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# Rover/Sitter Foraging Behavior in *Drosophila melanogaster:* Genetic Localization to Chromosome *2L* Using Compound Autosomes

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*Genetic control of the rover/sitter behavioral polymorphism in* Drosophila melanogaster *larvae was localized to the left arm of chromosome* 2. *Ten independent left and right compound second chromosomes were generated in isogenic rover and sitter strains by gamma irradiation and substituted into 25 different lines. Comparisons were made between lines to determine the chromosome arm contributions to rover/sitter phenotype expression.*

**KEY WORDS:** larval behavior; compound autosomes; genetic mapping; *Drosophila melanogaster.*

## INTRODUCTION

A naturally occurring polymorphism exists in the locomotor component of *Drosophila melanogaster* larval foraging behavior (Sokolowski, 1980). The behavior is assayed by measuring the distance a larva travels while foraging in a yeast-coated petri dish during a test period. The distance traveled while foraging (path length) is significantly longer in rover than in sitter larvae. The polymorphism has a genetic basis which we have localized to successively smaller regions of the *D. melanogaster* genome. Mendelian analyses have shown that rover/sitter fits a single-gene model of inheritance, with rover completely dominant over sitter (Bauer and Sokolowski, 1984; de Belle and Sokolowski, 1987). Analysis of crosses between isogenic rover and sitter strains showed that larval path length is autosomally inherited, and not significantly influenced by the *Y* chromosome, permanent cytoplasmic factors, transient maternal factors, or interactions

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between these hereditary components. A minor *X*-chromosome effect was also observed which may reflect *X*-linked gene modification of a major autosomal gene (de Belle and Sokolowski, 1987). Chromosome analyses of both laboratory (Sokolowski, 1980) and naturally derived strains (Bauer and Sokolowski, 1985) have revealed a second-chromosome genetic basis for rover/sitter. The third pair of chromosomes and second-by-third chromosome interactions do not significantly affect larval path length.

Further genetic localization of rover/sitter becomes difficult at this point. Accurate measurement of a behavioral phenotype requires precise control of biological and physical factors which contribute to variation during the rearing and behavioral testing of animals (Ehrman and Parsons, 1981). Previous attempts to localize rover/sitter using standard recombinational mapping techniques were not successful because penetrance of the single major gene became obscured by mutations introduced through crosses made to marked stocks. This was not surprising since genetic markers often influence the expression of behavioral phenotypes. For example, the *yellow* (*y*) mutation affects male wing display during courtship. Shorter and less frequent bouts of wing vibration by *y* males decrease their mating success (Bastock, 1956). Another example of the effect of a genetic marker on a behavioral phenotype was noted in the present study. The *jaunty* (*j*) mutation delayed larval development such that valid behavioral comparisons could not be made between developmentally asynchronous *j* and $j^+$ larvae of the same age (see Materials and Methods). In this report we describe a mapping method which uses compound autosomes rather than morphological markers to determine linkage of the rover/sitter gene to a chromosome arm.

Compound reverse metacentric chromosomes (CRM; also called compound autosomes) have two genetically identical arms symmetrically joined to one centromere (Chadov, 1970). These rearrangements are produced through a translocation mechanism (Hilliker, 1981) in which homologous centric and acentric free arms become attached (Holm, 1976).

Compound left and right autosome genes are physically unattached and segregate at meiosis in *D. melanogaster* females, producing two types of gametes (Grell, 1970). Segregation in males is random, resulting in four types of gametes (two segregational, one disomic, and one nullosomic) occurring at equal frequencies (Holm and Chovnick, 1975). Viable progeny are produced from the union of gametes, one of which carries a left compound autosome, and the other a right compound. Since both alleles of all CRM loci reside on either the left or the right compound, a recessive trait can be inherited from one parent (Holm, 1976).

In the present study, 10 compound second chromosomes were independently generated in irradiated rover and sitter strains. Twenty-five lines were then constructed with different left–right compound autosome combinations.

These were tested for rover/sitter behavior and were used successfully to localize this trait to a chromosome arm.

## MATERIALS AND METHODS

### Strains

Compound second compound-2 chromosome lines were constructed using three *Drosophila melanogaster* strains. EE [described by Sokolowski (1980), expressing a sitter phenotype] and B15B15 [described by Bauer and Sokolowski (1985), expressing a rover phenotype] are both isogenic for second and third pairs of chromosomes. A compound-2 chromosome strain [*C(2L)RM, j; C(2R)RM, px*] homozygous for the recessive markers *jaunty* (*j:* 2-48.7) on the left compound [*C(2L)RM, j*] and *plexus* (*px:* 2-100.5) on the right [*C(2R)RM, px*] was provided by J. C. Hall. RM is hereafter excluded from the nomenclature for convenience. Further details on the above mutants are given by Lindsley and Grell (1968) and Lindsley and Zimm (1985). Strains were maintained in plastic culture bottles on 45 ml of a dead yeast, sucrose, and agar (culture) medium at 24 ± 1°C, a 15 ± 1-mbar vapor pressure deficit, and a 12:12 L:D photocycle with lights on at 0800 h.

### The Construction of Compound Autosomes

The following protocol was used to generate compound autosomes in both the EE sitter (S) and the B15B15 rover (R) strains (hereafter referred to as strains of origin; Fig. 1). One thousand 3- to 7-day-old virgin females of each strain were treated with 2000 rads of gamma radiation from a $^{60}$Co source (Holm, 1976) and separately mated to 500 2- to 4-day-old *C(2L), j; C(2R), px* males (Bateman, 1968). The few larvae recovered from both crosses carried putative compound-2 chromosomes. These larvae were collected in individual vials over 6 days. Independent compound autosome lines were established by adding 10 *C(2L), j; C(2R), px* adult flies of opposite sex to the pupa in each vial. Progeny from these crosses were screened for *j* or *px* phenotypes identifying *C(2L), j* or *C(2R), px* chromosomes, respectively. Conversely, the absence of *j* or *px* identified newly generated *C(2L)* or *C(2R)* chromosomes (attached left or right arms) of EE sitter or B15B15 rover strain origin.

Compound autosomes of sitter strain origin were designated *C(2L), "S"* or *C(2R), "S"*, while those of rover strain origin were designated *C(2L), "R"* or *C(2R), "R"*. Lines are independent because they were initiated from individual larvae, each having a separately generated compound second chromosome. Ten lines were established: three *C(2L), "S"; C(2R), px,* three *C(2L), j; C(2R), "S"*, two *C(2L), "R"; C(2R), px,* and two *C(2L), j; C(2R), "R"*.

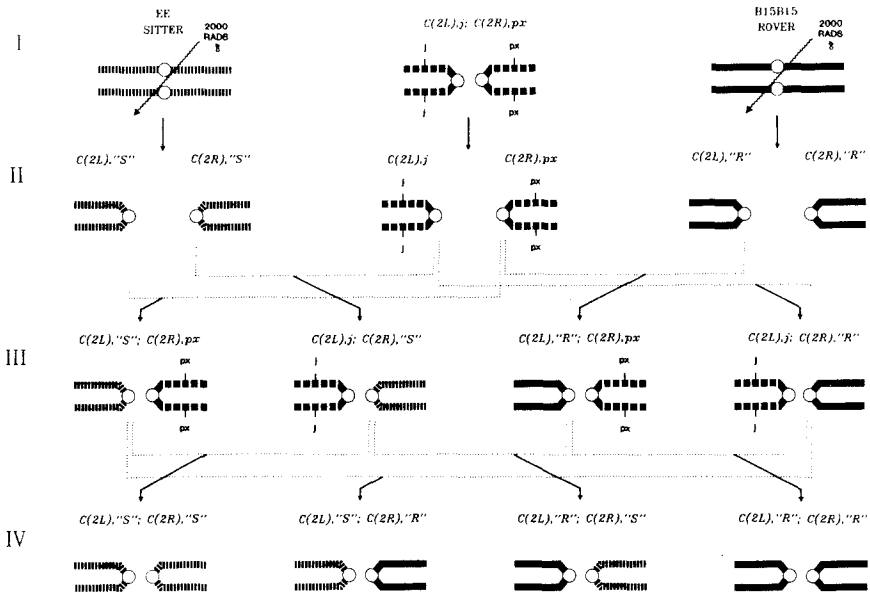


**Fig. 1.** Protocol for constructing compound-*2* lines from irradiated EE sitter (S; striped bars) and B15B15 rover (R; filled bars) *D. melanogaster* strains. Both irradiated strains were separately crossed to *C(2L), j; C(2R), px* (row I). Each strain had two types of viable compound-*2* gametes participating in these crosses (row II). Two types of progeny (marked with *j* or *px*) resulted from each cross (row III). Lines initiated from these progeny were crossed to eliminate the marked chromosomes [*C(2L), j* and *C(2R), px*]. Four groups of lines were then established which had combinations of compound-*2L* and compound-*2R* chromosomes of EE sitter (S) and B15B15 rover (R) strain origins (row IV).

CRM configurations were verified by cytological examination (A. J. Hilliker, personal communication).

We observed that developmental rates of all *C(2L), j* lines were considerably slower than those of *C(2R), px*. The importance of developmental synchrony (Sokolowski *et al.*, 1984) necessitated that we perform crosses to replace marked *C(2L), j* and *C(2R), px* chromosomes with unmarked ones (Fig. 1). Phenotypically wild-type progeny from these crosses were used to initiate 25 new compound-*2* lines: nine *C(2L), "S"; C(2R), "S"*, six *C(2L), "S"; C(2R), "R"*, six *C(2L), "R"; C(2R), "S"*, and four *C(2L), "R"; C(2R), "R"*.

## Behavioral Assay

The locomotor component of foraging behavior in third-instar larvae was quantified for each of the 25 compound-*2* lines along with the original EE sitter and B15B15 rover strains (standards) in a procedure similar to that described by de Belle and Sokolowski (1987). Approximately equal numbers of 2- to 7-

day-old adult flies from each compound-*2* line and both standard strains were provided with a Cream of Wheat and molasses egg-laying substrate. Synchronous first instar larvae were harvested (Sokolowski *et al.*, 1984) and 100 larvae per line were placed in petri dishes containing 35 ml of culture medium where they were incubated under standard conditions for 96 ± 3.0 h. Thirty foraging third instar larvae were randomly sampled from each dish with a moist paintbrush. These were individually tested in petri dishes (8.5 × 1.4 cm) coated with a thin homogeneous layer of aqueous yeast suspension (distilled water and Fleischmann's bakers' yeast at a 2:1 ratio by weight) applied with a glass spreading rod on a petri dish spinner. Path lengths made by larvae while foraging during a 5-min test period were measured. Subtle differences in the environment from one testing day to the next are known to have an affect on larval behavioral phenotypes (Bauer and Sokolowski, 1985). We therefore tested 30 larvae for each of the 25 lines on each day of testing. The testing order of all compound-*2* lines was randomized using a random number table. Lines were coded to conceal their identities during testing. We tested the standard rover and sitter strains both before and after all compound lines. All testing was done within a 6-h interval beginning at 1200 h at 22 ± 1°C and 14 ± 1-mbar vapor pressure deficit under homogeneous overhead illumination. The results of replicate experiments performed on each day did not differ significantly from those presented below.

## RESULTS

More than 95% of the variation in path length between compound-*2* lines is attributed to differences in *C(2L)* chromosomes [$F_{(1,734)} = 213.73$, $P < 0.0001$; Table I]. Effects of *C(2R)* chromosomes and *C(2L)* × *C(2R)* chromosome interactions are not significant. We also used a two-way nested model I ANOVA (Zar, 1984) to test the effects of independently generated compound-*2* chromosomes of different strain origin. As in the first ANOVA, a significant effect of *C(2L)* is found [$F_{(1,3)} = 43.45$, $P < 0.01$], while variation due to

**Table I.** ANOVA of Path Length of Compound-*2* Lines

| Source | df | SS | MS | *F* | *P* |
|---|---|---|---|---|---|
| Model (between lines) | 3 | 3389.73 | 1129.91 | 74.74 | <0.0001 |
| *C(2L)* chromosome | 1 | 3231.06 | 3231.06 | 213.73 | <0.0001 |
| *C(2R)* chromosome | 1 | 64.65 | 64.65 | 4.28 | NS[a] |
| *C(2L)* × *C(2R)* interaction | 1 | 94.01 | 94.01 | 6.22 | NS |
| Error (within lines) | 731 | 11050.80 | 15.12 | | |
| Total | 734 | 14440.53 | | | |

[a] Not significant.

*C(2R)* is not significant. These findings show a second chromosome left arm genetic basis for path length differences between rovers and sitters.

Mean larval path lengths (cm) ± 95% confidence intervals (CI) for all compound-2 lines grouped according to *C(2L)* and *C(2R)* chromosome origin are presented in Fig. 2. All 25 lines constructed with combinations of independently generated *C(2L)* and *C(2R)* chromosomes were developmentally synchronous, permitting valid behavioral comparisons. Both *C(2L), "S"; C(2R), "S"* and *C(2L), "S"; C(2R), "R"* groups have *C(2L)* chromosomes of sitter strain origin and, correspondingly, express sitter phenotypes. Notice that these groups are not affected by their different *C(2R)* chromosomes. Groups which have *C(2L)* chromosomes of rover strain origin [*C(2L), "R"; C(2R), "S"* and

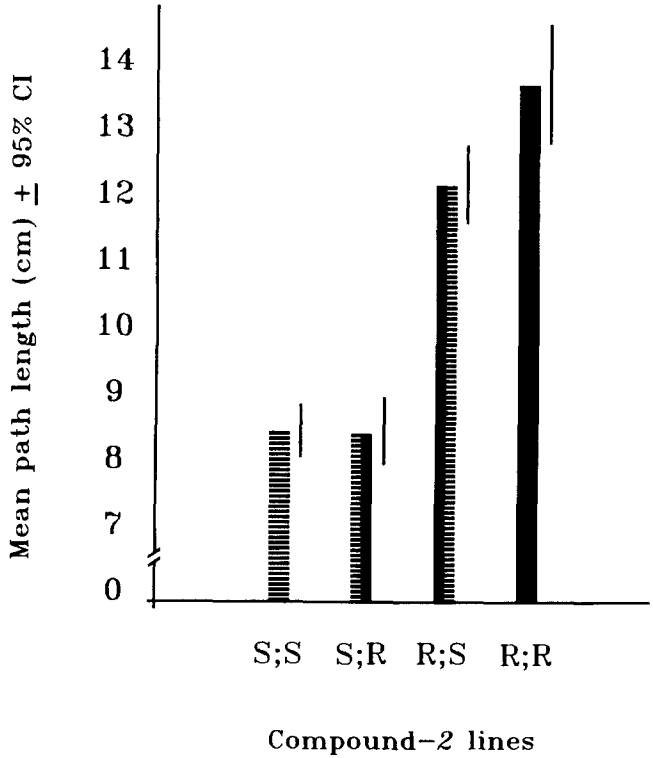


**Fig. 2.** Mean larval path lengths ± 95% CI of all compound-2 *D. melanogaster* lines grouped according to the four types of compound-2 left–right combinations shown in Fig. 1 (row IV). The left and right halves of each histogram correspond to the left and right chromosome-2 arm constitutions of each group. Chromosomes of S and R origin are represented by striped and filled bars, respectively. Groups with *C(2L)* of sitter strain origin (S;S and S;R) have sitter phenotypes, and those with *C(2L)* of rover strain origin (R;S and R;R) have rover phenotypes.

*C(2L), "R"; C(2R), "R"*] both express rover phenotypes. Table I illustrates well the ANOVA results indicating a close relationship between behavior and *C(2L)*-chromosome complement.

## DISCUSSION

The identification and genetic localization of a single major behavioral gene are important prerequisites for dissecting the mechanism of its expression (Hall, 1985). We previously showed that the rover/sitter larval foraging polymorphism can be attributed to a single major gene using a 16-reciprocal cross analysis (de Belle and Sokolowski, 1987). Accordingly, we attempted to determine the location of the gene using standard recombinational mapping techniques. However, this gene did not lend itself to genetic localization for the following reasons. First, rover/sitter is a quantitative character which does not express complete penetrance (de Belle and Sokolowski, 1987). Second, rovers and sitters specifically differ in locomotor activity while foraging. With this in mind, the reduced activity levels of larvae which carry multiple second chromosome morphological markers (de Belle and Sokolowski, unpublished) consequently obscure the probability of associating each marker class with being either rover or sitter. In view of this, accurate genetic localization of rover/sitter may be achieved only in a well-defined genetic background—one in which the effects of minor modifying genes are well controlled. In this investigation we combine techniques used in two previous studies to localize genetically the rover/sitter gene. Hirsch (1959) has used chromosome analyses to identify chromosomal contributions to differences in behavior; Hilliker *et al.* (1977) have used compound autosomes to verify the chromosome arm assignments of morphological markers. The present study is the first to use compound autosomes for identifying chromosome arm contributions to differences in a behavioral trait.

We were not surprised to find greater phenotypic variation in the rover and sitter compound lines compared with the standard rover and sitter strains. The compound autosome configuration increases variability in phenotypic measures for two reasons. First, compound autosome rearrangements probably have pleiotropic effects on rover/sitter phenotype expression. Known examples of pleiotropic effects resulting from compound autosome rearrangements include a 20% reduction in both egg-to-adult viability and fecundity (Leigh and Sobels, 1970). Second, Harger and Holm (1973) have reported a 3.4% probability of *X*-chromosome nondisjunction in compound autosome strains. Consequently it is possible that we tested a number of *XXX*, *XXY*, and *XO* larvae since these may survive to pupation (Fitz-Earle and Holm, 1978). Despite increased phenotypic variation, we obtained both consistent and highly significant results which demonstrate a second chromosome left arm designation for the rover/sitter trait.

The findings of this study provide further support for the notion that a quantitative behavioral phenotype may have a simple genetic basis (one major gene with minor modifiers) rather than being under the control of many minor genes distributed throughout the genome. The compound autosome technique has enabled us to circumvent the difficulties inherent to traditional methods of genetic mapping and may, therefore, have utility in localizing the genetic bases of other behavioral traits.

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