Testing transposable elements as genetic drive mechanisms using *Drosophila P* element constructs as a model system

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Abstract

The use of transposable elements (TEs) as genetic drive mechanisms was explored using *Drosophila melanogaster* as a model system. Alternative strategies, employing autonomous and nonautonomous *P* element constructs were compared for their efficiency in driving the ry^+ allele into populations homozygous for a ry^- allele at the genomic *rosy* locus. Transformed flies were introduced at 1%, 5%, and 10% starting frequencies to establish a series of populations that were monitored over the course of 40 generations, using both phenotypic and molecular assays. The transposon-borne ry^+ marker allele spread rapidly in almost all populations when introduced at 5% and 10% seed frequencies, but 1% introductions frequently failed to become established. A similar initial rapid increase in frequency of the ry^+ transposon occurred in several control populations lacking a source of transposase. Constructs carrying ry^+ markers also increased to moderate frequencies in the absence of selection on the marker. The results of Southern and *in situ* hybridization studies indicated a strong inverse relationship between the degree of conservation of construct integrity and transposition frequency. These finding have relevance to possible future applications of transposons as genetic drive mechanisms.

Introduction

Concurrent with the search for generalized transformation vectors, there is interest in exploring the use of 'loaded' transposons, along with other forms of parasitic DNA, as a method for driving engineered genes into natural populations for insect pest control and related purposes (Hastings, 1994). Although it has been more than a decade since the first use of *P* elements as highly efficient vectors for gene transformation in *Drosophila* (Rubin & Spradling, 1982; Spradling & Rubin, 1982), this technique has been applied almost exclusively in studies focused at the molecular and developmental levels (e.g., Spradling, 1986). Population studies have largely been neglected and therefore very little is known about how transposable element-gene constructs behave in real populations, over time.

The rationale for interest in transposable elements (TEs) as genetic drivers is based on computer simulation and empirical studies that indicate these elements have a natural proclivity for rapid spread in populations due to their non-Mendelian mode of replication by means of transposition. Encouraging results of computer simulations (Kidwell & Ribeiro, 1992; Ribeiro & Kidwell, 1994) suggested that transposons with a wide range of properties may be used successfully to drive genes into populations. Ribeiro and Kidwell (1994) used a three-parameter density-dependent growth equation to examine the critical parameter values that determine whether or not a mobile element will spread and become fixed in a finite diploid popu-

lation. Populations were simulated with parameter values affecting population size, reproductive rate, density dependence, and transposition frequency of the mobile element. Simulations indicated that an equilibrium value was reached quickly, typically in less than 50 generations. Even when present at 1% or less of a large population, the mobile element spread quickly and became fixed if transposition frequency was equal to unity and reproductive capacity was reduced by as much as 45%. These results were insensitive to the values of basic wild-type reproductive rates and density dependence, but population size, transposition efficiency, and reproductive rate of TE-bearing individuals did modify the outcome. As population size and transposition efficiency decreased in value, elements became fixed less easily. However, even in populations as small as n = 100, the results indicated that an element with a transposition efficiency higher than 0.75, that reduces fertility less than 25%, will become fixed when introduced at a frequency as low as 1% of the total population.

Consistent with the simulation results, a number of earlier empirical experiments (Good et al., 1989; Kidwell, Novy & Feeley, 1981; Kiyasu & Kidwell, 1984) documented the rapid spread of transposable elements in laboratory populations of *Drosophila* despite the negative fitness effects that the mobilization of these elements confers on their hosts (Fitzpatrick & Sved, 1986; Mackay, 1986). A dramatic natural example of the rapid rate of spread of transposable elements in natural populations is provided by the recent *P* element invasion of the cosmopolitan species *D. melanogaster* during the last half century (Anxolabéhère, Kidwell & Periquet, 1988; Engels, 1992; Kidwell, 1994). This rapid spread was consistent with the theoretical results of Uyenoyama and Nei (1985).

The demonstrated potential of *P* elements to spread rapidly in natural populations lead to the suggestion that it might be worthwhile to pursue possible ways of using these elements as carriers, or drivers, of engineered genes in populations (Curtis & Graves, 1988). Because of the many difficulties inherent in working directly with many pest species, such as mosquitoes, we have chosen to work with *D. melanogaster* as a model system. Although the current prospects of using the *P* element as a general insect transformation vector are rather poor due to its relatively narrow host range, no suitable alternatives were available at the time this study was initiated. On the contrary, the previously documented ability of *P* elements to spread rapidly through populations provides good justification

for using this well-studied element in a species which is highly tractable to genetic manipulation. Promising preliminary work with a model system would help make a case for more difficult and expensive experiments with genetically less tractable species.

The purpose of the present experiments is to begin to address a number of questions fundamental to the possible use of transposable elements as gene drivers, including: (1) Will 'loaded transposons' spread and increase in frequency over time in real populations? If so, what are the mechanisms responsible for their spread? (2) Will the genes introduced in this way be stable over time? (3) Do factors such as gene size, transposon structure, and site of insertion have an important effect on rate of increase of copy number and maintenance of construct stability? (4) What is the effect of population size and structure on transposon spread?

Answers to these and related questions should begin to provide guidance on the feasibility of this approach and, if the results are promising, for planning the most efficient strategies for introducing genes into populations for a number of different potential applications.
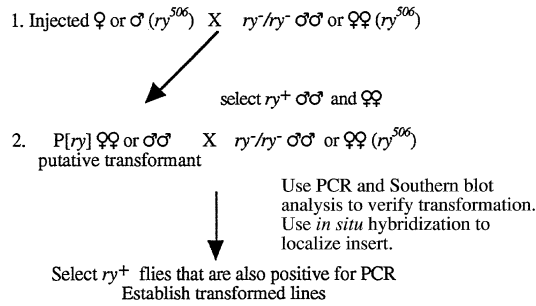
Materials and methods

Drosophila *stocks*

ry^{506}: a true M strain in the P-M system of hybrid dysgenesis (Kidwell, Kidwell & Sved, 1977) homozygous for ry^{506}, a recessive allele at the *rosy* locus. This allele has a large internal deletion at the distal end of the *rosy* locus and is normally located in cytological band 3R (87D). Flies homozygous for this allele have a reddish brown eye color that can be readily distinguished from wild type. The complete absence of any *P* elements in this mutant strain was confirmed by Southern hybridization and PCR analyses.

Harwich-*w* (*white*): a strong P strain carrying approximately 50 *P* elements per genome and used as a reference strain for the P-M system. The original strain was collected by Dr. M.L. Tracey in Harwich, Massachusetts in 1967; the *white* mutation carried by this strain was isolated by M.G.K. from the original Harwich strain.
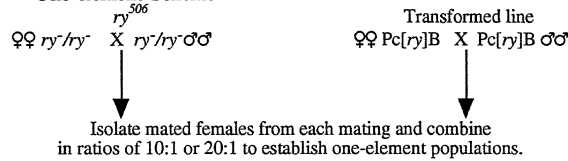
ry^{506}-dil*P*: a ry^{506} strain into which *P* elements were introduced by crossing Harwich-*w* (strong P) females to ry^{506} (M) males and backcrossing their progeny for three successive generations to ry^{506} (M) males (see Figure 1B – II). This scheme results in a P cytotype strain carrying a number of nonautonomous *P* elements

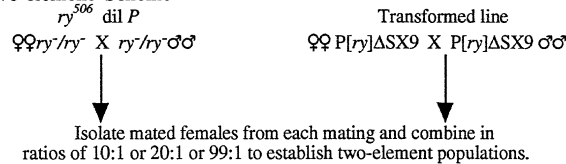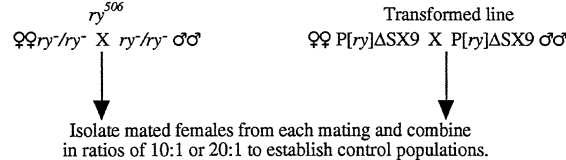**A. Establishment of Transformed Lines: One- and Two- Element Schemes**

1. Injected ♀ or ♂ (ry^{506})   X ry^-/ry^- ♂♂ or ♀♀ (ry^{506})

select ry^+ ♂♂ and ♀♀

2.   P[ry] ♀♀ or ♂♂   X ry^-/ry^- ♂♂ or ♀♀ (ry^{506})
putative transformant

Use PCR and Southern blot
analysis to verify transformation.
Use *in situ* hybridization to
localize insert.

Select ry^+ flies that are also positive for PCR
Establish transformed lines

B. Establishment of Populations

I.   One-element Scheme

ry^{506}

♀♀ ry^-/ry^- X ry^-/ry^- ♂♂

Transformed line

♀♀ Pc[ry]B   X   Pc[ry]B ♂♂

Isolate mated females from each mating and combine
in ratios of 10:1 or 20:1 to establish one-element populations.

II. Two-element Scheme

ry^{506} dil P

♀♀ ry^-/ry^- X ry^-/ry^- ♂♂

Transformed line

♀♀ P[ry]ΔSX9   X   P[ry]ΔSX9 ♂♂

Isolate mated females from each mating and combine in
ratios of 10:1 or 20:1 or 99:1 to establish two-element populations.

III. Control Lines

ry^{506}

♀♀ ry^-/ry^- X ry^-/ry^- ♂♂

Transformed line

♀♀ P[ry]ΔSX9   X   P[ry]ΔSX9 ♂♂

Isolate mated females from each mating and combine
in ratios of 10:1 or 20:1 to establish control populations.

Figure 1. Mating schemes used in Experiments I and II. (A) Establishment of transformed lines. Embryos were microinjected as described by Kim et al. (1994) and later, as adults, crossed to ry^{506} (M strain) flies. (B) Establishment of experimental and control populations. I. One-element scheme. The source of transposase is located in the same P element construct as the ry^+ marker. II. Two-element scheme. The source of transposase is provided by natural P elements introduced into the ry^{506}-dil$P$ strain (see Materials and methods). The ry^+ marker is located in the nonautonomous P[ry]ΔSX9 construct. III. Lines transformed with nonautonomous P[ry]ΔSX9 constructs, but lacking a source of transposase, were used to establish control populations.

and also a few autonomous P elements that provide a source of transposase. The number of both autonomous and nonautonomous P elements is expected to be reduced relative to those carried by the Harwich-w strain because of the backcrossing to an M strain.
ruP/TM6:   a third chromosome balancer strain.

Plasmids and constructs

Plasmid Pc[ry]B (Karess & Rubin, 1984) was kindly supplied by Dr. Roger Karess. The P element construct which it carries was used to transform the lines used in the one-element scheme. This construct (illustrated

in Figure 2) includes the ry^+ marker gene (the gene to be driven) integrated into an autonomous P element that provides a source of P element transposase (the drive mechanism). Plasmid Pc[ry]Δ2–3 (Laski, Rio & Rubin, 1986) was kindly supplied by Dr. Mark Benedict.

The plasmids pΔ2–3hsπ and pπ25.1 (O'Hare & Rubin, 1983) were kindly supplied by Drs. Y.-K. Kim and Bill Engels, respectively.

P[ry]ΔSX9 is a nonautonomous derivative of Pc[ry]B that was constructed by deleting a region of Pc[ry]Δ2–3 between the unique *Xho* I site at nucleotide position 728 and the unique *Sal* I site at position 2410
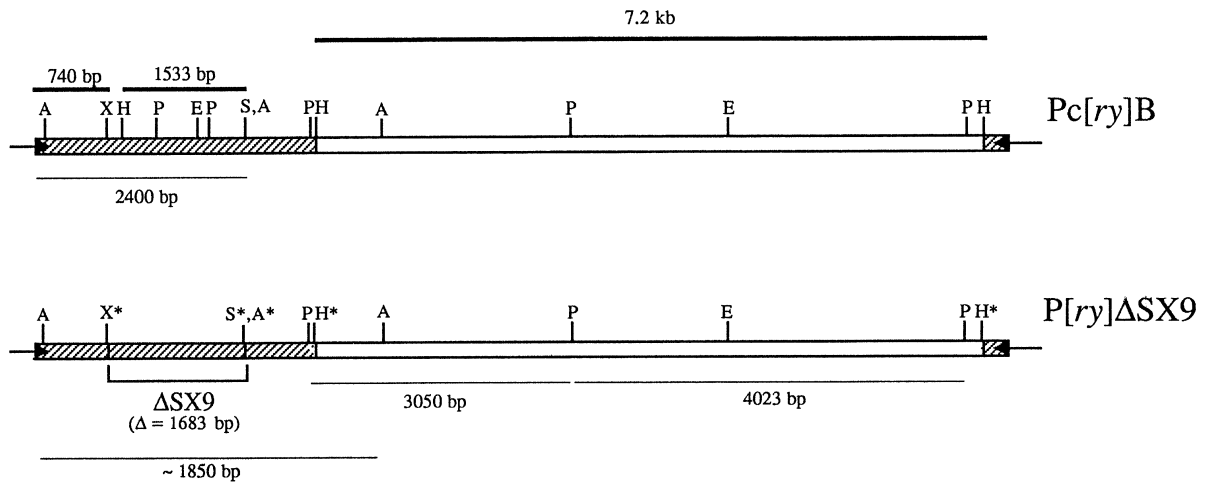
Figure 2. Structures of the two *P* element-*rosy* gene constructs used to establish transformed lines in Experiments I and II. Pc[*ry*]B carries a fully functional, autonomous *P* element. P[*ry*]ΔSX9 carries a nonautonomous *P* element. Horizontal lines above the constructs indicate the probes used in the restriction enzyme analysis. Horizontal lines below the constructs indicate the expected sizes of restriction fragments. * indicates a restriction site that was removed or altered. A = *Acc* I; E = *Eco* RI; H = *Hin*d III; P = *Pst* I; S = *Sal* I; X = *Xho* I.

in the canonical *P* element sequence from pπ25.1 (O'Hare & Rubin, 1983). This construct was used, along with a helper plasmid, to transform the lines used in the two-element scheme. The structure of this construct is illustrated in Figure 2.

Method of transformation

ry^{506} embryos were dechorinated and microinjected with Pc[*ry*]B DNA at a concentration of 250 μg/ml. The P[*ry*]ΔSX9 DNA was coinjected with the helper plasmid pΔ2–3hsπ, at 300 μg/ml and 50 μg/ml, respectively, as described previously (Kim et al., 1994).

Establishment of transformed lines

Flies emerging from injected embryos were mated with ry^{506} virgin females or males (Kim et al., 1994). Putative transformants were detected by wild-type eye color expression of the ry^+ allele present in the *P* element constructs. The presence of the relevant *P* element construct was confirmed by means of single fly PCR (Gloor & Engels, 1992) using the following *P* element-specific oligonucleotide primer sequences: #829, 5′-AACATAAGGTGGTCCCGTCG-3′, and #830, 5′-CGACTGGGCAAAGGAAATCC-3′. Individual lines were established from a number of transformed flies. A sample of these were used to establish the experimental and control populations (Figure 1).

DNA and plasmid isolation

Genomic DNA was isolated from single flies, or pools of 10 flies, by a modification of the method of Gloor and Engels (1992). Flies were homogenized in squishing buffer (10 mM Tris-HCl [pH 8.0], 10 mM EDTA, 50 mM NaCl) containing 0.5% SDS, incubated with Proteinase K (100 μg/ml) at 50°C for 20 min, and extracted once with phenol-chloroform (1:1) and once with chloroform alone. Nucleic acids were precipitated with 100% ethanol and washed once with 70% ethanol. Nucleic acids were redissolved, treated with RNase A (100 μg/ml), extracted with chloroform, and reprecipitated. Plasmid DNA was isolated by alkaline lysis methods and purified by centrifugation in cesium chloride–ethidium bromide density gradients prior to microinjection (Sambrook, Fritsch & Maniatis, 1989).

Southern hybridization analysis

Genomic DNA from individual and pooled fly samples was digested with restriction enzymes as indicated, electrophoresed in agarose gels, blotted to nylon membranes (Magnagraph, MSI) and hybridized with radioactively labeled probes essentially as described (Sambrook, Fritsch & Maniatis, 1989). DNA restriction fragments and dsPCR products used as probes were prepared by random primer labeling (Feinberg & Vogelstein, 1983) using the RadPrime Labeling System (GIBCO-BRL).

P element copy numbers were estimated in the Pc[ry]B and P[ry]ΔSX9 populations by extracting genomic DNA from pools of 10 ry^+ flies. DNAs were digested with Sal I, electrophoresed, blotted, and hybridized with the 1.5 kb Hind III–Sal I fragment of the P element from pπ25.1 or with a 740 bp dsPCR product derived from the 5′ end of the P element. The structural integrity of P element constructs was examined by extracting genomic DNA from pools of 10 ry^+ flies. This was digested with Acc I, electrophoresed, blotted, and hybridized with a P DNA specific probe (1.5 kb Hind III–Sal I fragment). A 2.4 kb band is expected for intact P element sequences.

The structural integrity of $rosy$ gene sequences in the Pc[ry]B and P[ry]ΔSX9 populations was examined by extracting genomic DNA from pools of 10 ry^+ flies. This was digested with Pst I, electrophoresed, blotted, and hybridized with a $rosy$ DNA specific probe (7.2 kb Hind III fragment from Pc[ry]B). Three bands of 3.1 kb and 4.1 kb (P-$rosy$ construct) and 6.5 kb (genomic $rosy$ locus) are expected.

In situ *hybridization to polytene chromosomes*

This method was performed as described previously (Kim & Kidwell, 1994; Lim, 1993) using DNA probes labeled with Bio- or Dig-modified analogs. Hybridization was detected by enzyme-linked immunoassay, using a specific coenzyme-antibody complex and subsequent enzyme-catalyzed color reaction. Cytological locations and numbers of individual insertions were determined using the 2.9 kb P element PCR product, or the entire pπ25.1 plasmid and the 7.2 kb Hind III fragment of the $rosy$ gene as specific DNA probes.

Results

Two initial experiments were performed to test the effects of several variables on the spread and integrity of the *P-rosy* constructs. These variables included the use of autonomous versus nonautonomous constructs (single- versus two-element strategies), different seed frequencies, different genomic insertion sites of the transformed lines, and different population sizes (half-pint milk bottles supporting up to about 600 flies versus population cages supporting up to several thousand flies). Control populations (described below) that carried the P[ry]ΔSX9 construct, but no source of P element transposase, were also established. In a third experiment, a transposon bearing the ry^+ marker gene

was placed in a ry^+ genetic background in order to estimate directly the effects of selection at the $rosy$ locus on transposon dynamics.

Experiment I

This experiment was designed to compare the efficiency of using a single autonomous P element construct with that using a two-element strategy to drive the wild-type ry^+ allele into homozygous ry^- populations (see Figure 1 for mating schemes). For each of the two strategies, we used two different transformed lines, twice replicated, to test the effect of different chromosomal insertion locations (Table 1). For the single element strategy, we used the B1-8 and B3-1 lines, transformed with Pc[ry]B (see Figure 2), with initial insertions at chromosome locations 3L (61F) and 2R (48E), respectively. For the two-element strategy, we used the SX27-2 and SX31-1 lines, transformed with P[ry]ΔSX9 (see Figure 2), with initial insertions sites at 3R (89C) and 2L (36B), respectively. In the latter case, the P[ry]ΔSX9 transformed lines were mated with females of strain ry^{506}-dilP that had been synthesized to incorporate natural functional P elements to provide a source of transposase for the nonautonomous P[ry]ΔSX9 element (see Figure 2).

Bottle populations were initially established with 100 individuals by combining non-virgin females of the ry^+ transformed lines with non-virgin ry^{506} females in ratios of 5% (5:95) and 10% (10:90) (Table 1). In addition to the bottle populations, four cage populations were also established at this time to determine the effect, if any, of population size on P element dynamics. The initial population size for all cage experiments was 200 individuals, twice as large as that of the bottles. The cages were established in the same manner as the bottle populations, except that only the nonautonomous transformed lines SX27-2 and SX31-1 were used to establish the cage populations.

Phenotypic assays

One hundred flies from each of the 16 bottle populations were randomly sampled every generation, for 40 generations, to estimate the frequency of flies with ry^+ eye color. This phenotype indicated the presence of at least one P element construct carrying the ry^+ marker gene. Figures 3A and 3B present the results for the eight populations seeded with individuals carrying the autonomous Pc[ry]B and the nonautonomous P[ry]ΔSX9 constructs, respectively (see Table 1). The
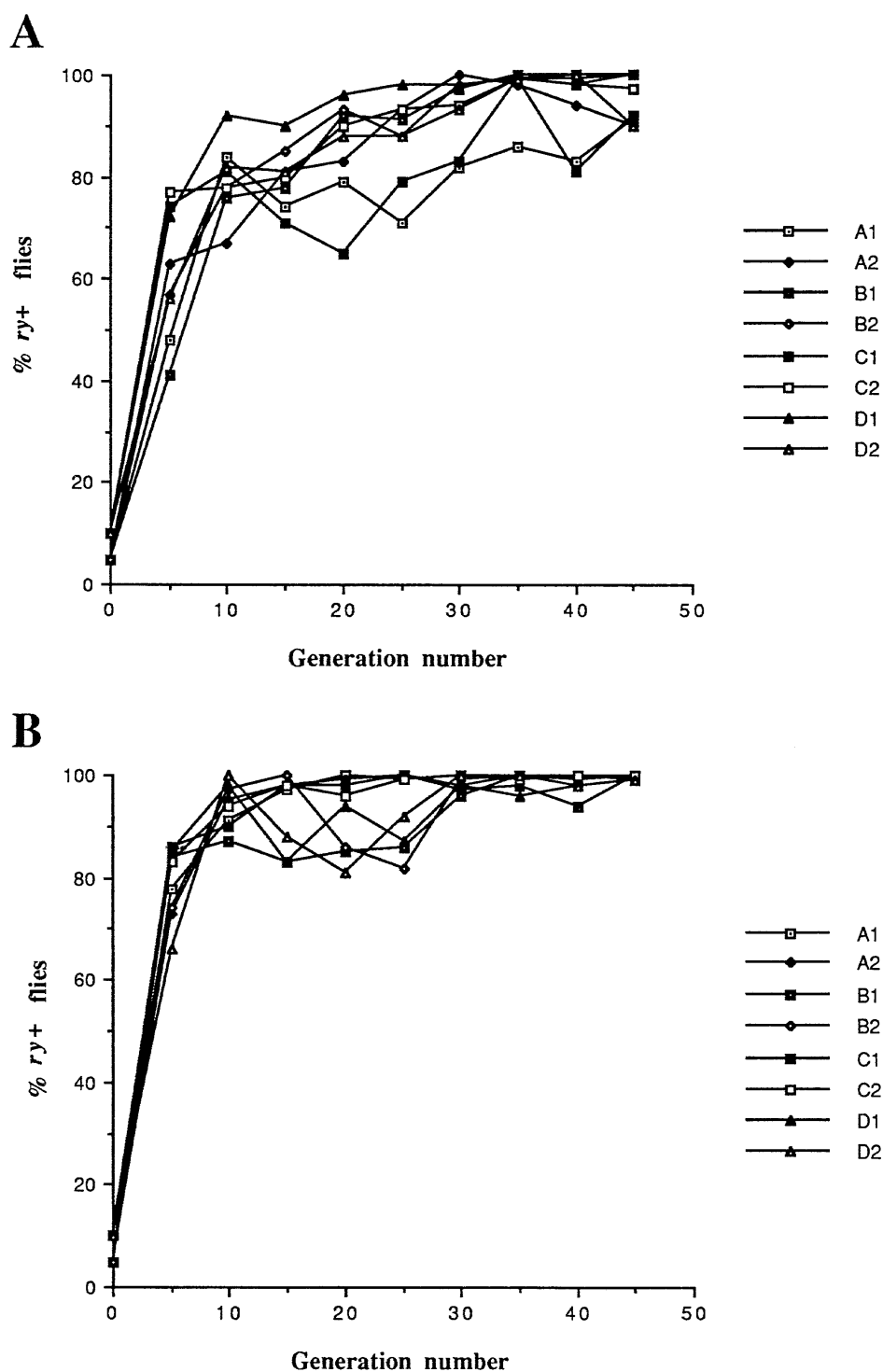
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Figure 3. Summary of the results of phenotypic assays for flies with *rosy* eye color in Experiment I. (A) Mean frequencies of flies carrying at least one *P-rosy* gene construct in eight populations seeded with individuals carrying the autonomous Pc[*ry*]B construct (see Table 1). (B) Mean frequencies of flies carrying at least one *P-rosy* gene construct in eight populations seeded with individuals carrying the nonautonomous P[*ry*]ΔSX9 construct (see Table 1).

Table 1. Identity of populations studied in Experiments I and II

| Identity of original transformed line | Chromosomal location | Experiment I | | | | Experiment II | | | Control |
		Bottles 5%	Bottles 10%	Cages 5%	Cages 10%	Bottles 1%	Bottles 5%	Cages 5%	Bottles 5%
B1-8 (autonomous)	3L (61F)	A1, A2	C1, C2						
B3-1 (autonomous)	2R (48E)	B1, B2	D1, D2						
Sx31-1 (nonautonomous)	2L (36B)	A1, A2	C1, C2	A	C				
Sx27-2 (nonautonomous)	3R (89C)	B1, B2	D1, D2	B	D	E1, E1 E3, E4	F1, F2	B, D	G1, G2 H1, H2
Sx28-4 (nonautonomous)	X (16D)					A1, A2 A3, A4	B1, B2	A, C	C1, C2 D1, D2
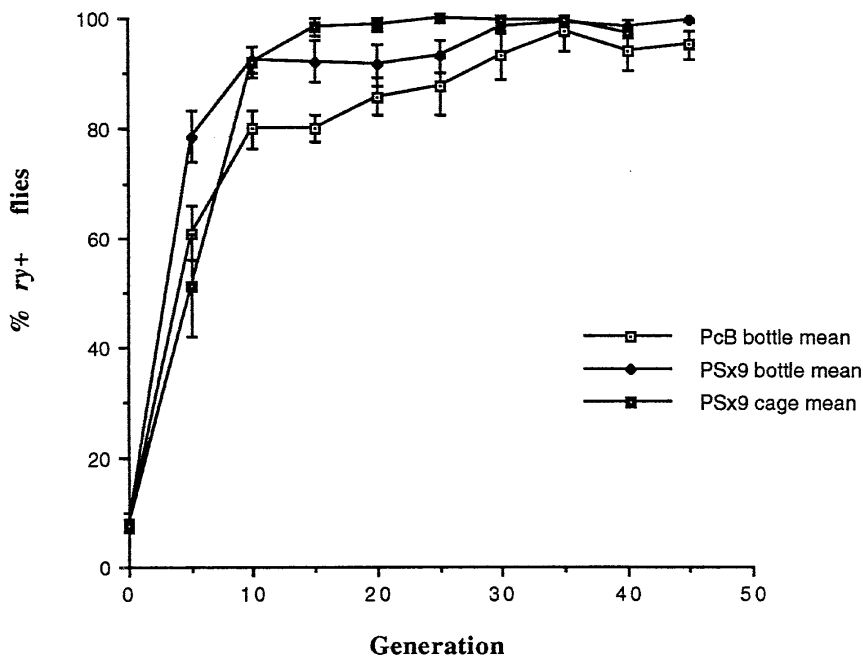
Figure 4. Comparison of one-element versus two-element schemes in Experiment I. Mean frequencies of flies carrying the ry^+ allele in PcB bottle populations seeded with the autonomous Pc[ry]B constructs versus ΔSX9 bottle and cage populations seeded with nonautonomous P[ry]ΔSX9 constructs.

graphs show a remarkably rapid and uniform increase in the frequency of P–ry^+ construct-bearing flies in both sets of populations. By the 10th generation, roughly 80% of all individuals in the Pc[ry]B populations and approximately 90% in the P[ry]ΔSX9 populations carried at least one copy of the construct.

Figure 4 compares the mean frequencies of ry^+ flies from the Pc[ry]B and P[ry]ΔSX9 populations. Similar dramatic increases in ry^+ frequency occurred in the first 10 generations in both sets of populations indicating only small differences between autonomous

and nonautonomous constructs. Mean frequencies of ry^+ flies in the four P[ry]ΔSX9 cage populations were very similar to those in the eight P[ry]ΔSX9 bottle populations, suggesting that population size had little or no effect on the increase of construct copy number. In a similar way, patterns of increase in ry^+ frequency between populations seeded with 5% versus 10% transformed flies showed no statistically significant differences (data not shown). This observation, combined with the fact that the construct was not lost in any of the 16 experimental populations, suggests that a seed fre-

quency as low as 5% may be adequate to ensure, with a reasonable probability, that a construct will not be lost during the critical generations following its introduction. Likewise, when the effects of the initial insertion site of the construct on subsequent rate of copy number increase were compared, the four different transformed populations showed only minor differences in pattern and rate of increase (data not shown). The similarity of phenotypic results between the behavior of the B1-8 versus B3-1 populations is particularly interesting in light of the striking underlying differences in transposition rate, P element copy number, and construct integrity that were revealed by the molecular and cytological analyses (see below).

Experiment II

Experiment II employed only the two-element strategy (Table 1) and included the comparison of a transformed line initially carrying a single autosomal insertion, SX27-2, with initial insertion at 3R (89C), with one carrying a sex-linked insertion, SX28-4, with initial insertion at X(16D). The experimental populations were established with 1% and 5% seed frequencies in the same manner as in Experiment I (see Figure 1). At the same time, control populations that lacked an endogenous source of transposase were also initiated. These populations were started in the same way as those in Experiment I, using the P[ry]ΔSX9 nonautonomous P element construct at a 5% initial seed frequency. In this case however, the ry^{506} M strain that lacked a source of transposase was substituted for the ry^{506}-dil$P$ strain (see Figure 1). Hereafter the term 'control populations' will refer to populations carrying a P[ry]ΔSX9 construct, but lacking a source of transposase. In this experiment, bottle and cage populations were initiated with 200 and 400 flies, respectively.

Phenotypic analysis

Samples of 100 flies per population were scored for ry^+ eye color each generation using the same method as in Experiment I. The populations initiated with only 1% seed frequencies provided highly variable results. About 50% of these populations failed to maintain the transposon after a few initial generations. Combining this result with those from Experiment I, it appears that, in general, 5% seed frequencies are adequate to ensure introduction of the construct, but that 1% frequencies are not satisfactory. There was no clear effect of pop-

ulation size (bottles versus cages) on the population dynamics of the construct.

As shown in Figure 5, there was little difference in the frequencies of the ry^+ allele between the SX27-2 experimental and control means. The SX28-4 (X-linked) control populations showed a slower initial increase, but by generation 20 the frequencies were not significantly different. Possible contamination of the control populations with autonomous P elements was ruled out by *in situ* hybridization analysis of samples of larvae from the control lines, C1, C2, G1, G2, H1, and H2 (see Table 1). From three to five larvae per line were examined at the 16th generation for hybridization to either a *rosy* DNA specific probe (7.2 kb *Hin*d III fragment) or the pπ25.1 P element probe. Out of a total of 36 control larvae examined, none showed hybridization at any site other than at the initial construct insertion site. These results, together with the consistently rapid initial increases in frequency of the ry^+ allele, suggest that positive selection was acting on the ry^+ construct. However, results from additional experiments, described below, strongly suggest that selection on the ry^+ allele was not the only factor responsible for the high frequencies observed.

Estimates of construct copy number using Southern hybridization analysis

Because the phenotypic assays could not differentiate between individual flies carrying one or more copies of the ry^+ construct, it was necessary to carry out a more detailed molecular analysis to provide an estimate of changes in copy number over time. At five generation intervals, genomic DNA from pools of 10 ry^+ flies was digested with *Sal* I, electrophoresed, blotted, and hybridized with the internal 1.5 kb *Hin*d III–*Sal* I fragment of the P element from pπ25.1. DNA samples from Harwich (P strain) and ry^{506} (M strain) were included as positive and negative hybridization controls, respectively.

Southern hybridization results for four individual Pc[ry]B populations in Experiment I are presented in Figure 6. The autoradiograms shown in Figures 6A and 6C indicate only a few major bands, indicating the presence of insertions at the same one or two chromosomal sites in most individuals and a limited number of minor bands, suggesting the presence of an insertion site in only a few individuals sampled. This pattern is typical for those Pc[ry]B populations having the transposon initially inserted at cytological position 3L (61F). Estimates of construct copy number at generation 35 were
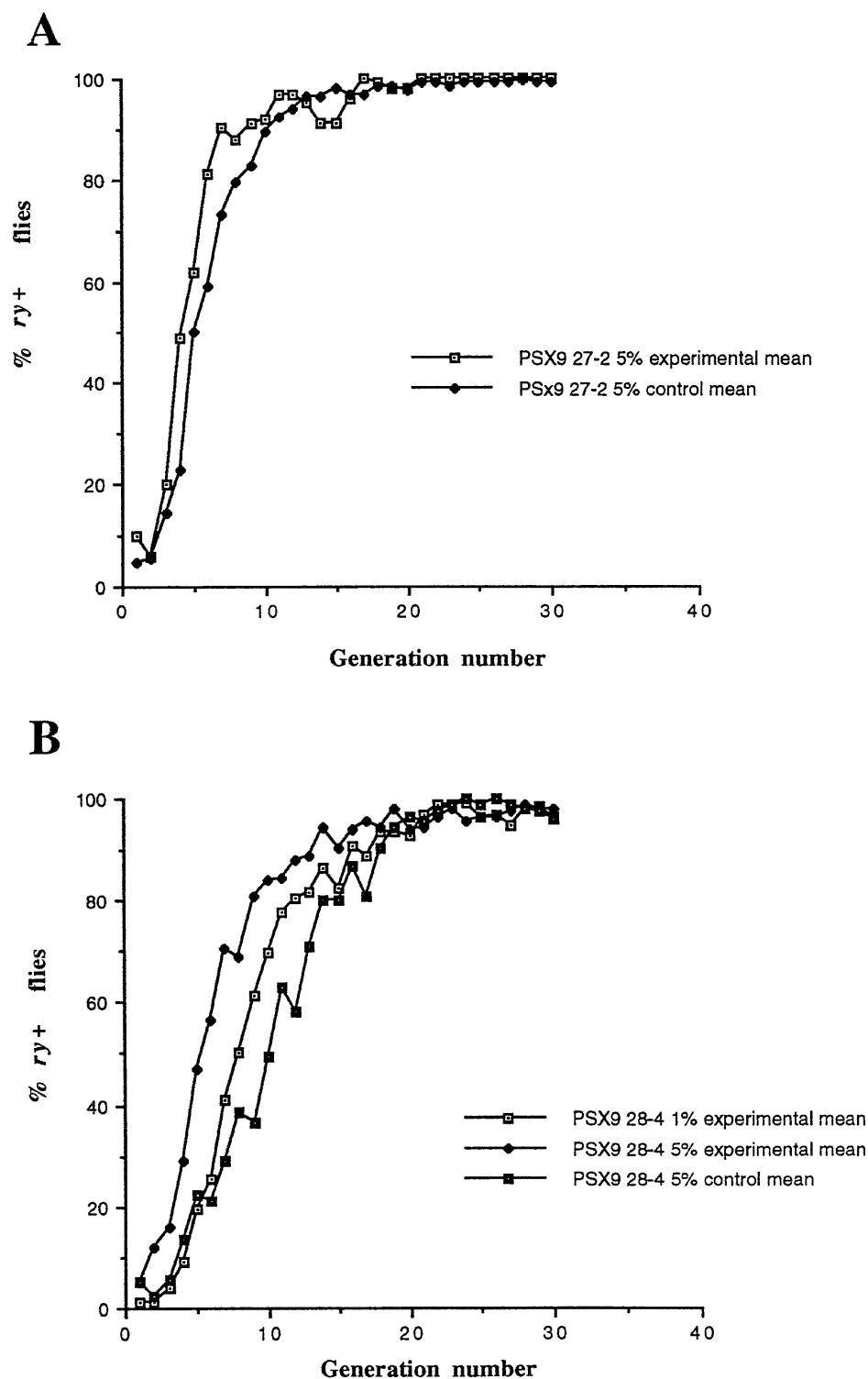
A

B

Figure 5. Comparison of experimental and control populations in Experiments I and II. (A) Mean frequencies of flies carrying the ry^+ allele in experimental and control SX27-2 populations seeded with the nonautonomous construct originally inserted at location 3R (89C).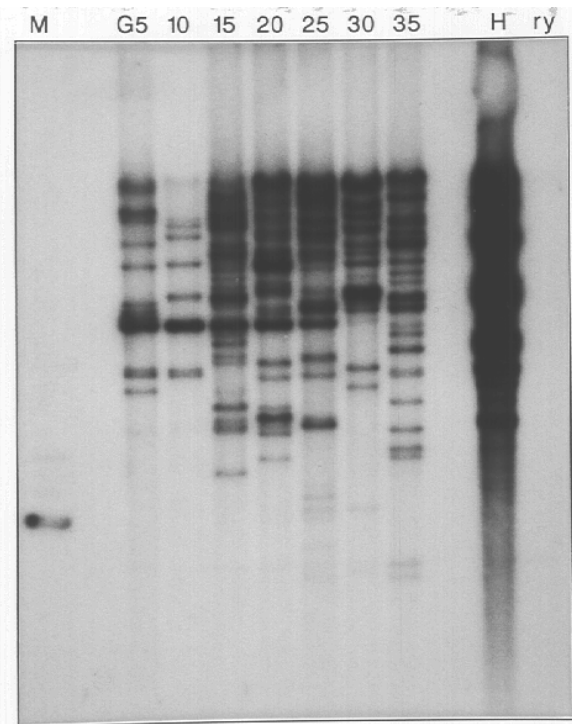 (B) Mean frequencies of flies carrying the ry^+ allele in experimental and control, SX28-4, populations seeded with the nonautonomous construct originally inserted at location X(16D).
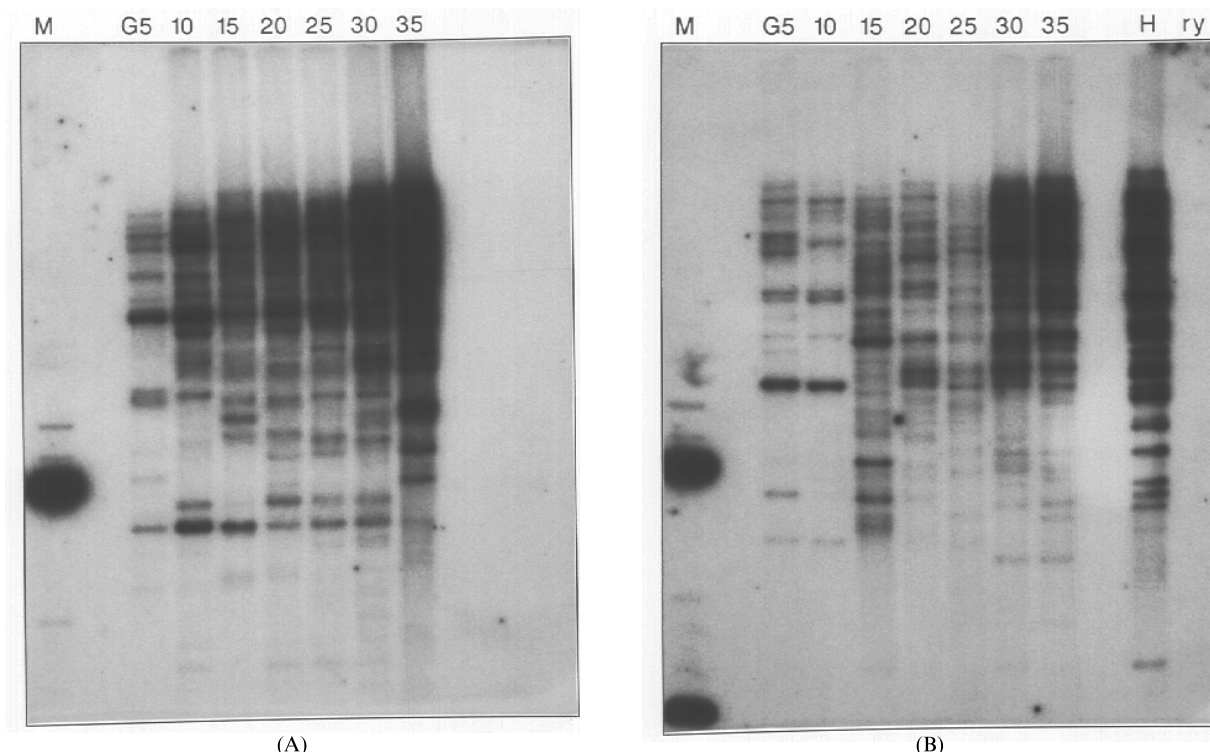
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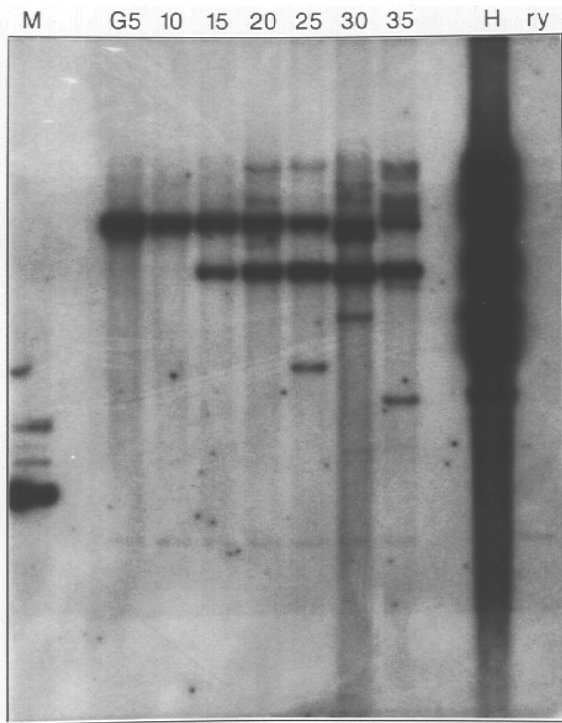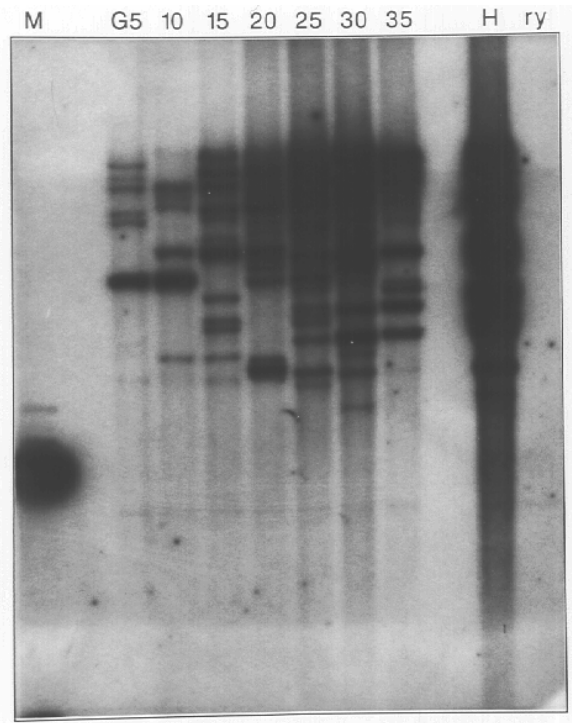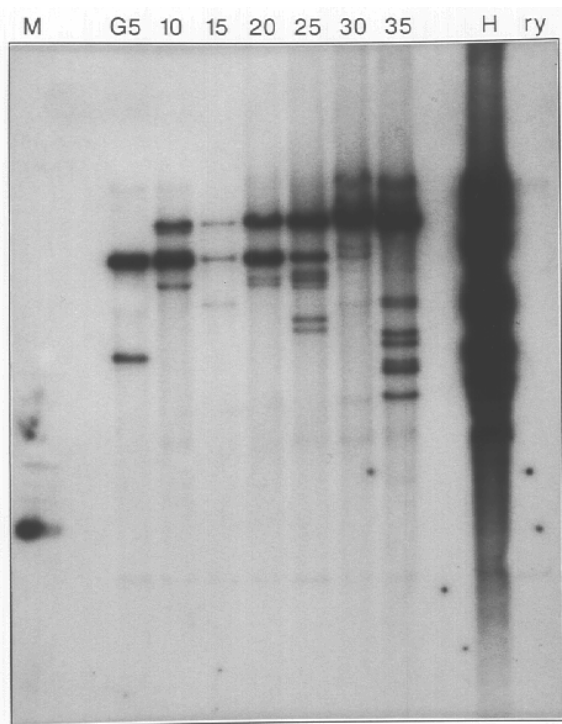
Figure 6. Estimation of Pc[*ry*]B construct copy number over time in Experiment I. Autoradiograms compare the number of genomic fragments hybridizing to the 1.5 kb *Hin*d III–*Sal* I *P* element probe at five generation intervals in four of the eight populations carrying this autonomous construct. Genomic DNA from pools of 10 *ry*⁺ flies was digested with *Sal* I. (A) Line A2 [initial genomic insertion site: 3L (61F)]; (B) Line B2 [initial genomic insertion site: 2R (48E)]; (C) Line C2 [initial genomic insertion site: 3L (61F)]; (D) Line D2 [initial genomic insertion site: 2R (48E)]. Lanes: M, DNA size markers, 1 kb ladder; G5-35, experimental lines tested at generations 5, 10, 15, 20, 25, 30, and 35; H, Harwich *white* (*P* strain control); ry, *ry*^506 (M strain control).

Figure 7. Estimation of P[*ry*]ΔSX9 construct copy number over time in Experiment I. Autoradiograms compare the number of genomic fragments hybridizing to the 740 bp dsPCR *P* element probe at five generation intervals in two of the eight populations carrying this nonautonomous construct. Genomic DNA from pools of 10 *ry*⁺ flies was digested with *Pst* I. (A) Line A1 [initial genomic insertion site: 2L (36B)]. Lanes: M, DNA size markers, 1 kb ladder; G5-35, experimental lines tested at generations 5, 10, 15, 20, 25, 30, and 35; (B) Line D1 [initial genomic insertion site: 3R (89C)]. Lanes: M, DNA size markers, 1 kb ladder; G5-35, experimental lines tested at generations 5, 10, 15, 20, 25, 30, and 35; H, Harwich *white* (*P* strain control); ry, *ry*⁵⁰⁶ (M strain control).

2 major and 6-7 minor bands for line Pc[*ry*]B A2, and 1 major and 11 minor bands for line Pc[*ry*]B C2. In contrast, the autoradiograms shown in Figures 6B and 6D show numerous bands indicating multiple different insertions resulting from frequent transpositions. This pattern is typical for those Pc[*ry*]B populations having the transposon initially inserted at cytological position 2R (48E). Construct copy number estimates at generation 35 were 3 major and more than 10 minor bands for line Pc[*ry*]B B2, and 2 major and more than 20 minor bands for line Pc[*ry*]B D2. Note that the initial rate of spread of the autonomous construct was similar for both high and low copy number lines (Figure 3A).

Results for two individual P[*ry*]ΔSX9 populations are presented in Figure 7. In both the P[*ry*]ΔSX9 A1 (Figure 7A) and P[*ry*]ΔSX9 D1 (Figure 7B) populations, the increase in number and intensity of bands indicates an increase in overall *P* element copy number over 35 generations. However, these blots cannot distinguish between the endogenous or native *P* elements

introduced into the background of this *ry*⁵⁰⁶-dil*P* strain from the Harwich-*w* strain (see Figure 1) and the *P-rosy* constructs that were introduced by transformation.

Examination of construct integrity using Southern hybridization analysis

We examined the integrity of the *P* element and *rosy* gene portions of the constructs in two separate assays (see Materials and methods). At generation 35, pooled DNA samples of 10 *ry*⁺ flies were examined from all 16 experimental populations in Experiment I. As seen in Figure 8A, all Pc[*ry*]B populations carry a prominent band of the expected 2.4 kb size, strongly suggesting the presence of intact *P* element sequences. In populations Pc[*ry*]B A1, A2, C1, and C2, at most a few weak bands of other sizes are seen, suggesting there is very little breakdown of the *P* sequences. In contrast, populations Pc[*ry*]B B1, B2, D1, and D2 show a number of both strong and weak bands of various

(A)

(B)
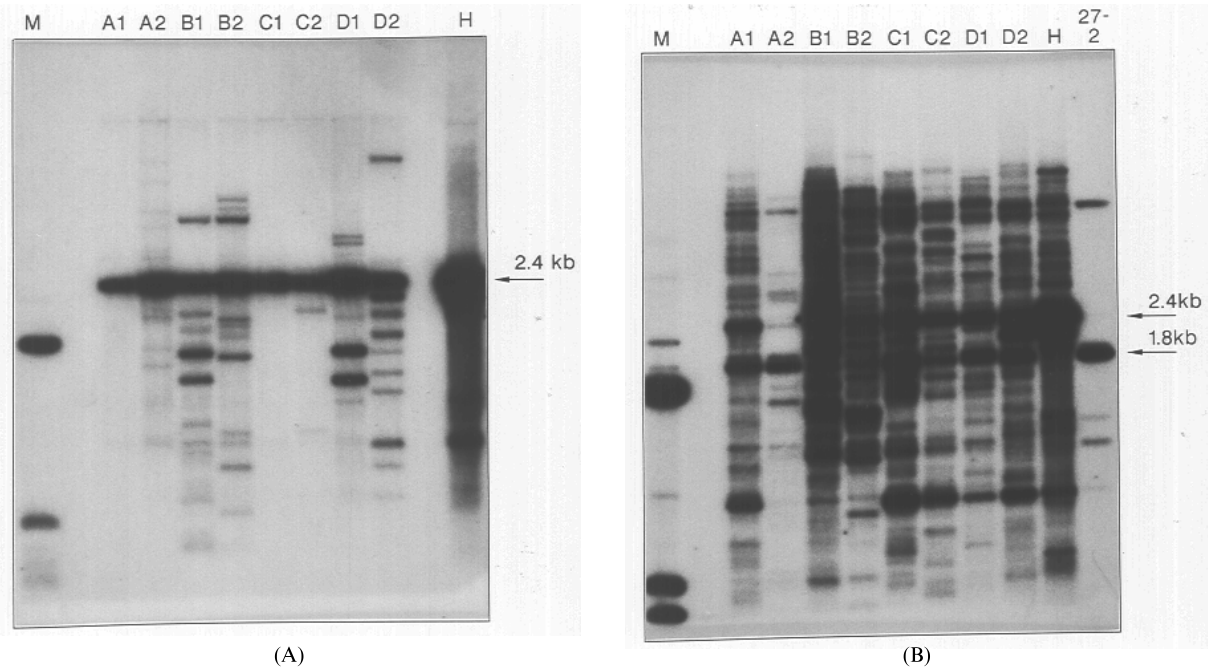
Figure 8. Autoradiograms illustrating the integrity of *P* element sequences at generation 35 of Experiment I. (A) Pc[*ry*]B populations. Genomic DNA from pools of 10 *ry*+ flies was digested with *Acc* I and hybridized with the 1.5 kb *Hin*d III–*Sal* I fragment of the *P* element. Lanes: M, DNA size markers, 1 kb ladder; A1 through D2, Pc[*ry*]B experimental populations; H, Harwich *white* (*P* strain control). The position of the 2.4 kb band, corresponding to the *Acc* I internal fragment of intact *P* element, is indicated. (B) P[*ry*]ΔSX9 populations. Genomic DNA from pools of 10 *ry*+ flies was digested with *Acc* I and hybridized with a 740 bp dsPCR *P* DNA-specific probe. Lanes: M, DNA size markers, 1 kb ladder; A1 through D2, P[*ry*]ΔSX9 experimental populations; H, Harwich *white* (*P* strain control); 27-2, SX 27-2 control line. The positions of two bands of sizes 1.8 kb and 2.4 kb are indicated. A 2.4 kb *Acc* I band is expected from natural autonomous *P* elements. A 1.8 kb *Acc* I band is expected from the P[*ry*]ΔSX9 nonautonomous construct.

sizes, especially bands less than 2.4 kb in length. This suggests that, although some intact constructs are still present, there has also been fairly extensive breakdown of construct sequences in these populations. A striking overall inverse relationship between transposition frequency (as reflected in copy number) and construct breakdown is revealed by these data.

Examination of the P[*ry*]ΔSX9 populations (Figure 8B) revealed a strong 1.85 kb band. An *Acc* I band of this size is expected in all individuals carrying the P[*ry*]ΔSX9 construct (see Figure 2). A prominent 2.4 kb band is also observed in all the experimental populations, except A2, in which it is weak. Bands of 2.4 kb are expected from the presence of intact naturally occurring *P* elements in these populations. Also, as expected, the 2.4 kb band is completely absent in the control SX27-2 line that carries only the P[*ry*]ΔSX9 construct but no autonomous, native *P* elements.

Tests of the integrity of the *rosy* gene sequences were carried out for all Pc[*ry*]B and P[*ry*]ΔSX9 populations at generation 35. The results are presented in

Figure 9. The 4.1 kb *Pst* I band that is expected from an intact *rosy* gene in both constructs is observed in all experimental populations. This 4.1 kb band is also observed in the Harwich-*w* and *ry*506 control lanes, as expected from the presence of the genomic copy of the *rosy* gene. Likewise, the 3.1 kb *Pst* I band is present, as expected, in the sixteen experimental populations. This band was neither expected, nor observed, in the Harwich-*w* and *ry*506 control lanes.

A *Pst* I band, approximately 6.5 kb in size, is both expected and observed in the Harwich lanes of Figures 9A and B because of the presence of the wild-type allele at the genomic *rosy* locus. The absence of this 6.5 kb band in the *ry*506 lanes is expected because the genomic copy of the *rosy* locus in this mutant strain carries a large deletion in the promoter-distal region (Keith et al., 1987; M.F. Wojciechowski, unpublished results). A band of similar size and intensity to the 6.5 kb band in the Harwich-*w* lanes was also unexpectedly observed in the Pc[*ry*]B B1, B2, and D2 lanes (Figure 9A) and the P[*ry*]ΔSX9 B1 and B2 lanes (Fig-

(A)

(B)
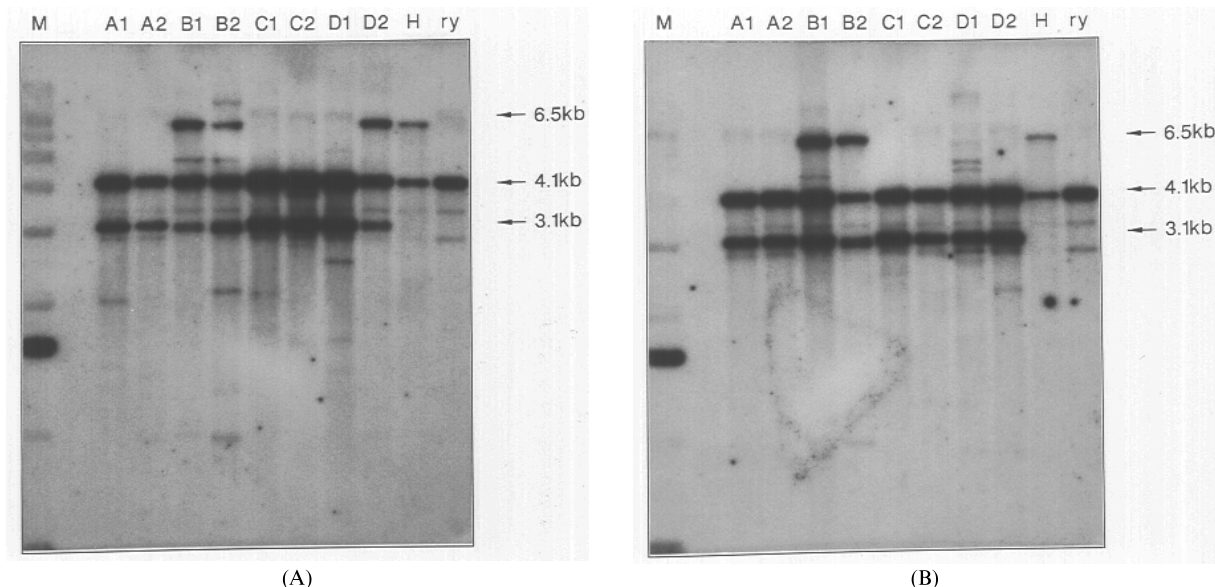
Figure 9. Integrity of *rosy* gene sequences in Pc[*ry*]B and P[*ry*]ΔSX9 populations at generation 35, in Experiment I. Genomic DNA from pools of 10 *ry*⁺ flies per population was digested with *Pst* I and hybridized with a *rosy* DNA specific probe (7.2 kb *Hin*d III fragment). (A) Pc[*ry*]B populations. (B) P[*ry*]ΔSX9 populations. Lanes: M, DNA size markers, 1 kb ladder; A1-D2, experimental populations A1, A2, B1, B2, C1, C2, D1, D2; H, Harwich *white* (*P* strain); ry, ry^{506} (M strain). Positions of bands of expected sizes, approximately 3.1 kb and 4.1 kb (*P-rosy* constructs) and 6.5 kb (genomic *rosy* locus), are indicated.

ure 9B). The most likely explanations for this anomalous result are either contamination with wild-type (ry^+) flies, or gene conversion of the mutant ry^{506} allele, using ry^+ sequences from the *P-rosy* constructs as template. To distinguish between these possibilities, additional Southern hybridization analyses were performed on the experimental populations. Using as probe a DNA restriction fragment that flanks the wild-type allele and is not present in the ry^{506} allele or in the constructs, we observed the presence of a band of the appropriate size in populations Pc[*ry*]B B1, B2, and D2, and also P[*ry*]ΔSX9 B1, B2, but not in any of the remaining populations (results not shown). Therefore, the most likely explanation of the presence of the 6.5 kb band in the five experimental populations of Figures 9A and B is contamination by ry^+ flies. Such contamination appears to have had little influence on the population dynamics of the constructs because these five populations do not appear to have differed significantly in any obvious respect from the common patterns of construct dynamics observed in all 16 experimental populations (e.g., Figures 3 and 4).

In situ *hybridization analysis*

Random samples of 3 to 10 third instar larvae from each of the 16 experimental populations were examined every fifth generation for copy number and genomic location of *P* and ry^+ sequences. Figure 10 presents a summary of the *in situ* hybridization results for the eight autonomous Pc[*ry*]B populations in Experiment I. This graph shows the number of *P* element hybridization sites, observed at five generation intervals, using the 1.5 kb *Hin*d III/*Sal* I fragment of the pπ25.1 plasmid as probe. It is evident that the copy number of *P* hybridizing elements in populations Pc[*ry*]B A1, A2, C1, and C2 is relatively low over all generations surveyed, varying between only one and two copies. In contrast, populations B1, B2, D1, and D2 show marked increases in copy number over time; those in B1 and B2 rose as high as 25 to 30 copies per genome by generation 40.

Figure 11 provides a histogram that compares the results of *in situ* analyses of the eight Pc[*ry*]B populations in Experiment I, at generation 40, using both *rosy* (7.2 kb *Hin*d III fragment) and *P* (complete pπ25.1 plasmid) sequences as probes. In all cases, the number of *P* hybridization sites is greater than the number of
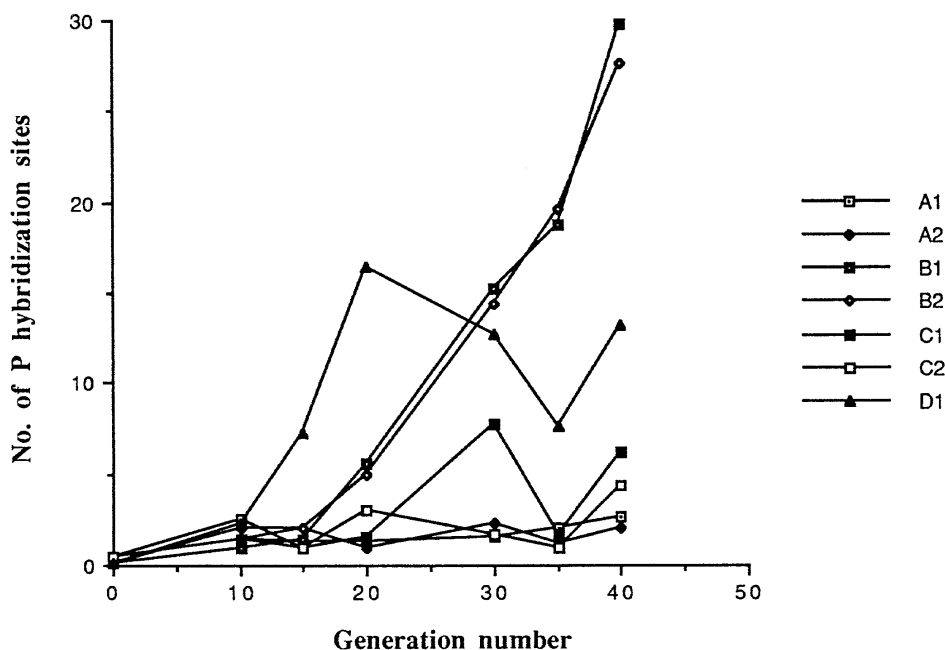
Figure 10. Summary of *in situ* hybridization results for the eight autonomous Pc[*ry*]B populations in Experiment I. Numbers of *in situ* hybridization sites observed at five generation intervals using the 1.5 kb *Hin*d III/*Sal* I fragment of the pπ25.1 plasmid as probe.
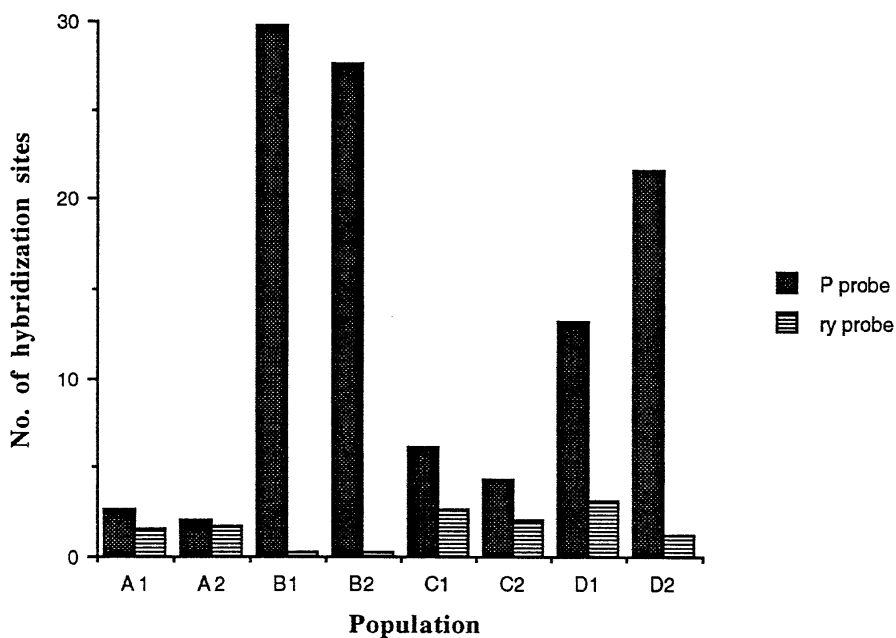

Figure 11. Histogram summarizing the *in situ* hybridization results for the eight autonomous Pc[*ry*]B populations at generation 40, Experiment I. Numbers of *in situ* hybridization sites were estimated using both *P* element (complete pπ25.1 plasmid) and *rosy* gene (7.2 kb *Hin*d III *rosy* DNA fragment) probes.
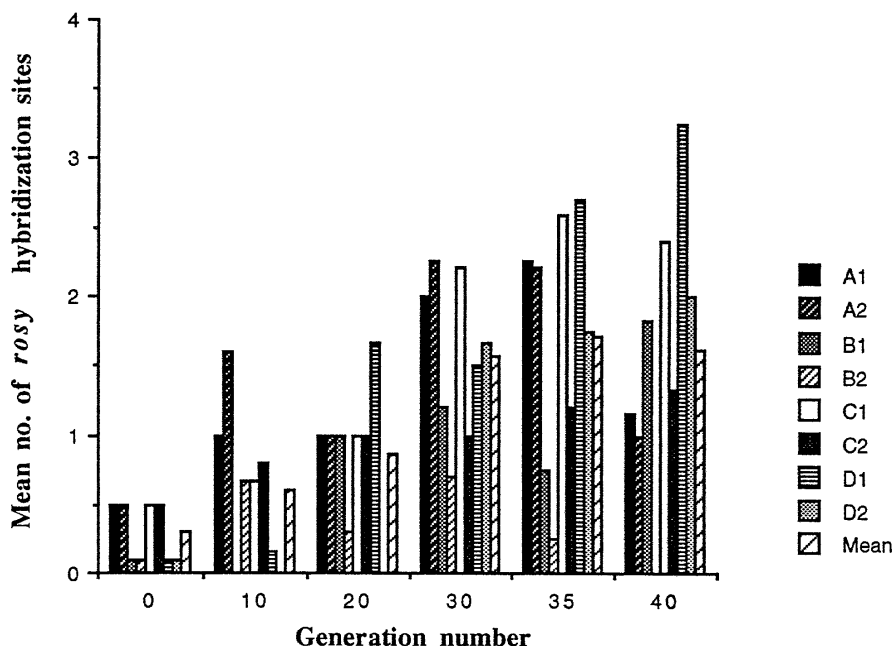
Figure 12. Summary histogram of *in situ* hybridization results for the eight nonautonomous P[*ry*]ΔSX9 populations in Experiment I. The numbers of *in situ* hybridization sites observed at five generation intervals were estimated using a *rosy* gene probe (7.2 kb *Hin*d III *rosy* DNA fragment).

rosy hybridization sites. This difference is very large for populations Pc[*ry*]B B1, B2, D1, and D2, strongly suggesting extensive breakdown of the constructs associated with a high transposition rate. The number of sites hybridizing to *rosy* sequences is at most four in all populations that were examined. Populations A1, A2, C1, and C2, carrying autonomous constructs initially inserted at location 3L (61F), appear to have low copy number and high integrity. In contrast, populations Pc[*ry*]B B1, B2, D1, and D2, carrying autonomous constructs initially inserted at location 2R (48E), have high *P* copy number and low construct integrity. Overall the *in situ* hybridization data confirm the pattern of construct breakdown correlated with copy number increase that was observed earlier. In turn, this appears to be closely related to the identity of the original transformed populations.

Figure 12 provides a summary of the *in situ* hybridization results of the eight nonautonomous P[*ry*]ΔSX9 populations in Experiment I, at five generation intervals, using the 7.2 kb *Hin*d III *rosy* fragment as a probe. This gives a much better estimate of construct copy number than the number of *P* hybridizing sites, which are not informative on this point because of the presence of native *P* elements introduced when

the populations were started. Overall, the histogram in Figure 12 shows a very modest increase in mean *rosy* copy number over time for the P[*ry*]ΔSX9 populations. Construct copy number at generation 40 varies between one and three per genome, with one exception: based on a sample of five larvae, line B2 appears to have lost all its copies. The mean *rosy* copy number of 1.62 is remarkably close to that of 1.72 for the Pc[*ry*]B lines at the same generation 40.

In summary, the *in situ* hybridization analyses suggest that for the single autonomous *P* element populations B1, B2, D1, and D2, a high transposition rate was accompanied by extensive construct break down, resulting in a large increase in number of *P* element sequences, but no parallel large increase of *rosy* sequences. In the remaining autonomous populations, and in all nonautonomous populations, transposition frequency was apparently lower, resulting in lower *P* sequence copy number. In these populations, the integrity of the *rosy* gene in the constructs was largely maintained. Despite the large differences in *P* copy number, the mean genomic copy number of *rosy* sequences showed little variation between these two classes.
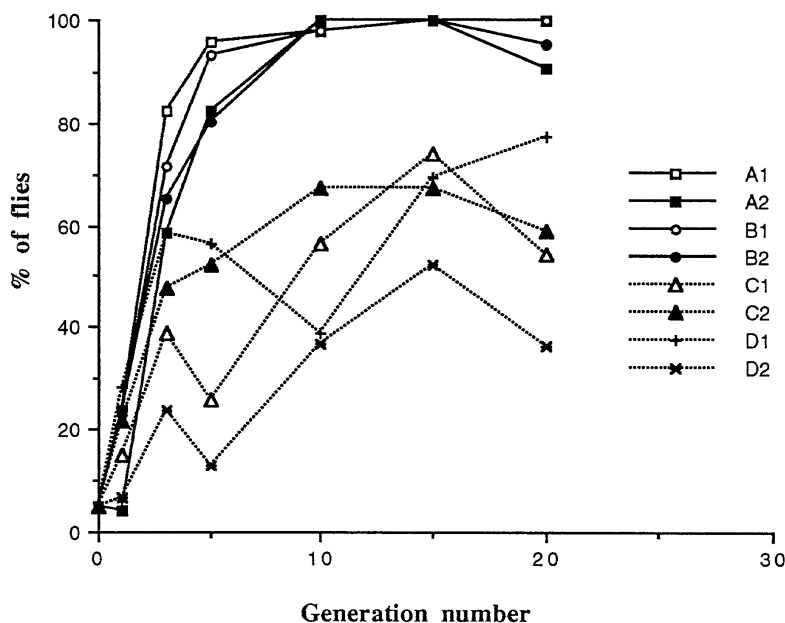
Figure 13. Frequency (determined by PCR analyses) of flies carrying *P* element sequences in the eight Pc[*ry*]B1–8CS populations, sampled every five generations over 20 generations.

Experiment III

The results of Experiments I and II indicate that *P* transposable elements, loaded with a wild-type copy of the *rosy* allele, spread rapidly in all populations examined. Because positive selection on the ry^+ marker is a likely explanation for at least part of this rapid spread, another set of experiments was designed in which ry^- was replaced by ry^+ at the genomic *rosy* locus of the recipient strain. The ry^+ marker gene, carried on the *P-rosy* construct, should therefore be selectively neutral following introduction into recipient populations of a homozygous ry^+ strain. In these experiments, we again used the transformed line B1-8 carrying the autonomous construct that was originally inserted into genomic location 3L (61F).

Four generations were necessary to introduce the third chromosomes carrying the *P* element-*rosy* gene construct Pc[*ry*]B into a ry^+ Canton-S background. A single B1-8 transformant male was crossed to ry^{506} virgin females, and ry^+ males were selected and crossed to a *ru*P/TM6 balancer stock (Materials and methods). *ru*P G2 males were then selected and crossed to Canton-S females. Progeny that were ry^+, but not *ru*P, were chosen to establish a number of individual lines. From each line, non-virgin mating pairs selected from G4 were used to establish four Pc[*ry*]B1-8CS bottle populations, each twice replicated. Ten B1-8CS non-virgin females were place in bottles with 190 Canton-S non-virgin females for five days for laying eggs; thus, these populations were started with 5% of premated transformants. During the first, third, and fifth generations and every fifth generation thereafter, a sample of 100 flies was taken from each population to evaluate the frequencies of the *P* element construct, using both single-fly PCR (Gloor & Engels, 1992) with *P* (5'-CCCCACGGACATGCTAAGGG, #2749R) and *rosy* gene-specific (5'-CATTCGAATTGCTTGTCAGG, #6530) oligonucleotide primers, and *in situ* hybridization methods as described above. These latter primers hybridize to sequences that flank the 5' junction of the *P* element sequence and the *rosy* gene fragment in the Pc[*ry*]B construct in these lines, producing an approximately 700 bp PCR product when this region is intact.

The results of experiments using the *P* element primers again indicate a rapid increase in the frequency of flies carrying *P* element sequences in all eight populations during the first 5 to 10 generations (Figure 13). The results were similar, overall, to the dramatic increase observed in Experiment I. However, assays for the presence and frequency of *rosy* gene sequences, using *rosy* specific primers, indicated an initial increase of these sequences in most lines, often followed by a
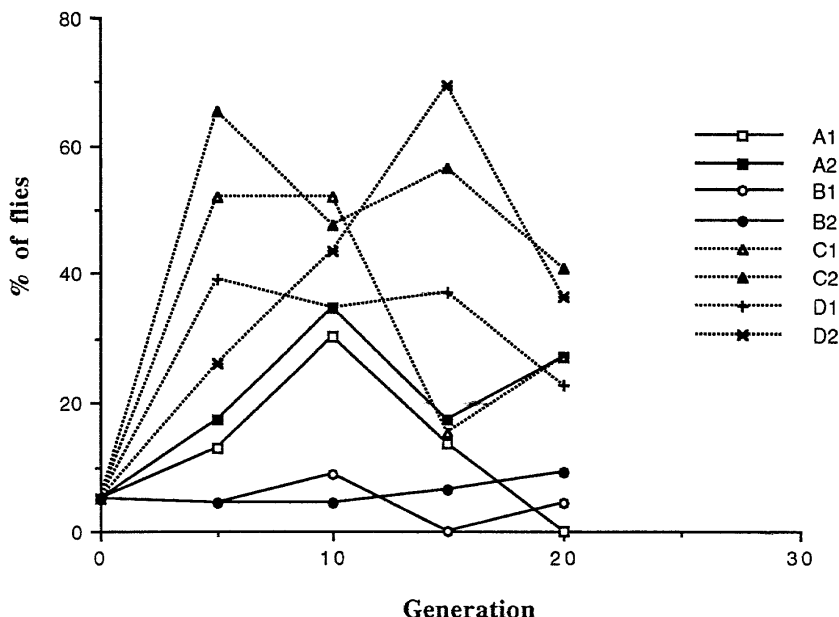
Figure 14. Frequency (determined by PCR analyses) of flies carrying *P–rosy*$^+$ sequences in the eight Pc[*ry*]B1–8CS populations, sampled every five generations over 20 generations.

subsequent leveling off and later decline (Figure 14). The similarity of results of replicate lines (e.g., A1 compared to A2) suggests that there was a significant effect of the chromosomal insertion site on subsequent transposition frequency and construct stability within each line. Four of the lines showed high levels of transposition, accompanied by high construct instability and loss of the *rosy* marker in the construct. The remaining four lines had modest levels of transposition accompanied by markedly higher stability and maintenance of *ry*$^+$ sequences in the construct. Figure 15 provides a comparison of the mean frequencies of flies carrying *P* and *P-rosy* junction sequences in Experiment III. These frequencies are also compared with the mean frequencies of *ry*$^+$ flies in Experiment I for the four sublines that originated from the same transformed line B1-8 (having the autonomous construct originally inserted into genomic location 3L (61F)). In the latter experiment, the overall average frequency of flies with a *rosy*$^+$ phenotype is given for samples from the first 20 generations.

The lines that have the highest frequency of *rosy* are also the ones with the slowest increase in the frequency of flies with the *P* construct. In those lines in which *P* became rapidly fixed (A1, A2, B1, and B2), the frequency of *ry*$^+$ was never very high. This result is consistent with a high incidence of construct break-

down that separates the *ry*$^+$ marker from the *P* element driver. The level of breakdown appears to be correlated with the frequency of transposition, as in Experiment 1. This finding is also consistent with our knowledge of *P* element biology (e.g., Gloor et al., 1991).

Discussion

The results of Experiments I and II indicate that *P* element constructs carrying a functional *rosy*$^+$ allele spread rapidly through almost all the *D. melanogaster* populations tested. The rate and pattern of spread was unexpectedly uniform in the initial generations, regardless of whether or not the construct itself carried an active *P* element (one- versus two-element schemes), or whether or not a source of functional transposase was present in the population (experimental versus control populations). In fact, a similar increase of the *ry*$^+$ transposon was also observed in control populations that carried the *P-rosy* construct, but lacked a source of transposase, suggesting that selection on the *ry*$^+$ allele was the cause of the rapid increase in frequency of this allele. However, when selection on the *ry*$^+$ allele was removed in Experiment III, frequencies of *P*-hybridizing sequences reached moderate to high levels in all eight lines examined. Further, pre-
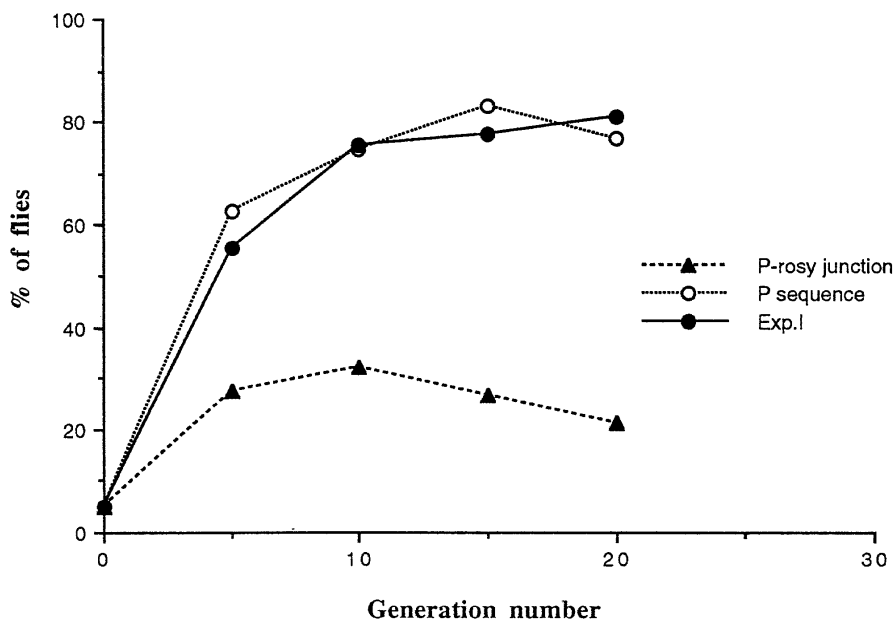
Figure 15. Comparison of the mean frequencies of flies from Pc[*ry*]B1–8CS populations carrying *P* and *P–rosy*[+] sequences, and flies from Pc[*ry*]B1–8 populations with *rosy*[+] phenotype (data from Experiment I).

liminary results from another control experiment (C. Babcock, unpublished results) indicated that, in the absence of any *P* element sequences, the *ry*[+] genomic allele from the Canton S stock did not reach fixation after being introduced by backcrossing into the genetic background of the *ry*[506] strain used in these experiments. On the contrary, in four replicate backcross lines, the *ry*[+] allele appeared to reach an equilibrium frequency of about 70% in combination with the *ry*[506] allele. Overall, these observations suggest that positive selection was acting on the *P-rosy* construct in a *ry*[−] background, but that selection alone cannot account for the increase in frequency of the *P-rosy* construct to near fixation values.

Under the conditions of Experiments I and II, it was not possible to distinguish between the results from populations having initial seed frequencies of 5% versus those with 10% construct-bearing flies. However, when introductions of only 1% construct-bearing flies were attempted in Experiment II, the constructs were quickly lost in several populations. This suggests that a minimum seed frequency higher than 1% is required to provide a high probability of construct spread. Comparison of bottle populations (initiated with 100 individuals) versus cage populations (initiated with 400 individuals) also indicated no detectable differences. Therefore, population size, within the range tested,

appeared to have very little effect on the outcome of these experiments. This conclusion is consistent with the computer simulation results of Ribeiro and Kidwell (1994), which suggested that differences in initial release frequencies of transposable elements were only critical in determining fixation rates when they were in the range of 10% or less in small populations and occurred over only a fairly narrow range of element reproductive rates.

The use of two different methods, Southern hybridization and *in situ* hybridization to polytene chromosomes, provided fairly consistent results concerning the copy number and integrity of the constructs in Experiment I. The 16 tested populations can be divided into two groups on the basis of results of molecular and cytological assays. The first group consists of all four populations seeded with the autonomous construct inserted in chromosomal location 3L (61F) and all eight populations seeded with the nonautonomous construct (see Table 1). In these 12 populations, transposition was modest and construct copy number rose slowly, never exceeding a total of about three or four copies per line up to 40 generations. In these populations, with only a few exceptions, there was little construct breakdown and *P* element integrity was apparently maintained (e.g., Figure 7A). The second group consists of all four populations initiated with the

autonomous construct inserted in chromosomal location 2R (48E) (see Table 1). In contrast to the first class, these four populations showed early indications of high transposition frequencies and corresponding high rates of *P* sequence breakdown (Figure 8A). This was confirmed by *in situ* hybridization studies that showed that the number of *P* element hybridization sites rose as high as 30 copies in some of these populations. However, the number of *rosy* hybridization sites did not rise correspondingly, but remained at a modest level, similarly to the populations carrying more stable constructs (see Figure 12). Apparently in these populations, high transposition rate is coupled with or accompanied by the internal deletion of constructs and the loss of *rosy* sequences. However, it is not known whether bursts of transposition occurred only after the *rosy* sequences were lost or whether the *rosy* sequences were lost in the process of frequent transposition. The former scenario seems most plausible because smaller *P* elements are expected to transpose at a faster rate than when present in a larger construct (Spradling, 1986). There may also have been selection acting to prevent the increase in copy number of functional ry^+ sequences.

The coincidence of increased transposition rates and initial insertion site of the construct at 2R (48E) in Experiment I suggests very strongly that site of insertion may have been a very important factor in determining subsequent behavior. However, because we only tested a very limited number of insertion sites, further data are needed to determine whether nonautonomous elements might display this instability as well. Another possible explanation for the low frequency of transposition in the populations seeded with nonautonomous constructs is the presence of P cytotype due to *P* element deletion derivatives that were almost certainly co-introduced with autonomous elements during the construction of the ry^{506}-dil*P* strain. These deletion derivatives may have acted as repressors of transposition.

The range of copy numbers observed in the present study appears to be similar to those observed in two earlier studies. Scavarda and Hartl (1984) examined a line of *Drosophila simulans* that had been co-transformed with a *P* element-*rosy* gene transposon in addition to autonomous *P* elements. After 40 generations, the number of *P* elements per individual was observed to have increased to 8-12 and the number of *rosy* transposons to the level of 4-12. There was also molecular evidence for deletions and rearrangements. More recently, four experimental populations of *D. melanogaster* were monitored into which a *P* element-

Adh gene construct had been introduced (Meister & Grigliatti, 1994). These authors reported that, despite an approximately three-fold increase in size compared to the unloaded *P* element, these constructs were capable of 'rapid dispersal to other individuals in the population'. In addition, they showed that many copies of the inserted *Adh* gene continued to produce an active gene product during the period monitored.

There is an increasing need to develop generalized transformation vectors that can be used in insects other than *Drosophila* for many purposes, including methods of biological control. Class II mobile elements (Finnegan, 1992) appear to offer better prospects than Class I elements for this purpose (O'Brochta & Atkinson, 1996), because their rates of transposition tend to be higher than those of Class I elements and they are not subject to the technical difficulties associated with Class I elements (Kaiser, Sentry & Finnegan, 1995). The early promise of *P* elements was not realised because of their narrow host range (Clark, Maddison & Kidwell, 1994). In contrast, the prospect of using members of the Class II *mariner-Tc1* family as transformation vectors is attractive because these elements are taxonomically widespread (Robertson, 1993). However, although there has been success in transformation of the *D. melanogaster* genome with loaded *mariner* elements, they appear to have low mobility following insertion (Lohe & Hartl, 1996). One likely possibility is that these elements are at the small end of the range in TE length and may not be able to remain mobile when loaded with DNA sequences large enough to encode a gene. The *D. hydei* element *Minos* also belongs to the *mariner-Tc1* family and appears to hold high promise as an alternative for existing systems for transposon tagging and enhancer trapping, not only in *Drosophila* but also in other insects including the Mediterranean fruit fly, *Ceratis capitata*, a major pest on fruit crops (Loukeris et al., 1995). In addition, the *hobo* element from *D. melanogaster* (O'Brochta et al., 1994), the *Hermes* element from the house fly, *Musca domestica* (Warren, Atkinson & O'Brochta, 1994) and *piggy-Bac/IFP2*, a member of the TTAA-specific family of elements isolated from the Lepidopteran species *Trichoplusia ni* (Fraser et al., 1995) have all recently been shown to have potential as insect transformation vectors (O'Brochta & Atkinson, 1996).

Despite the difficulties in separating out the effects of selection on the ry^+ allele from the effects of drive due to construct transposition, the results of this series of experiments do provide valuable information regarding the future potential use of transposable elements

for biological control of insects. We can now begin to answer some of the questions posed in the introduction to this paper: (1) The results of Experiment III, in which selection on the marker allele was absent, suggest that the frequency of a gene can be increased significantly by transposition above a low initial level in a time period as short as five to 10 generations. Because of dominance of the ry^+ phenotype, the copy number increases observed in Experiment 1 can also be ascribed to transposition rather than selection. (2) The question about stability of introduced genes over time turns out to be a critical one. On the basis of the negative correlation between transposition rate and construct stability, which was a prominent feature of the present results, it appears that high transposition frequencies, with their high attendant levels of instability, are to be avoided. In this regard, the possible co-introduction of repressors of transposition, along with the original constructs may reduce transposition and increase stability. This was possibly accomplished in the present experiments by introduction of the nonautonomous constructs into a dilute P strain. (3) From the present results, no comparisons of different gene sizes or different transposon structures were made, but there did appear to be strong evidence for the importance of genomic insertion location. Consistently different results were obtained from the sublines in which the Pc[ry]B autonomous element was inserted at the two different genomic locations 3L (61F) and 2R (48E). Populations with inserts in 3L (61F) had low copy number and high integrity, in contrast to populations with inserts at 2R (48E), which had high P copy number and low construct integrity. (4) No effects of population size or population structure (continuous versus discrete generations) were observed in these experiments, but only two population sizes were examined.

Many questions remain to be answered. The present results, although not conclusive, do appear to warrant further experimentation. To this end, an autonomous P element construct has been engineered with a multiple cloning site (MCS) in the noncoding 3′ region (Prokchorova et al., 1995) allowing the easy insertion of alternative markers. One such marker that is being tested in current ongoing experiments is the *opd* gene sequence (Benedict, Salazar & Collins, 1995) under the control of the heat shock promoter *hsp 70*. The frequency of the construct will be monitored following heat shock but, during routine maintenance in the absence of heat shock, the marker is not expected to be subject to direct selection. In the future, the behavior of other elements such as *hobo* as genetic drivers is planned.

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