A Stable Genomic Source of *P* Element Transposase in *Drosophila melanogaster*

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ABSTRACT

A single *P* element insert in *Drosophila melanogaster*, called $P[ry^+\Delta 2\text{-}3](99B)$, is described that causes mobilization of other elements at unusually high frequencies, yet is itself remarkably stable. Its transposase activity is higher than that of an entire P strain, but it rarely undergoes internal deletion, excision or transposition. This element was constructed by F. Laski, D. Rio and G. Rubin for other purposes, but we have found it to be useful for experiments involving *P* elements. We demonstrate that together with a chromosome bearing numerous nonautonomous elements it can be used for *P* element mutagenesis. It can also substitute efficiently for "helper" plasmids in *P* element mediated transformation, and can be used to move transformed elements around the genome.

THE syndrome of genetic effects known collectively as P-M hybrid dysgenesis is now understood to be related to the activity of the *P* element family of transposons (reviewed by BREGLIANO and KIDWELL 1983; ENGELS 1983, 1988). Briefly, these effects include male recombination, the generation of mutations and chromosomal rearrangements, and the destruction of the germline at high temperatures yielding a type of sterility known as gonadal dysgenic (GD) sterility. These effects result from the mobilization of *P* elements when they are released from regulation by P cytotype, the repressive condition characteristic of P strains. Because P cytotype is partially maternally inherited, dysgenesis occurs primarily in the cross of a male bearing several complete (autonomous) elements as well as numerous internally deleted defective (nonautonomous) elements (P strain), to a female with no elements (M strain). When the complete elements enter the permissive M cytotype from the M strain female they express their transposase in the germline of the progeny, mobilizing both autonomous and nonautonomous elements, and hybrid dysgenesis results.

Complete *P* elements are 2.9 kb in length with inverted terminal repeat structures thought to be required for transposition (O'HARE and RUBIN 1983). Four open reading frames (0,1,2 and 3) code for an 87-kD transposase polypeptide (KARESS and RUBIN 1984; RIO, LASKI and RUBIN 1986). Restriction of *P* element activity to the germline results from differential splicing of the mRNA such that the third (2-

3) splice occurs only in the germline (LASKI, RIO and RUBIN 1986). The nature of P cytotype remains unclear, although it may be related to failure of this splice and the resultant production of a truncated 66-kD polypeptide (RIO, LASKI and RUBIN 1986; H. M. ROBERTSON and W. R. ENGELS, unpublished results).

In addition to the study of their interesting properties, *P* elements have been developed as an important tool of genetics and molecular biology in *Drosophila melanogaster*. One application is insertional mutagenesis (ENGELS 1985a; KIDWELL 1986), primarily for transposon tagging of genes for cloning (*e.g.*, SEARLES *et al.* 1983). Unfortunately the very characteristics that make *P* elements suitable for mutagenesis, their transposable nature and multiple copies, also cause problems. Novel insertion mutations must usually be stabilized in the *P* cytotype, where numerous other inserts complicate cloning. *P* elements are also used as transformation vectors (SPRADLING and RUBIN 1982; RUBIN and SPRADLING 1982). Because transformed genes are inserted at various locations in the genome, their expression is subject to position effects (*e.g.*, SPRADLING and RUBIN 1983; DANIELS *et al.* 1986). To compensate, it is useful to collect many inserts. This has been accomplished by large scale transformation (*e.g.*, LAURIE-AHLBERG and STAM 1987), and by the subsequent mobilization of inserts by reinjection of a "helper" element (*e.g.*, LEVIS, HAZELRIGG and RUBIN 1985). These methods are effective but time-consuming. A more efficient alternative is to use a genomic transposase source. For example, COOLEY, KELLEY and SPRADLING (1988) have used an element they call "Jumpstarter" for this

purpose, and others (MOUNT, GREEN and RUBIN 1988; F. SPENCER, personal communication) have used a progenitor of Jumpstarter. Ideally such an element should not be mobile itself.

In this report we describe a modified P element that is particularly capable of mobilizing other elements, yet is itself remarkably stable. To examine the somatic effects of P element activity (ENGELS *et al.* 1987), and for studying the P element repressor (H. M. ROBERTSON and W. R. ENGELS, unpublished results), we had obtained three lines from F. LASKI, D. RIO and G. RUBIN that had been transformed with the $P[ry^+\Delta 2\text{-}3]$ element. This element, abbreviated $\Delta 2\text{-}3$, is marked with a wild-type sequence of the *rosy* gene and is missing the third (2-3) intron of the P element. It was constructed *in vitro* by LASKI, RIO and RUBIN (1986) to demonstrate that the normal restriction of P element activity to the germline results from lack of splicing of the third intron in somatic tissues. This element caused somatic mosaicism when combined with a mutable P insert allele, indicating production of transposase in somatic tissues. Although these $\Delta 2\text{-}3$ inserts were unstable, as might be expected from their production of transposase, one of the lines we received now has a remarkably stable insert. It also has high levels of transposase activity. While the molecular basis of these properties remains under investigation, we demonstrate that this element can be used to improve the efficiency of experimental procedures requiring P element transposase.

MATERIALS AND METHODS

Drosophila stocks: Genetic symbols not otherwise explained are in LINDSLEY and GRELL (1968).

ry^{506} $P[ry^+$ $\Delta 2\text{-}3](99B)$: LASKI, RIO and RUBIN (1986) describe the construction of this modified P element, its transformation into ry^{506} flies, and the demonstration that it produces transposase both germinally and somatically. The three lines sent to us contained elements mapping to the third chromosome. Since the inserts were usually unstable, they had been maintained for a year by repeated back-crossing to a stock containing the *TM3* ry^{RK} *Sb* balancer, with selection for *TM3*/$P[ry^+$ $\Delta 2\text{-}3]$ flies. As described below, this subline was derived from one of these lines, designated "3-2" in their report.

$w;P[ry^+$ $\Delta 2\text{-}3](99B)$: The $\Delta 2\text{-}3$ element from the above subline was substituted into a *white* mutant stock.

sn^w; ry^{506} (*M*): An M cytotype stock of a P element insertion allele at the X-linked *singed* locus (ENGELS, 1979a, 1981, 1984), with the ry^{506} allele on the third chromosome. In the presence of transposase sn^w mutates at high rates to an almost wild-type allele, $sn^{(+)}$, and a more extreme allele, sn^e (ENGELS 1979a, 1984; SPRADLING and RUBIN 1982; ROIHA, RUBIN and O'HARE 1988).

Birm2; Sb / TM6, Ubx: A stock with the second chromosome isolated from a strain known as Birmingham, and dominantly marked M-derived third chromosomes. Despite bearing numerous P elements, the Birmingham strain behaves like an M strain in standard tests and is therefore classified as an M' strain (BINGHAM, KIDWELL and RUBIN

1982; SIMMONS and BUCHOLZ 1985; SIMMONS *et al.* 1987). This second chromosome bears 17 defective elements that produce neither transposase nor repressor (ENGELS *et al.* 1987; H. M. ROBERTSON unpublished results).

TM3, ry^{RK} Sb / ry^{506}: A third chromosome balancer dominantly marked with *Sb* and carrying a recessive x-ray induced *ry* allele (see KARESS and RUBIN 1984), allowing convenient scoring of the presence of ry^+ marked elements.

w; TM3, Ser / TM6B, Tb: A *white* stock with both third chromosomes balanced. *TM6B, Tb* is a variant of *TM6, Ubx* with the dominant marker *Tubby* (CRAYMER 1984).

C(1)DX, yf / shi^{ts}; π_2: A compound X stock with the P cytotype (ENGELS 1985b), and the free X chromosome bearing a temperature sensitive lethal (*shibire^s*) to facilitate virgin collection.

Rearing conditions: Flies were maintained and experiments conducted on standard yeasted cornmeal-molasses-agar food at room temperature (21–24°) unless otherwise indicated.

In situ **hybridizations:** Hybridizations to salivary gland chromosome squashes were performed using the biotin labeling method as described elsewhere (ENGELS *et al.* 1986). P element sequences such as the plasmid pπ25.7wc were used as a probe for P elements (KARESS and RUBIN 1984). This plasmid also contains sequences from the 17C region of the X chromosome, and hybridization there served as a control. Chromosomes were examined as heterozygotes with the M-derived chromosomes of a *w m f* stock that yields excellent cytology.

Transformation: Two genetically marked P element constructs were used for transformation. V. PIRROTTA (personal communication) provided one called CaSpeR that contains a 5-kb P element with a modified w^+ gene inserted into the Carnegie 4 vector (RUBIN and SPRADLING 1983). The other plasmid, p$P[ry^+$ $\Delta 2\text{-}3$ *SalI*], was modified from the original p$P[ry^+$ $\Delta 2\text{-}3]$ plasmid for other purposes by induction of a frameshift mutation at the *SalI* site in the fourth open reading frame of the P element, following KARESS and RUBIN (1984).

Microinjection was by a modification of the method of SPRADLING and RUBIN (1982), in that the embryos were not dechorionated or desiccated, and a much smaller volume of more concentrated (0.5 mg/ml) DNA was injected with a green food dye to aid visualization. This simplified procedure increases the speed of injection, however the lowered volume of injected material might affect transformation efficiency.

RESULTS

The $P[ry^+$ $\Delta 2\text{-}3](99B)$ strain: One of the three lines sent to us was used for studying P element regulation and the somatic effects of hybrid dysgenesis, since it destabilized sn^w at high rates (LASKI, RIO and RUBIN 1986). *In situ* hybridization using a P element probe to two chromosomes from this stock revealed only a single position on the third chromosome, 99B7-10, suggesting that this insert had remained as a single copy and was stable. A homozygous stock was created by repeated pair matings with selection for both the ry^+ marker and the presence of $\Delta 2\text{-}3$ activity, as assayed by wild type eye color and somatic mosaicism in half-sib sons from matings to sn^w; ry^{506} females. This stock and its insert, both of which we call $P[ry^+$
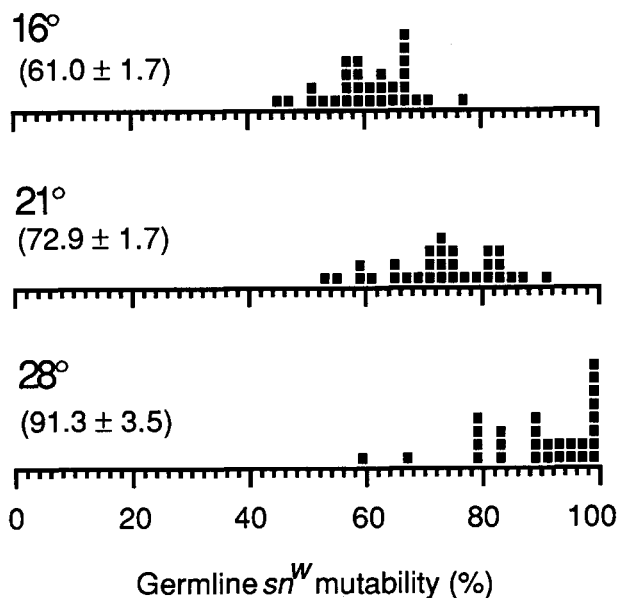
16°
(61.0 ± 1.7)

21°
(72.9 ± 1.7)

28°
(91.3 ± 3.5)

0   20   40   60   80   100

Germline *sn*^{*W*} mutability (%)

FIGURE 1.—Germline mutability of *sn*^{*w*} induced by the Δ2-3(99B) element. Each block represents the sons of one *sn*^{*w*}; Δ2-3(99B) */ry*^{*506*} mosaic male crossed to *C(1)DX, y f*; π₂ females with the P cytotype. The P cytotype suppresses Δ2-3(99B) in somatic tissues as well as the germline, facilitating scoring of the bristle phenotypes of the sons since they were not mosaic. The average number of sons scored per mosaic male was 111, 55 and 62 for the three temperature groups. Mutation rates are given as the total of *sn*⁽⁺⁾ and *sn*^{*e*}. The unweighted means and standard errors are in parentheses (ENGELS 1979b).

Δ2-3](99B) and abbreviate here as Δ2-3(99B), are characterized in detail below.

Transposase production by Δ2-3(99B): The hypermutable *sn*^{*w*} allele was used to evaluate the production of transposase by this element. When Δ2-3(99B) males were crossed to *sn*^{*w*}, *ry*^{*506*} females at 16°, 21° and 28°, all sons had multiple mosaic patches of *sn*⁽⁺⁾ and *sn*^{*e*} bristles indicating the presence of transposase in somatic tissues. Germline transposase was assayed by crossing these mosaic males to compound *X* females and scoring the bristle phenotype of the sons. The results in Figure 1 show that there was high germline mutability at all temperatures, with the production of *sn*⁽⁺⁾ and *sn*^{*e*} sons exceeding 90% at 28°.

These results can be compared to those of LASKI, RIO and RUBIN (1986) who found *sn*^{*w*} mutation rates of 21%, 38%, and 81% for three Δ2-3 strains, the last being the progenitor strain of Δ2-3(99B). These authors and KARESS and RUBIN (1984) also tested four inserts of the complete *P* element from which Δ2-3 was derived. Their measurements of *sn*^{*w*} mutability induced by these elements ranged from 12% to 38%. However, since these are unstable elements, it is not clear that all tested individuals carried a transposase making element, and some might have had multiple copies. Despite these uncertainties, it appears that Δ2-3(99B) and its progenitor strain

produce more transposase activity than other *P* elements tested.

The mutation rates observed for Δ2-3(99B) are even considerably higher than those caused by strong *P* strains such as π₂ (*e.g.*, ENGELS 1984), which are usually in the range of 20–60%. In addition, the pronounced increase with temperature was not seen when the π₂ genome supplied the transposase (ENGELS 1979a, 1981). Finally, this already high mutation rate increased as the males were aged. For example, a group of males had a mutation rate of 76% (1183 of 1554) from their first mating immediately after eclosion, which increased to 91% (605 of 665) in a second mating 20 days later, and to 100% (212 of 212) in a third mating after 27 days. This is another difference from π₂ where no effect of age was seen (ENGELS 1979a). We conclude that Δ2-3(99B) produces at least as much transposase activity as a strong *P* strain, but with some qualitative differences.

Stability of Δ2-3(99B): The Δ2-3(99B) stock was repeatedly reexamined by *in situ* hybridization for a year after it was made homozygous. Again, only a single hybridization site at 99B was seen in each of eight chromosomes indicating that this insertion of Δ2-3 was indeed stable, undergoing neither excision nor transposition at appreciable rates. In addition, all individuals in the stock were *ry*⁺, indicating lack of excision of the Δ2-3(99B) element.

To quantify its stability we attempted to detect excisions of the element by crossing Δ2-3(99B) males to *sn*^{*w*}; *ry*^{*506*} females and scoring the sons for their bristle morphology and eye color. Normally sons would display mosaicism in their bristles, and wild-type eye color due to the *ry*⁺ gene carried by Δ2-3(99B). Excisions of Δ2-3(99B) would yield nonmosaic sons with mutant eye color, while partial excisions (internal deletions) might result in loss of only one of these traits. No complete or partial excisions of Δ2-3(99B) were detected among 8507 sons from 196 such crosses, indicating that each of the 196 males from the stock was homozygous for Δ2-3(99B) and that excision in the germline is rare or nonexistent.

Another means of examining the stability of Δ2-3(99B) was provided by the observation that when it is placed with chromosomes of the M' strain Birmingham, the G₁ progeny die as pupae (ENGELS *et al.* 1987). This pupal lethality is temperature sensitive and is thought to be the somatic equivalent of GD sterility. It provides a powerful method for selection of loss of Δ2-3(99B), since only flies that do not receive the element should survive. In one experiment, Δ2-3(99B) females were mated to *Birm2*; *Sb*/*TM6* males at 28°, a temperature that leads to almost complete lethality. Since the lethality occurs primarily at the pupal stage, the number of progeny tested was estimated by counting pupae on the sides of the vials. From 50 crosses, 9 survivors (all male) were obtained

from 5267 pupae. These survivors were weak, malformed, and GD sterile, phenotypes consistent with the continued presence of Δ2-3(99B) (ENGELS *et al.* 1987). Another experiment using the third chromosome from the Birmingham strain yielded only three deformed and sterile survivors from approximately 8000 pupae. Hence we were again unable to detect any excisions of the Δ2-3(99B) element.

Nevertheless we do not believe that the Δ2-3(99B) element is completely stable. We have used it extensively in numerous other experiments with Birmingham chromosomes (ENGELS *et al.* 1987), and have observed occasional survivors of pupal lethality that might represent excisions of the element. To date three such events have been documented. Each involved loss of Δ2-3 activity, but two retained ry^+ expression and an *in situ* hybridization site at 99B. These two were therefore probably internal deletions. Rare cases of loss and transposition were also observed in some subsequent large scale experiments designed to investigate other aspects of Δ2-3(99B) behavior. These results are described below.

The stability of Δ2-3(99B) allows its use as a genomic source of transposase in the following experiments, where it is introduced genetically for a single generation, and then allowed to segregate away to ensure stability of the *P* element products that result.

P element insertional mutagenesis: The chromosomes of a P strain can be used to provide both the transposase and elements for insertional mutagenesis, while the target chromosomes are best derived from an M strain (ENGELS 1985a; KIDWELL 1986). For mutagenesis using Δ2-3(99B) a chromosomal supply of nonautonomous elements to be mobilized was required. There were two considerations in the choice of this supply. First, the elements should be entirely defective, producing neither transposase nor repressor which might interfere with Δ2-3(99B) activity. Second, the chromosome should have enough elements to give a reasonably high mutation rate, but not so many as to cause harmful effects such as pupal lethality and GD sterility in the presence of Δ2-3(99B). We have chosen the second chromosome from the Birmingham strain. It has 17 non-autonomous elements, produces no repressor, and can survive the presence of Δ2-3(99B) at temperatures below 21° (ENGELS *et al.* 1987). Survival and fertility are excellent when larval development occurs at 16°. The X and third chromosomes from this strain are usually too harmful when combined with Δ2-3(99B) (ENGELS *et al.* 1987). We have examined the chromosomes from two other strains classified as M' by standard tests (*Tübingen* and *UK4*), but they are unsuitable, either because they do not bear enough elements or because their elements produce sufficient P cytotype to repress Δ2-3(99B) (H. M. ROBERTSON and W. R. ENGELS, unpublished results).

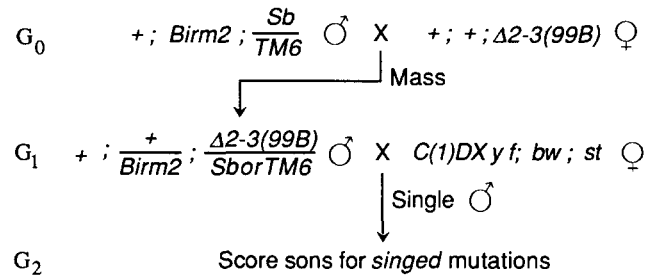$$G_0 \qquad +; Birm2; \frac{Sb}{TM6} \; \male \quad X \quad +; +; \Delta 2\text{-}3(99B) \; \female$$

$$G_1 \qquad +; \frac{+}{Birm2}; \frac{\Delta 2\text{-}3(99B)}{Sbor\,TM6} \; \male \quad X \quad C(1)DX\,y\,f;\, bw;\, st \; \female$$

Mass → Single ♂

$$G_2 \qquad \text{Score sons for } singed \text{ mutations}$$

FIGURE 2.—Mutagenesis of the *X* chromosome using Δ2-3(99B) and *Birm2*. G_2 males of interest were again mated to *C(1)DX, y f; bw; st* females to create a stock homozygous for *bw* and *st*, thus eliminating both Δ2-3(99B) and *Birm2* chromosomes. Mutagenesis of other chromosomes could be accomplished with analogous schemes.

TABLE 1

Mutation rate of the *singed* locus

Temperature	G_1 males[a]	G_2 males[a]	*Singed* males	Mutation rate ± SE (×10³)
16°	100	5396	12	2.22 ± 1.03
19°	109	3829	10	2.61 ± 1.30

All 22 *sn* males were fertile and transmitted the mutation, and all the mutations failed to complement an extreme allele, sn^{x2}. At 16° there were eight independent events with one cluster of five males, and at 19° there were five independent events with two clusters of two and one of four. The standard errors were calculated as in ENGELS (1979b) to take clustering into account.

[a] See Figure 2.

The mating scheme used is in Figure 2, where the objective was to recover transpositions of elements from the *Birm2* chromosome onto the M-derived *X* chromosome of the Δ2-3(99B) stock. Second generation males were examined for mutations at the *singed* locus, a hotspot for *P* element insertion mutations (GREEN 1977; ENGELS 1979a). Each mutant male recovered was crossed to compound *X* females to establish stocks. To examine an unselected set of chromosomes, stocks were also made from ten wild type males from separate cultures. The experiment was set up with replicates at 16° and 19°, temperatures that allowed survival of the males, although at 19° about three quarters of the G_1 males were sterile.

The results of the mutation screen in Table 1 show that *singed* mutants were recovered at an average frequency of 2.4 × 10^{-3}. In addition, an eye color mutation was recovered at 16°, and complementation tests indicated that it was at the *prune* locus. These rates for *P* element insertion mutations at the *singed* locus are near the upper limit of those observed with P-M dysgenic crosses, which range from 2.9 × 10^{-4} to 4.9 × 10^{-3} (SIMMONS *et al.* 1984; KIDWELL 1988).

To determine the positions and numbers of insertions, *in situ* hybridizations of a *P* element probe were made to the ten wild-type chromosomes from each
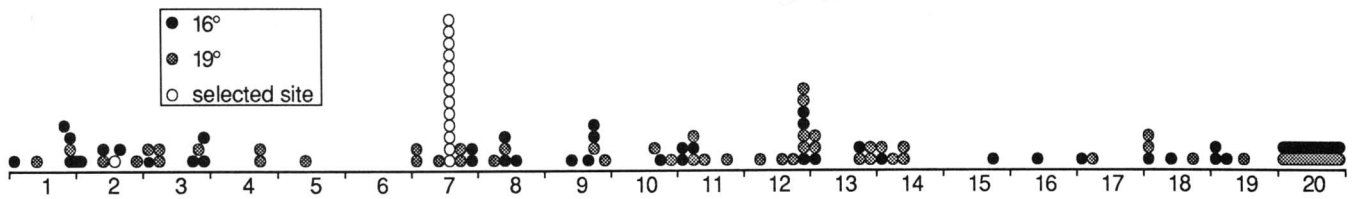
FIGURE 3.—Positions of novel *P* element hybridization sites on the *X* chromosome. Altogether 100 sites from 29 chromosomes are plotted. Note that no inserts would have been detected at 17C since the probe had unique flanking sequences from that region. The range of uncertainty of each site is indicated by the width of the oval.
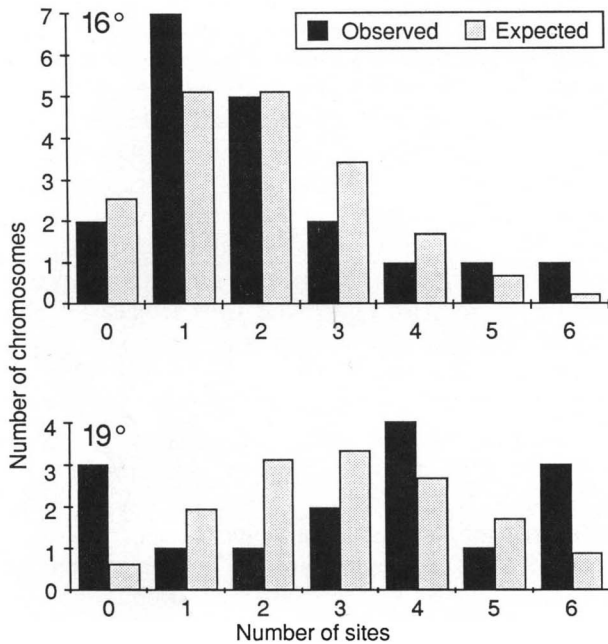

FIGURE 4.—Observed and expected distributions of the number of insertions per *X* chromosome. Included are the ten wild type chromosomes from each temperature, and the mutant chromosomes (9 from 16° and 5 from 19°), from which the selected sites at 7D and 2D are excluded. The expected distributions were calculated as Poisson distributions.

temperature, and to the 14 independent mutant chromosomes. All of the *singed* mutants had hybridization to the 7D region corresponding to the *singed* locus, and the *prune* mutant had a site at its known cytological position (2D). Thus all the mutations appeared to be caused by insertions of *P* elements. Furthermore, the presence of P hybridization at all visible mutation sites suggests that most or all of the elements coming from the *Birm2* chromosome are sufficiently large for detection by *in situ* hybridization, the lower limit being approximately 600 bp by our methods. There were many other sites in addition to those at *singed* and *prune*, and these were distributed in a nearly uniform fashion along the *X* chromosome (Figure 3).

Figure 4 shows the distribution of the number of insertions per *X* chromosome at the two temperatures. The sites at 7D and 2D are excluded because they were selected. At 16° there was a good fit to the Poisson distribution, with an average of two insertions per chromosome per generation. The distribution at 19° deviated significantly from Poisson expectations ($P = 0.011$ by a χ^2 test), suggesting some lack of independence among the insertion events. The average was 3.2 insertions per chromosome per generation. Since rates obtained with P-M dysgenic crosses average one novel insertion per chromosome arm per generation (BINGHAM, KIDWELL and RUBIN 1982; EGGELSTON, JOHNSON-SCHLITZ and ENGELS 1988; W. K. BENZ, unpublished results), we conclude that the combination of Δ2-3(99B) and the *Birm2* chromosome provides potent mutagenesis.

Transformation using Δ2-3(99B): When using a "helper" plasmid such as pπ25.7wc to provide transposase for *P* element mediated germline transformation, the helper must be taken up and expressed simultaneously with uptake of the vector (RUBIN and SPRADLING 1982). The potential advantage of using Δ2-3(99B) for transformation is that the presence of transposase is ensured in any germ cell that takes up the plasmid vector DNA. We tested this method using a vector called CaSpeR, which is marked with a modified sequence of the w^+ gene (V. PIRROTTA, personal communication). Vector DNA was injected into w^- embryos that were heterozygous for Δ2-3(99B) (Figure 5a). Transformants were recovered with either the Δ2-3(99B) chromosome or the balancer (TM3 or TM6). The latter would usually be more useful since the $P[w^+]$ element will have experienced only one generation in the presence of Δ2-3(99B), thus minimizing the likelihood of secondary transpositions and internal deletions.

The results in Table 2 show that transformation occurred at high frequencies, with 16 transformed germlines among 39 fertile adults. Progeny from eight of these 16 adults were analyzed in detail by segregation patterns, as well as differential expression of the w^+ marker, which is subject to strong euchromatic position effects. They yielded at least 28 independent insertions. Approximately one quarter of the transformed individuals proved to carry multiple insertions, which may have been produced by secondary transpositions. As expected, flies receiving both w^+ and Δ2-3(99B) had mosaic eyes and might normally be discarded unless further transpositions were immediately desired (see below).

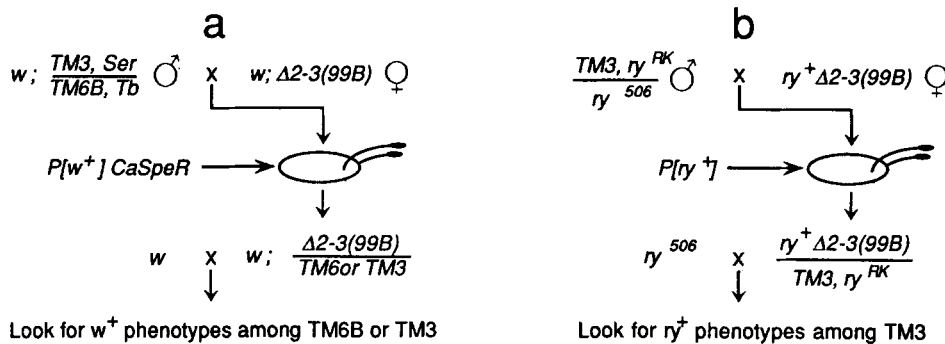FIGURE 5.—Transformation of the (a) $P[w^+]$ and (b) $P[ry^+]$ constructs using Δ2-3(99B). These experiments were conducted at 19°.

TABLE 2

Transformation using Δ2-3(99B)

Plasmid	Embryos injected	Larvae hatched	Adults eclosed	Fertile adults	Transformed germlines
p$P[w^+]$	264	62	45	39	16
p$P[ry^+]$	491	135	98	33[a]	8

[a] Only 41 of the eclosed adults were TM3 and hence useful (see Figure 5b).

We also used this technique with a vector marked with ry^+ instead of w^+. In this case, however, the recovery of transformants was complicated by the presence of the ry^+ marker on the Δ2-3(99B) element, and only half the injected individuals could be used (Figure 5b). Nevertheless, eight transformed germlines were obtained among 33 fertile adults (Table 2), and they yielded at least 12 independent transformants as determined by segregation analysis.

These results demonstrate that Δ2-3(99B) is an effective source of transposase for transformation experiments. Despite the lowered volume of DNA in our injection method, the use of Δ2-3(99B) yielded transformation frequencies similar to those obtained using helper plasmids and microinjection techniques that involve dechorionation and dehydration of the embryos (SPRADLING 1986). We have not determined the efficiency of using Δ2-3(99B) with microinjection of dechorionated embryos.

Transposition of other marked elements: We also tested whether Δ2-3(99B) could be used as a transposase source to mobilize other marked nonautonomous elements that had been introduced by microinjection. We recovered transpositions of several w^+ and ry^+ marked elements from their original locations on the X chromosome to autosomal sites. These elements had been introduced by transformation for other purposes (KARESS and RUBIN 1984; HAZELRIGG, LEVIS and RUBIN 1985; F. LASKI, personal communication). Transpositions of the w^+ elements were recovered by a method similar to that of LEVIS, HAZELRIGG and RUBIN (1985) except that Δ2-3(99B) was used for transposase instead of injected pπ25.7wc helper plasmid. Males of the genotype w $P[w^+]$; Δ2-

3(99B) / + were mated to $C(1)DX, y$ w f females and the progeny scored for sex and eye color. Excisions and internal deletions of the $P[w^+]$ element were identified as white eyed males, and transpositions to autosomal sites were recovered as red eyed females. The excision rate per element per generation was estimated as the ratio of white to total males. The transposition rate was computed as the proportion of red eyed females multiplied by 9/4, because only four of the nine chromosome arms would be recovered by this method.

The procedure also worked for $P[ry^+]$ elements, but was again complicated by the presence of ry^+ on the Δ2-3(99B) element. These experiments also afforded another opportunity to detect excisions of Δ2-3(99B), as well as the opportunity to detect transpositions of Δ2-3(99B) (see below). The mating scheme is in Figure 6, where events in the germline of males of the genotype $P[ry^+]$; Δ2-3($99B$) / TM3, ry^{RK} Sb were recovered in the progeny of matings to ry^{506} females. Scoring was similar to the above except that the sexes were reversed and only the Sb progeny were included in the estimates of excision and transposition rates.

The results in Table 3 show that Δ2-3(99B) was an effective source of transposase for mobilization of other elements. The elements varied considerably in their activity with a strong positive correlation between transposition and excision rates. This variability suggests that stability of an insert is at least partly dependent on its position. Excisions were consistently more common than transpositions, a difference that may result from high rates of partial excisions (internal deletions). One insert, $P[w^+$ B1-2](17E), proved to be nearly as stable as Δ2-3(99B) itself. It had previously been resistant to mobilization in P-M dysgenic crosses using the P strain π2, but was mobilized slightly more successfully with Δ2-3(99B), especially at higher temperatures (Table 3). Again the effectiveness of Δ2-3(99B) was comparable to that of a complete P strain, except that its effects were temperature dependent.

Stability of Δ2-3(99B) revisited: As noted above, the experiments with the ry^+ elements also allowed detection of excisions and transpositions of Δ2-3(99B)

TABLE 3

Δ2-3(99B)-induced excision and transposition of marked elements

Original insert	N	Excision			Transposition		
		Nonexcision	Excision	Rate (% ± SE)	Nontransposition	Transposition	Rate (% ± SE)
$P[w^+cx1](4E)$	9	391	39	9.1 ± 3.3	921	5	1.2 ± 0.5
$P[w^+cx2](7D6-9)$	11	674	22	3.2 ± 1.2	1090	4	0.8 ± 0.3
$P[w^+cx3](7D1-2)$	7	301	29	8.8 ± 1.9	622	6	2.2 ± 1.0
$P[w^+cx5](7D1-2)$	25	1079	260	19.4 ± 2.5	2319	28	2.7 ± 1.4
$P[w^+cx6](2F)$	20	500	230	31.5 ± 4.0	1318	44	7.3 ± 1.6
$P[w^+](9B)$	6	188	497	66.6 ± 9.1	851	16	4.1 ± 1.8
$P[w^+B1-2](17E)$	34	4368	39	0.893	3208	1	0.031
$P[w^+B1-2](17E)^a$	19	1400	18	1.290	1138	2	0.176
$P[w^+B1-2](17E)^b$	265	11442	18	0.157	8544	2	0.023
$P[ry^+Xho41](14B)$	20	654	257	28.2 ± 2.2	778	47	12.8 ± 0.5
$P[ry^+R1\ 8](19B)$	20	518	280	35.1 ± 2.4	707	71	20.5 ± 1.8
$P[ry^+hspSal](3A)$	30	1944	203	9.5 ± 0.1	2109	25	3.2 ± 1.0

Details of the crosses are in the text and Figure 6. The computation of excision and transposition rates is described in the text; standard errors were again calculated as in ENGELS (1979b). N is the number of single male crosses. Experiments were conducted as usual with Δ2-3(99B) except: ^{a}Δ2-3(99B) at 28° and ^{b}*P-M* dysgenic crosses with π_2.

$$P[ry^+]; \frac{ry^{506}\,P\,[ry^+\,\Delta 2\text{-}3]}{TM3,\,ry^{RK}Sb}\ \male \quad \times \quad ry^{506}\ \female$$

phenotype		sex	interpretation
Sb⁺	ry⁺	male	normal
Sb	ry	male	normal
Sb⁺	ry⁺	female	normal
Sb	ry⁺	female	normal
Sb⁺	ry	male	excision of Δ2-3(99B) element
Sb	ry	female	excision of X chromosomal ry⁺ element
Sb⁺	ry	female	excision of both ry⁺ elements
Sb	ry⁺	male	transposition of either ry⁺ element

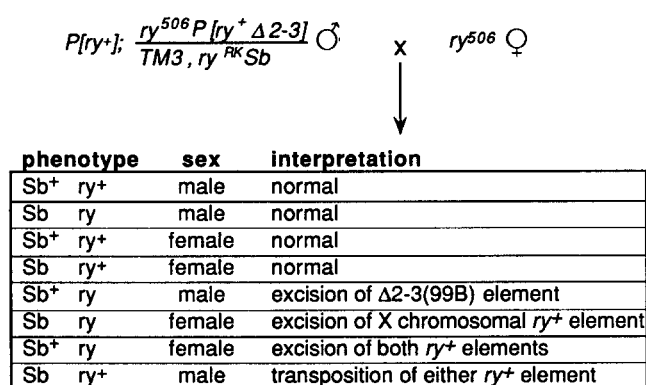FIGURE 6.—Transposition of $P[ry^+]$ elements from the X to the autosomes. Two generations of preliminary crosses produced the parental males. The Sb ry⁺ male progeny were testcrossed to sn^w; ry^{506} females to determine whether they were transpositions of Δ2-3(99B), which would make the sn^w sons mosaic. Analogous schemes could be used to mobilize elements on other chromosomes.

(Figure 6). As before, no excisions were observed among a total of 5252 males. Each of the 143 transpositions of a ry^+ marker onto an autosome failed to induce sn^w mosaicism, indicating that they were indeed transpositions of the non-autonomous $P[ry^+]$ elements as opposed to Δ2-3(99B). Thus no transpositions of Δ2-3(99B) were recovered among a total of 3737 progeny where they could have been observed.

As additional controls for the possible movement of Δ2-3(99B), we performed another series of crosses similar to Figure 6 except without a $P[ry^+]$ element on the X. In this case transpositions of Δ2-3(99B) could be observed in all Sb progeny and excisions in all remaining progeny. No transpositions were ob-

served among a total of 6972 progeny, but two potential excisions (loss of the ry^+ marker) were observed among a total of 8970 progeny. Both were females in one family and therefore presumably came from one event. One was sterile, and the other showed no evidence of Δ2-3 activity in subsequent crosses, suggesting that a complete excision had occurred.

Yet another experiment of this kind involved a dominantly marked ry^{506} Sb $P[ry^+$ Δ2-3]($99B$) chromosome that was constructed to facilitate monitoring of Δ2-3(99B) in experiments where the ry^+ marker is not being used. Males with this chromosome opposite the ry^{506} chromosome were mated to ry^{506} females. Excisions could be observed in the Sb progeny with transpositions recovered in the remaining progeny. Five losses of the ry^+ marker were observed in three families among 3907 progeny. These were not recovered and may have been the products of male recombination rather than excision of Δ2-3(99B), since the Sb marker is not closely linked to Δ2-3(99B). In addition, two independent potential transpositions of Δ2-3(99B) were observed among 3710 Sb⁺ progeny. Both were ry⁺ Sb⁺ males, one of which was sterile while the other carried a transposition of the $P[ry^+$ Δ2-3] element to position 1C on the X chromosome. This new insert at 1C retains its transposase expression as demonstrated by sn^w mutability both germinally and somatically, and the induction of pupal lethality with Birmingham chromosomes. The poor viability and fertility of this insert have thus far prevented extensive examination of its stability.

In summary, we have recovered only one confirmed excision of Δ2-3(99B) from approximately

36,100 chromosomes in our quantitative experiments, and another three excisions in numerous other experiments. One transposition was recovered among 14,420 progeny in which such events could have been observed. We conclude that Δ2-3(99B) is quite stable and can be kept as a stock without special attention and readily used in experiments of the kind described herein.

DISCUSSION

Characteristics of Δ2-3(99B): An unusual characteristic of this Δ2-3 element is its high transposase activity. This activity appears to be enormous, with the levels of destabilization of sn^w in the germline higher than the 20–60% observed with an entire P genome in P-M dysgenic crosses (ENGELS 1979a, 1984). Similarly, in combination with non-autonomous elements of the *Birmingham* strain, Δ2-3(99B) induces GD sterility at even lower temperatures than in *P-M* dysgenic crosses (ENGELS *et al.* 1987).

Several explanations for this high activity can be considered, primarily position effects and intrinsic properties of the Δ2-3(99B) element. The possibility of euchromatic position effects is supported by the observation that the Δ2-3(99B) element causes higher germline sn^w mutability than other tested Δ2-3 inserts (LASKI, RIO and RUBIN 1986). Position effects observed for other transformed genes with large 5' buffering regions have typically been rather small (*e.g.* SPRADLING and RUBIN 1983; DANIELS *et al.* 1986; LAURIE-AHLBERG and STAM 1987). By contrast, the *P* element promoter is not protected by such a buffering region and may be more sensitive to the effects of flanking sequences. The w^+ gene on the CaSpeR vector used here has a reduced 5' region leading to considerable variation in expression (V. PIRROTTA, personal communication), which might be more indicative of the position effects *P* elements experience.

Another possibility is that the splicing of the 2-3 intron may be a limiting step in expression of transposase from normal *P* elements, leading to enhanced expression of Δ2-3 elements. Furthermore, the 66-kD truncated polypeptide produced when this intron is not spliced may be a repressor of *P* element activity (RIO, LASKI and RUBIN 1986; H. M. ROBERTSON and W. R. ENGELS unpublished results), so Δ2-3 elements might be incapable of regulating their own activity. In P-M crosses there are also additional non-autonomous *P* elements that probably produce repressor (P cytotype), and limit transposase activity (H. M. ROBERTSON and W. R. ENGELS, unpublished results). In P-M dysgenic crosses there are also many defective elements that can titrate transposase, as shown by SIMMONS and BUCHOLZ (1985).

The absence of repressor may also explain the increase in sn^w mutability induced by Δ2-3(99B) with age and temperature, effects not seen in P-M crosses. P cytotype (repressor) is expected to increase with age in P-M crosses (ENGELS 1983) and has been reported to increase with temperature (RONSSERAY, ANXOLABÉHÈRE and PÉRIQUET 1984), offsetting any direct effect of age or temperature on transposase activity. The temperature effect on Δ2-3(99B) might involve increased expression and/or activity of transposase. In addition, the Δ2-3(99B) element does not show the reciprocal cross effects characteristic of P-M crosses, presumably because there is no P cytotype to cause such effects.

The other unusual attribute of Δ2-3(99B) is its extreme stability, which was surprising in view of its high transposase activity. Several large scale attempts to detect complete or partial excisions of the element yielded only one event, demonstrating that loss of Δ2-3(99B) is extremely rare. Furthermore, only one transposition to another chromosome has been recovered from more than 14,000 gametes. The stability of Δ2-3(99B) might result from its position of insertion or changes in its structure. Examination of transposed copies of Δ2-3(99B) and molecular analysis of these elements will distinguish between these possibilities. In our experience no other insert has proven as resistant to mobilization. Although properly considered to be an autonomous element, meaning one capable of mobilizing itself, Δ2-3(99B) lies at the extremes of high transposase activity but low mobility.

Utility of Δ2-3(99B): There are several advantages to the use of Δ2-3(99B) for mutagenesis, transformation, and transposition. For mutagenesis, it minimizes the problem of additional *P* element sites that complicate subsequent cloning. If there are inserts other than the one of interest on the target chromosome, they can be removed by recombination. The stability of Δ2-3(99B) allows its removal by genetic crosses, after which the lack of transposase-producing elements ensures that all inserts will be sufficiently stable for such genetic manipulations and stock keeping. Similar techniques for *P* element mutagenesis involving the Jumpstarter element have been used by COOLEY, KELLEY and SPRADLING (1988).

Once a mutation has been obtained, reversion using Δ2-3(99B) should allow confirmation of a *P* insertion. It should also be possible to use Δ2-3(99B) to catalyze imprecise excisions for use in deletion analysis of flanking DNA. A similar procedure was used by DANIELS *et al.* (1985) and SALZ, CLINE and SCHEDL (1987), but without the advantage of a stable genomic transposase source.

A disadvantage of Δ2-3(99B) for mutagenesis is that it can cause pupal lethality and GD sterility in combination with Birmingham chromosomes. Therefore, mutagenesis is best done at 16° where such

effects are minimized. The insertion and mutation rates obtained with this method under these conditions are at least as high as those obtained with P-M dysgenic crosses.

Use of Δ2-3(99B) for transformation ensures the presence of transposase in any cell taking up vector DNA and is as efficient as standard transformation techniques making use of helper plasmids. Subsequent transposition of marked elements to other positions in the genome is easily accomplished by crosses. For some purposes, such as obtaining inserts to mark chromosomes that are too poorly viable for microinjection (*e.g.*, ring chromosomes), this may be the only practical method. Other applications are also imaginable, such as the generation of small somatic mosaic patches of expression of transformed genes or P insert mutants.

In conclusion, we have demonstrated that Δ2-3(99B) is an unusually stable element with high transposase activity. It can therefore be used to enhance the efficiency of various experimental techniques that use *P* element transposase for mutagenesis, transformation and mobilization.

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Communicating editor: A. SPRADLING