Mapping of Drosophila Mutations Using Site-Specific Male Recombination

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Manuscript received November 1, 1997
Accepted for publication January 8, 1998

ABSTRACT

Although recombination does not usually occur in the male Drosophila germline, site-specific recombination can be induced at the ends of *P* elements. This finding suggested that male recombination could be used to map Drosophila mutations. In this article, we describe the general method and its application to the mapping of two EMS-induced female-sterile mutations, *grauzone* and *cortex*. Within two months, the *grauzone* gene was mapped relative to seven different *P*-element insertion sites, and *cortex* was mapped relative to 23 different *P*-elements. The results allowed us to map *grauzone* to a region of about 50 kb, and *cortex* distal to the chromosomal region 33E. These experiments demonstrate that *P*-element-induced site-specific male recombination is an efficient and general method to map Drosophila autosomal mutations.

RECOMBINATION does not normally occur at significant frequencies in the Drosophila male germline. However, in crosses involving wild-type strains containing *P* elements, male recombination can occur at a frequency of up to 1% (Hiraizumi 1971; Kidwell and Kidwell 1976). In fact, it was the early studies of male recombination that led to the discovery of the hybrid dysgenesis syndrome (Kidwell *et al.* 1977) and the identification of *P* elements (Bingham *et al.* 1982; Rubin *et al.* 1982; Engels 1996). Since then, *P* elements have been the subject of intensive research and become indispensable for manipulating the Drosophila genome (Spradling 1986, 1995; Sentry and Kaiser 1992; Engels 1996).

Although much was known about *P*-element transposition and its regulation, the mechanism by which male recombination occurs was only recently resolved (Gray *et al.* 1996; Preston and Engels 1996). Preston and Engels (1996) studied male recombination at an autosomal site and found that ~0.5–1% of the progeny were recombinants. The great majority of the crossovers occurred within a 4-kb region containing the *P* element. Most recombinants retained a mobile *P* element at the site of the recombination, usually with either a deletion (1/3) or a duplication (1/3) immediately adjacent to the *P* end at which the crossover occurred. The sizes of these deletions and duplications ranged from a few base pairs to over 100 kb. These structures can be explained by the "hybrid element insertion" model of male recombination, in which the two *P* element copies on sister chromatids combine to form a "hybrid element" whose termini insert into a nearby position on the homolog (Svoboda *et al.* 1995; Gray *et al.* 1996).

The site-specific feature of *P*-element-induced male recombination events suggested a new way to map Drosophila mutations. We reasoned that a mutation could be mapped relative to a *P*-element insertion by isolating *P*-element-induced recombinants and determining whether the mutation segregated with proximal or distal markers. An advantage of this strategy is that the flanking markers do not have to be closely linked in order to be informative. Instead, the resolution of this method is defined by the density of *P* elements in the region of interest. Furthermore, this method has the potential to rapidly localize mutations in the Drosophila genome, given the large number of already characterized *P*-element insertion sites in the Drosophila genome, and the fact that many more *P* element lines are being mapped both genetically and physically by the Berkeley Drosophila Genome Project (BDGP).

To explore the feasibility of exploiting *P*-element-induced male recombination for gene mapping, we used this method to map two genes, *grauzone* and *cortex*. These genes were identified in a screen for EMS-induced female-sterile mutations on the second chromosome and have been phenotypically characterized (Schüpbach and Wieschaus 1989; Lieberfarb *et al.* 1996; Page and Orr-Weaver 1996). When these experiments were started, *grauzone* had been roughly localized by the inability of the large chromosomal deficiency *Df(2R) Pu-D17* (extending from 57B2-5 to 58B) to complement the mutant alleles. Using male recombination, we mapped *grauzone* relative to seven different *P*-element insertions in the 57 region. The data placed the gene within a region between two *P*-element insertion sites, a region of ~50 kb. By meiotic recombination *cortex* had been genetically mapped to 2-49, which

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roughly corresponds to polytene chromosomal region 34. Thirty-three deficiencies from region 30 to 38 were tested and all complement the *cortex* mutation. We mapped *cortex* relative to the 23 *P*-element insertions throughout the region from 33 to 36. The mapping results placed the gene distal to the chromosomal region 33E. Our data demonstrate that *P*-element-induced male recombination is an efficient method to map autosomal mutations into a molecularly defined interval of the Drosophila genome.

MATERIALS AND METHODS

Drosophila stocks: Flies were raised on standard Drosophila medium and grown at room temperature (21–24°) unless otherwise noted. The five *grauzone* alleles ($grau^{RM61}$, $grau^{RG1}$, $grau^{QQ36}$, $grau^{QF31}$ and $grau^{QE70}$) and two *cortex* alleles ($cort^{QW55}$ and $cort^{RH65}$) were obtained from Trudi Schüpbach (Schüpbach and Wieschaus 1989) and Terry Orr-Weaver (Page and Orr-Weaver 1996). The transposase stock *Sp[1]/CyO; Sb[1] Delta2-3 /TM6 Ubx [P15]* and all the *P*-element stocks were obtained from the Bloomington Fly Stock Center or from Amy Beaton and Todd Laverty in the Drosophila Genome Center at the University of California at Berkeley. The information about locations of *P* elements and P1 clones is from Flybase (1997). All genetic symbols not described in the text are in the Drosophila reference works (Lindsley and Zimm 1992; Flybase 1997).

Drosophila crosses: For male recombination experiments to map the *grauzone* mutation, flies containing the *P*-element chromosome (cn^+ *P* bw^+/*CyO*) were mated to flies that were *cn grau bw*/*CyO*; *Sb[1] Delta2-3*/*ry*. Male flies that were cn^+ bw^+/*cn grau bw*; *Sb[1] Delta2-3*/+ were crossed to female *cn bw* flies. The letter *P* represents either *P{lacW}* or *P{ry[+7.2]}*. About four male flies and 10 female flies were put into one vial. For each chosen *P*-element line, 20 to 40 crosses were set up, and 1000–2000 progeny were screened for recombinants. The recombinants had cinnabar or brown eye color. For mapping the *cortex* mutation, the scheme was similar to *grauzone*, except that the flanking markers were different. For *P* elements proximal to *black* (*b*) (34D4-6), male *b cort cn*/b^+ *P* cn^+; *Sb[1]Delta2-3*/+ flies were generated and crossed to female *b cn* flies. The recombinants had either black body with wild-type eye color or wild-type body color with cinnabar eye color. For *P* elements distal to *black*, male *dp cort cn*/dp^+ *P* cn^+; *Sb[1] Delta2-3*/+ flies were generated and crossed to female *dp cn* flies. The recombinants had either dumpy wing with wild-type eye color or wild-type wing with cinnabar eye color.

Fertility tests of *P*-element-induced male recombinants: The male recombinants (cn^+ *bw*/*cn bw* and *cn* bw^+/*cn bw* for *grauzone*; b^+ *cn*/*b cn*, *b* cn^+/*b cn*, dp^+ *cn*/*dp cn* and *dp* cn^+/*dp cn* for *cortex*) were crossed to *cn grau bw*/*CyO* or *b cort cn*/*CyO* females, respectively. The female progeny carrying a recombinant chromosome and a *grauzone* or *cortex* chromosome were collected and mated to *Canton-S* male flies at both room temperature and at 29°. For mapping *cortex*, female recombinants were also collected and mated to *b cort cn*/*CyO* and *dp cort cn*/*CyO* males. The female progeny carrying a recombinant chromosome and a *cortex* chromosome were mated to *Canton-S* males and tested individually for fertility. The vials were scored daily for embryo hatching and larvae.

RESULTS

Scheme for mapping Drosophila mutations using *P*-element-induced male recombination: Figure 1 illustrates the general scheme for mapping a mutation using

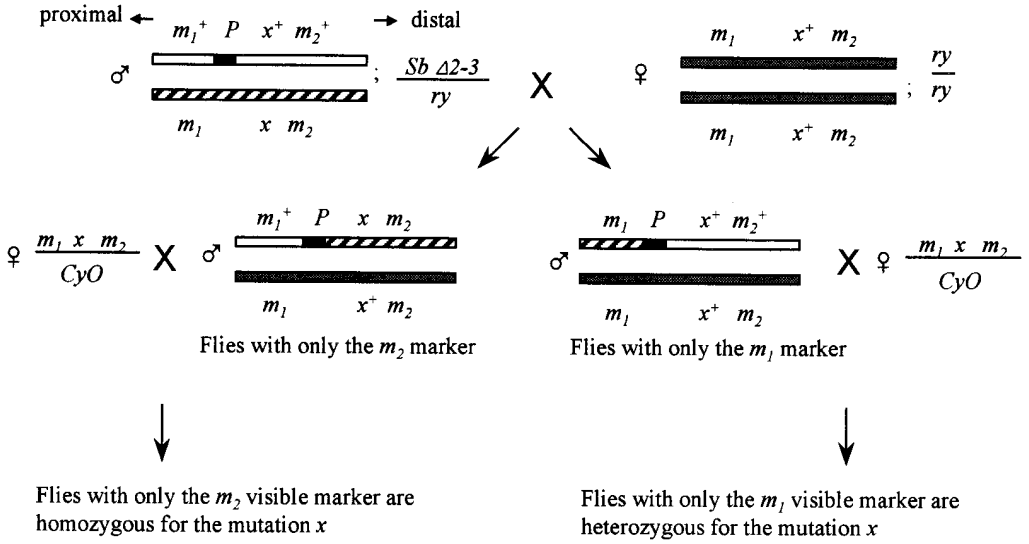

Figure 1.—Scheme for mapping mutation *x* using *P*-element-induced male recombination. m_1 and m_2 are proximal and distal flanking markers that flank the *P* element. A chromosome containing *x* is put in *trans* to a chromosome containing the *P* element. The transposase source (*Delta2-3*) is provided by a different chromosome. Recombination events are induced at the ends of the *P* element in the male flies in the presence of transposase. In the next generation, most flies are of parental phenotype; recombinants are identified by the presence of only the m_1 or m_2 visible marker. Male recombinants are crossed to *x* females to test whether the mutation *x* is present or absent on the recombinant chromosomes. If *x* is distal to the *P* element, the recombinant chromosomes m_1^+ m_2 do not complement *x*, and m_1 m_2^+ do complement *x* (shown here). If *x* is proximal to the *P* element, m_1^+ m_2 recombinants complement *x*, whereas m_1 m_2^+ do not (not shown).

TABLE 1

Male recombination mapping of the *grauzone* gene

P element	Cytological location	Estimated MR frequency[a] (percent)	Male recombinant[b]	Complements *grauzone*	*grauzone* distal or proximal to the *P* element
l(2)00629	57B1-3	~1	cn^+ *bw* (2/7)	no	distal
			cn bw^+ (4/7)	yes	
l(2)05475	57B2-3	~10	cn^+ *bw* (13/56)	no	distal
			cn bw^+ (34/56)	yes	
l(2)k12405	57B1-20	~5	cn^+ *bw* (10/16)	no	distal
			cn bw^+ (4/16)	yes	
l(2)07806	57B4-6	~2	cn^+ *bw* (5/10)	no	distal
			cn bw^+ (2/10)	yes	
l(2)03050	57B13-14	~1	cn^+ *bw* (1/6)	yes	proximal
			cn bw^+ (4/6)	no	
l(2)k03401	57B13-14	~3	cn^+ *bw* (8/15)	yes	proximal
			cn bw^+ (3/15)	no	
l(2)k10220[c]	57B13-14	~3	cn^+ *bw* (1/10)	yes	proximal
			cn bw^+ (1/10)	no	
			cn^+ *bw* (3/10)	no	distal
			cn bw^+ (3/10)	yes	

[a] Estimated male recombination (MR) frequency = total number of male and female recombinants / estimated total number of progeny.

[b] Number in parentheses refers only to male recombinants (total number of male recombinants that were fertile and tested with *grauzone* / total number of male recombinants).

[c] This line contains two *P* elements (see text for details).

P-element-induced male recombination. Two visible markers (m_1 and m_2) are selected that flank the *P* element, and a chromosome containing the mutation of interest (*x*) is put in *trans* to a chromosome containing the *P* element. In this case, the flanking markers m_1 and m_2 are present on the same chromosome as the mutation *x*. The transposase source (*Delta2-3*) is provided by another chromosome. A *P*-element-induced recombination event in such male flies results in the *x* mutation cosegregating with either m_1 or m_2, depending on the relative position of *x* and the chosen *P* element. In the next generation, recombinants are identified by the presence of either the m_1 or m_2 visible marker. Male recombinants are crossed to *x* females to test whether the mutation *x* is present or absent on the recombinant chromosomes. By determining whether *x* cosegregates with m_1 or m_2, one can infer whether *x* is proximal or distal to the *P*-element insertion tested (Figure 1).

To map the *grauzone* gene, *P* elements mapped to the second chromosome region 57-58, where *grauzone* had been mapped previously, were chosen for the analysis. The eye color loci *cinnabar* (*cn*) and *brown* (*bw*) were used as visible markers flanking the *P* elements. Male recombinants were crossed to *grauzone* females, which carry *cn* and *bw* on the *grauzone* chromosome. Female progeny carrying both the recombinant chromosome and the *grauzone* chromosome were mated to *Canton-S* males for a fertility test; sterility indicated that the recombinant chromosome contained the *grauzone* mutation. The cosegregation of *grauzone* with *cn* or with *bw* indicated whether *grauzone* is proximal or distal to the *P* elements.

The *cortex* gene was mapped relative to the *P*-element insertions throughout the 33–36 region. The loci *black* (*b*) or *dumpy* (*dp*) were used as distal markers and the *cn* locus was used as a proximal marker. The cosegregation of *cortex* with *b/dp* or with *cn* indicated whether *cortex* was distal or proximal to the *P* element tested.

Mapping the *grauzone* gene: The *grauzone* gene was initially mapped to 57B2-5; 58B in complementation tests with deficiency *Df(2R)Pu-D17* (Schüpbach and Wieschaus 1989). This deficiency identifies an interval of 1600 kb (Flybase 1997, http://flybase.bio.indiana.edu). To refine the mapping of this gene, we mapped *grauzone* relative to seven *P*-element insertions within the 57 region. The *P*-element insertion sites were mapped by *in situ* hybridization by the BDGP. The male recombination data are summarized in Table 1.

Among all the chromosomes tested, recombinants from all but the *l(2)k10220 P*-element insertion line allowed us to unambiguously map *grauzone* to one side of the *P*-element insertion or the other (Table 1; Figure 2A). From the four *P*-elements that have been mapped to the region from 57B1 to B6, the cn^+ *bw* recombinant chromosomes always contained the *grauzone* mutation, whereas the *cn* bw^+ recombinant chromosomes always retained the wild-type gene (Table 1), indicating that *grauzone* is distal to these *P*-elements (Figure 2A). Con-

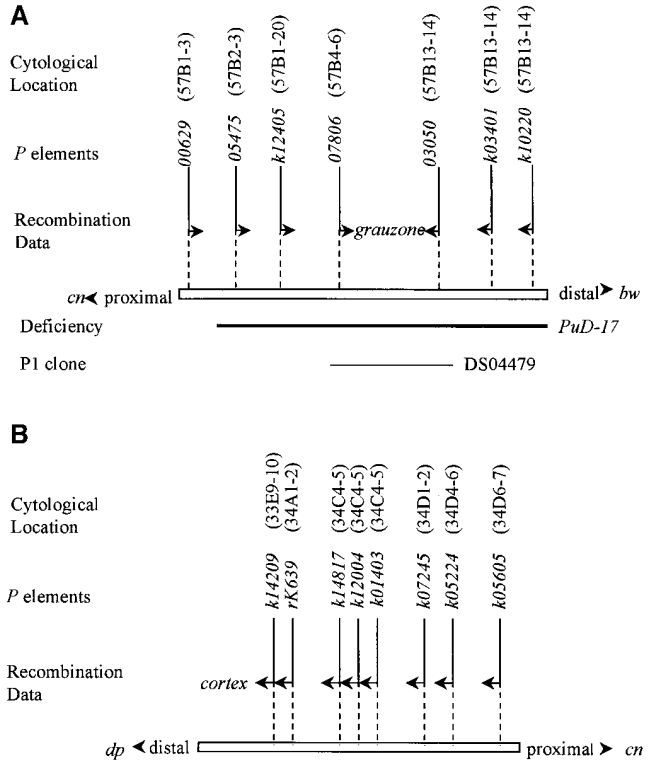

Figure 2.—The mapping of *grauzone* and *cortex*. The locations of the *P* elements are shown. The arrows indicate the positions of *grauzone* and *cortex* relative to that *P* element determined by male recombination. The thick lines represent deficiencies. The thin line represents the P1 clone. The figure is not drawn to scale. (A) The *grauzone* gene is mapped between *l(2)07806* and *l(2)03050*. The relative position of the two *P* elements *l(2)05475* and *l(2)k12405* was not determined. (B) The *cortex* gene is mapped distal to the 33E region. The relative positions of *P* elements *l(2)k14817*, *l(2)k12004* and *l(2)k01403* were not determined.

versely, on recombinant chromosomes from the two *P*-element insertion lines that have been mapped to 57B13-14, *grauzone* always cosegregated with *cn* and was unlinked to *bw* (Table 1), indicating that *grauzone* is proximal to these two *P*-element insertions (Figure 2A). These results allowed us to place *grauzone* between 57B6 and 57B13-14. The ambiguous male recombination results using the *l(2)k10220 P*-element line are discussed below.

We refined these genetic data by determining the relative position of the *P* elements to each other by various methods. These methods included complementation tests with deficiencies, and Southern hybridization of P1 clones using *P*-element flanking sequences as probes. Of the two *P* elements that are distal to *grauzone*, *l(2)03050* is proximal to *l(2)k03401* (Flybase 1997). Among the other four *P* elements, *l(2)07806* is distal to *l(2)00629*, *l(2)05475*, and *l(2)k12405* (B. Chen, unpublished results). These results established the proximal limit of the *grauzone* locus as *l(2)07806* and the distal limit as *l(2)03050* (Figure 2A). PCR and Southern hybridization data demonstrated that the flanking sequences of these two *P* elements are contained within the P1 clone DS04479. Further analysis of this P1 clone identified that the region between these two *P* elements is between 40 and 50 kb. These data thus mapped *grauzone* to this well-defined molecular interval of the Drosophila genome.

Male recombination to map the *grauzone* mutation using the *P*-element *l(2)k10220* line generated two groups of recombinants (Table 1). The first group contained two male recombinants. The presence of the *grauzone* mutation on the *cn bw*$^+$ recombinant chromosome and not on the *cn*$^+$ *bw* chromosome indicated that *grauzone* is proximal to the *P*-element insertion site associated with the recombination event. The second group contained six male recombinants. Within this group the presence of the *grauzone* mutation on the *cn*$^+$ *bw* recombinant chromosome suggested that *grauzone* is distal to the *P*-element recombination site. Analysis of the starting *l(2)k10220* line revealed two different *P*-element insertions in the second chromosome (B. Chen, unpublished results). One is inserted in the 57B13-14 region and is allelic to *l(2)k03401* (Flybase 1997). The other is proximal to the 57B region. The *grauzone* gene is thus proximal to the 57B13-14 *P* element and distal to the *P* element that is proximal to the 57B region.

Male recombination frequencies using these different *P* elements were relatively high, varying from ~1 to 10%. For each *P*-element insertion line used, there were always some recombinants that were sterile, and the complementation tests between them and the *grauzone* chromosome could not be done. The basis for this sterility is not understood but presumably is because of other events associated with *P*-element transposition (Kidwell *et al.* 1977).

Mapping the *cortex* gene: The *cortex* gene was initially mapped by meiotic recombination to 2-49 on the left arm of the second chromosome (Schüpbach and Wieschaus 1989). In order to refine the mapping of *cortex*, we used *P*-element-induced male recombination with 23 *P*-element insertion lines throughout the 33-36 region. The data are summarized in Table 2.

Results from these *P*-element-induced male recombination experiments place *cortex* in a region distal to the *P* element *l(2)k14209* (33E9-10) (Figure 2B). This is an unexpected result because the data from meiotic recombination suggested that *cortex* is very close or proximal to *b* (Schüpbach and Wieschaus 1989). We performed three additional meiotic recombination experiments, one with *b* (2-48.5) and *pr* (2-54.5), another with *b* and a *P* element *l(2)k14608* (35F11-12), and a third with *dp*(2-13) and *b* (data not shown). The combined data from these experiments suggested that *cortex* is indeed distal to *b* and proximal to *dp*. Further mapping of the gene using *P* elements distal to 33E is currently

TABLE 2

Male recombination mapping of the *cortex* gene

P element	Cytological location	MR frequency[a] (percent)	Number of recombinants[b]	Complements *cortex*[b]	*cortex* distal or proximal to the *P* element
l(2)k08819	36A12-14	0.36	b^+ *cn* (2/4)	yes	distal
			b cn^+ (1/4)	no	
l(2)k00301	36A11-12	0.50	b^+ *cn* (1/7)	yes	distal
			b cn^+ (5/7)	no	
l(2)k05627	36A6-7	0.41	b^+ *cn* (0/5)	—[c]	distal
			b cn^+ (3/5)	no	
l(2)k16215	36A4-5	0.42	b^+ *cn* (2/4)	yes	distal
			b cn^+ (1/4)	—[c]	
rk(2)364	36A1-2	0.20	b^+ *cn* (2/9)	yes	distal
			b cn^+ (4/9)	no	
cact255	35F6-12	0.23	b^+ *cn* (0/3)	—[c]	distal
			b cn^+ (2/3)	no	
l(2)k14608	35F11-12	0.63	b^+ *cn* (3/8)	yes	distal
			b cn^+ (1/8)	—[c]	
l(2)k00809	35F1-2	0.57	b^+ *cn* (3/4)	yes	distal
			b cn^+ (0/4)	—[c]	
l(2)k09033	35D6-7	0.27	b^+ *cn* (3/3)	yes	distal
			b cn^+ (0/3)	—[c]	
l(2)k14423	35D5-7	0.053	b^+ *cn* (1/1)	yes	distal
			b cn^+ (0/1)	—[c]	
l(2)k02602	35D3-4	0.26	b^+ *cn* (2/3)	yes	distal
			b cn^+ (1/3)	no	
l(2)k05007	35D3-4	1.11	b^+ *cn* (3/8)	yes	distal
			b cn^+ (2/8)	no	
l(2)k07904	35B8-9	0.84	b^+ *cn* (0/6)	—[c]	distal
			b cn^+ (4/6)	no	
l(2)k00811	35A1-2	0.33	dp^+ *cn* (4/8)	yes	distal
			dp cn^+ (3/8)	no	
l(2)k06321	34D6-7	0.07	dp^+ *cn* (0/1)	—[c]	distal
			dp cn^+ (1/1)	no	
l(2)k05605	34D6-7	0.07	dp^+ *cn* (3/3)	yes	distal
			dp cn^+ (0/3)	—[c]	
l(2)k05224	34D4-6	0.09	dp^+ *cn* (3/3)	yes	distal
			dp cn^+ (0/3)	—[c]	
l(2)k07245	34D1-2	0.31	dp^+ *cn* (3/4)	yes	distal
			dp cn^+ (1/4)	no	
l(2)k01403	34C4-5	0.79	dp^+ *cn* (0/9)	—[c]	distal
			dp cn^+ (9/9)	no	
l(2)k12004	34C4-5	0.22	dp^+ *cn* (2/3)	yes	distal
			dp cn^+ (1/3)	no	
l(2)k14817	34C4-5	0.38	dp^+ *cn* (1/3)	yes	distal
			dp cn^+ (2/3)	no	
rK(2)639	34A1-2	0.21	dp^+ *cn* (3/7)	yes	distal
			dp cn^+ (4/7)	no	
l(2)k14209	33E9-10	0.09	dp^+ *cn* (2/2)	yes	distal
			dp cn^+ (0/2)	—[c]	

[a] MR = Male recombination; MR frequency = total number of male and female recombinants/total number of progeny.

[b] Because the recombination rate is low, both male and female recombinants were tested for complementation over *cortex* whenever possible (see materials and methods). Number in parentheses is the number of recombinants tested for complementation/total number of recombinants.

[c] Fertility tests of the recombinant chromosome could not be done or no recombinants were obtained.

being performed to identify the two *P* elements that delimit the *cortex* region.

The male recombination frequency in this region is lower than that at 57B. However, since a large collection of *P*-element insertion lines were used, and only a few recombinants from one *P* element are needed to map the mutation relative to the *P* element, this low frequency has not been problematic. The low frequency of *P*-element-induced recombination in this region suggested there are positional effects on the accessibility of the transposase. In addition to the 23 *P* elements listed in Table 2 from which recombinants were obtained, seven other *P*-element insertion lines were used for the mapping, and no recombinants were recovered.

DISCUSSION

In this article we mapped two Drosophila genes using *P*-element-induced site-specific male recombination. Of the 37 *P*-element insertion lines used in our experiments, we were able to obtain recombinants from 30, and we obtained unambiguous results from all lines except the one that contained two *P* elements. These results suggest that most *P*-element insertion lines from the Fly Stock Center and BDGP can be utilized for this method. Most *P*-induced male recombination chromosomes (2/3) contain a deletion or duplication of the genomic sequence immediately flanking the *P*-element insertion (Preston *et al.* 1996). We have not screened the recombinant chromosomes for chromosomal deletions or duplications, but such alterations did not complicate the interpretation of the data.

Compared to the traditional meiotic recombination mapping, there are several advantages for the male recombination method. Any mutation can be quickly mapped to the genetic and physical map of the Drosophila genome, provided it is known on which chromosome the mutation is situated, and there are two usable markers present on the mutant chromosome. Because there is normally no meiotic recombination in male flies, the configuration of the flanking markers in the progeny will normally be of the parental type. The relatively rare recombination events can be quickly recognized using the visible markers and tested for the presence or the absence of the mutation. Because the recombination event is targeted to a particular chromosomal site, this method allows high resolution mapping of new mutations, with the added advantage that the *P*-element insertions allow rapid identification of the DNA surrounding the region of interest. Also, as the map position becomes more precise, further refinement via meiotic recombination relies on exceedingly rare events; in contrast, the male recombination frequency is much higher. This is an added advantage over the traditional restriction fragment linkage polymorphism mapping performed in Drosophila and in other organisms. Male recombination data also provide valuable information and reagents for further analysis. For example, the *P* elements closest to the gene of interest can be identified and used to generate deficiencies or *P*-element insertion alleles of the gene. The closest *P* elements can also serve as the markers for molecular polymorphism analysis.

There are limitations for the male recombination method. Even though male recombination is an efficient way to map autosomal genes, it usually is not suitable for mapping mutations on the *X* and *Y* chromosome. It is preferable that the mobile *P* element comes from one parent and that the transposase source and the target gene comes from the other to avoid premature mobilization of the *P* element. If the gene of interest is on chromosome *3*, a transposase source on chromosome *2* (Flybase 1997) can be utilized. In the >100 recombinants obtained from our experiments, we have not encountered evidence for events where mobilization of the *P* elements was followed by male recombination.

Both genetic and molecular tools are required to decipher genomic information and ultimately to understand gene function. With the rapid emergence of sequence data from the BDGP, it is important to find efficient methods to correlate sequences with gene functions. One current approach is a massive gene disruption project that uses individual, genetically engineered *P* transposable elements to target open reading frames throughout the Drosophila genome (Spradling *et al.* 1995). However, inherent site specificity may preclude the *P* elements from mutating some loci. Chemical mutagenesis screens provide a less biased method for isolating mutations that disrupt a biological process of interest. However, these mutations are frequently not associated with readily identifiable physical landmarks and are difficult to clone. The male recombination method provides an efficient way to map these and any other mutations on the Drosophila autosomes relative to a series of *P*-element insertion lines available from BDGP. As the size of the BDGP gene disruption library grows, the density of insertions along the physical map will increase, along with the power and precision of the male recombination mapping method.

We are especially grateful to Paul Bingham and Zuzana Zachar for invaluable discussion. We thank JoAnne Engebrecht, Marshal Lieberfarb, Tian Xu, and the members from the Strickland lab for insightful discussions and/or comments on the manuscript. We thank the following for Drosophila stocks and reagents: Amy Beaton, Rachel Kraut, Todd Laverty, Janis O'Donnell, Terry Orr-Weaver, Andrea Page, John Roote, Trudi Schüpbach, Dianne Stewart, John Tower, E. M. Underwood, Bloomington and Umeå Drosophila Stock Center. This work was supported by grant GM-51584 from the National Institutes of Health to S.S.

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Communicating editor: K. Anderson