the 153 tested heteroallelic combinations were either semilethal or viable at one or both temperatures (Table 4A). Representative alleles $l(2)g^{D4}$ and $l(2)g^{H10}$ were fully viable with alleles of *slp* and generated fertile offspring in combinations with alleles of *capu* (L. J. Manseau, personal communication). All mutations of this group were fully viable with *T(Y;2)D6*, separating *l(2)g* from both *l(2)d* and *slp* complementation groups in subinterval III.

TABLE 4. Complementation matrices* for *l(2)g* heteroalleles†

(A) Semilethal and viable combinations of *l(2)g* alleles

	$l(2)g^{C5}$	$l(2)g^{D4}$	$l(2)g^{H21}$	$l(2)g^{117}$
$l(2)g^{D5}$				
29°C	–	–	–	sl
25°C	–	–	–	sl
$l(2)g^{F5}$				
29°C	–	–	sl	–
25°C	+	sl	+	+
$l(2)g^{H19}$				
29°C	–	sl	–	–
25°C	–	sl	–	–
$l(2)g^{H24}$				
29°C	–	–	–	–
25°C	+	–	–	–
$l(2)g^{117}$				
29°C	–	–	–	
25°C	+	–	–	

(B) Lethal and semilethal combinations of *l(2)g* and *ed* alleles‡

	$l(2)g^{D5}$	$l(2)g^{H14}$	$l(2)g^{H21}$	$l(2)g^{117}$
Df(2L)M11				
29°C	+	–	–	sl
25°C	+	–	–	+
$T(2;3)ed^{A16}$				
29°C	+	+	+	sl
25°C	+	+	+	+
ed^{lA11}				
29°C	+	+	+	sl
25°C	+	+	+	+
ed^{slA12}				
29°C	+	+	+	sl
25°C	+	+	+	+
ed^{lB6}				
29°C	+	+	+	–
25°C	+	+	+	+
ed^{lC2}				
29°C	+	sl	+	sl
25°C	+	+	+	+
ed^{lC4}				
29°C	–	+	+	–
25°C	+	+	+	+
ed^{lF20}				
29°C	+	+	+	sl
25°C	+	+	+	+
ed^{lH5}				
29°C	+	+	+	–
25°C	+	+	+	+
ed^{slH8} §				
29°C	+	sl	+	sl
25°C	+	+	+	+
ed^{lH15}				
29°C	+	–	+	–
25°C	+	–	+	+
ed^{lH23}				
29°C	+	sl	+	sl
25°C	+	+	+	+

*Critical class viabilities: +, viable (>10% of progeny); sl, semilethal (<10% of progeny); –, lethal.

†Seven *l(2)g* alleles ($l(2)g^{B15}$, $l(2)g^{c7}$, $l(2)g^{E9}$, $l(2)g^{F1}$, $l(2)g^{F18}$, $l(2)g^{H10}$, and $l(2)g^{H11}$) were lethal in combination with all *l(2)g* alleles and fully viable with all tested alleles of other complementation groups. Three alleles ($l(2)g^{G7}$, $l(2)g^{H21}$, and $l(2)g^{13}$) were semilethal in combination with $T(2;3)slp^{A10}$ (see Results).

‡All *l(2)g/ed* heterozygotes not listed here were completely viable and visibly wild type.

§All $l(2)g/ed^{slH8}$ heterozygotes were visibly wild type.

Four *l(2)g* alleles represent a puzzling class of mutants. $l(2)g^{H14}$ and $l(2)g^{I17}$ were lethal or semilethal at 29°C, respectively, in combination with *Df(2L)M11* and with some lethal alleles of *ed*, the single complementation group in subinterval IV (see below; Table 4B). The alleles designated $l(2)g^{H21}$ and $l(2)g^{D5}$ were lethal with *Df(2L)M11* and with a single *ed* allele at 29°C, respectively (Table 4B). However, only one heteroallelic combination of a *l(2)g* allele and an *ed* allele failed to complement at both 25 and 29°C, $l(2)g^{H14}/ed^{lH15}$.

Complementation groups of subinterval IV (24D3-4)

Mutations within subinterval IV fail to complement with the deletions $Df(2L)ed^{Sz}$, $Df(2L)ed\ dp^{h1}$, and *Df(2L)M11* but not $Df(2L)sc^{19\text{-}8}$ (maintained with an insertional duplication, which is viable in combination with *Df(2L)M11*; Table 1 and Fig. 1). All subinterval IV mutations are members of the *echinoid* complementation group (Reuter and Szidonya 1983; Szidonya and Reuter 1988*a*). *T(2;3)7* is a previously described rearrangement with a lethal breakpoint in 24D2-E1 (Table 1).

We recovered 11 new *echinoid* mutants (Fig. 1), 4 of which were generated with gamma radiation and 7 with EMS. $T(2;3)ed^{A16}$ is gamma induced with a breakpoint in 24D2-5 (Table 1). The other new *ed* alleles are cytologically normal. ed^{lA11} and ed^{lB6} are both recessive lethal and ed^{slA12} is semilethal. Six EMS-induced mutants are recessive lethal and one is semilethal, ed^{slH8}. Hemizygous ed^{slH8} flies have extreme echinoid visible phenotypes.

Among *ed* alleles, all failed to complement with the following exceptions. Crosses that included ed^{slH8} were semilethal; surviving *trans*-heterozygotes had extreme echinoid visible phenotypes. ed^{slA12} was viable with $T(2;3)ed^{A16}$ at 25°C only and semilethal with ed^{lH5} at both temperatures. The new *ed* alleles gave rise to viable phenotypically wild-type progeny in combinations with alleles of all other complementation groups within the *for* microregion, excepting some alleles of *l(2)g* (see above; Table 4B).

Discussion

In an earlier study we used lethal tagging to generate a number of putative lethal alleles of *for*. We were not able to conclude from this study whether the three noncomplementing lethal mutations were lethal alleles of *for* or small deletions which removed adjacent genes that carried vital and behavioural functions. The present study was designed to address this issue and to generate chromosome rearrangements in the *for* microregion to facilitate cloning of the *for* gene. To this end, we undertook gamma radiation screens to generate chromosomal rearrangements in the vicinity of *for* and EMS screens to attempt to generate lethal mutations. We reasoned that if *for* is a vital locus, *bona fide* lethal mutants of *for* should show altered behavioural phenotypes. We generated three new *for* alleles associated with chromosome rearrangements with gamma radiation, two EMS-induced lethal alleles of *for* and another lethal allele of *for* associated with a chromosomal rearrangement *T(2;3)8r4* that we had available (Eberl *et al.* 1989). Lethality and behaviour did not complement in all of these *for* strains. The latter rearrangement enabled us to narrow the *for* breakpoint to 24A3-5. All of the rearrangements should be useful for molecular analysis of *for*.

How strong is the evidence that *for* is a vital gene? Let us first consider the three gamma radiation induced *for* lethal mutations. The three gamma induced point mutants reported in de Belle *et al.* (1989) may have been small cytological (indiscernible) deletions. Of the four lethal *for* mutations reported in the present study, one is associated with a chromosomal rearrangement *(T(2;3)8r4)* that had a heterochromatic breakpoint that may have resulted in simultaneous inhibition of the expression of for^R and an adjacent vital locus owing to position-effect variegation. However, the other three *for* lethal mutations reported in this study are associated with cytological rearrangements that are most easily explained as having breaks within the *for* locus and would unlikely affect an adjacent locus. For these rearrangements of *for* to also carry a deletion of *for* and an adjacent vital gene, they would all have to be multibreak rearrangements, which is very unlikely (Eberl *et al.* 1989). Thus the overall analysis of the gamma-induced *for* lethals does not provide compelling support for the notion that the *for* lethality is due to the effects on an adjacent gene.

The best evidence that *for* is a vital gene is the behavioural change in the two EMS-induced for^1 mutations. EMS, when employed as a mutagen in *Drosophila melanogaster*, under the conditions used in this study, produces almost exclusively point mutations, i.e., mutations confined to one gene (see Hilliker *et al.* 1980, and references therein). Thus, the probability that both EMS *for* alleles are deletions is small. Taken together, the data from our nine lethal *for* mutants strongly argue that *for* is a vital gene.

The *cutlet* locus is adjacent to *foraging*. We have yet to determine the relative order of these two loci and we cannot say with confidence that *ctl* represents a genetic element truly separable from *for*. Three of the five *ctl* alleles showed clearly reduced path lengths when heterozygous in combination with for^5 (i.e., they at least partially failed to complement the sitter behaviour associated with the hypomorphic for^5 allele). Consequently, *for* and *ctl* may together constitute a complex genetic locus. This issue is further clouded by the complex and conditional patterns of complementation among *ctl* alleles involving lethal and visible phenotypes. Complex patterns of complementation and multiple phenotypes are proving to be common features of many loci known to influence behaviour (e.g., *dunce* (review Tully 1991 and references therein); *Shaker* (Ferrus *et al.* 1990); *optomotor-blind* (Pflugfelder *et al.* 1990; Brunner *et al.* 1992); and *miniature-dusky* and *Andante* loci (Newby *et al.* 1991; Konopka *et al.* 1991)). As has been shown to be the case in these examples, we anticipate that molecular and further genetic analyses will give us insight into the function of the *foraging* gene and help clarify the relationship between *foraging* and *cutlet*.

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