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The fitness consequences of *P* element insertion in *Drosophila melanogaster*

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Summary

In this study we estimate the frequency at which *P*-element insertion events, as identified by *in situ* hybridization, generate lethal and mild viability mutations. The frequency of lethal mutations generated per insertion event was 0·004. Viability dropped an average of 1% per insertion event. Our results indicate that it is deletions and rearrangements resulting from the mobilization of *P* elements already in place and not the insertions *per se* that cause the drastic effects on viability and fitness observed in most studies of P–M dysgenesis-derived mutations. Elements of five other families (*I, copia, 412, B104,* and *gypsy*) were not mobilized in these crosses. Finally, we contrast the density of *P* elements on the *X* chromosome with the density on the four autosomal arms in a collection of thirty genomes from an African population. The relative number of *P* elements on the *X* chromosome is too high to be explained by either a hemizygous selection or a neutrality model. The possible reasons for the failure to detect selection are discussed.

1. Introduction

The material nature of mutation has long been an issue in evolutionary biology (see Provine, 1971). The demonstration that DNA is the heritable material, the deciphering of the genetic code, and our increased understanding of the mechanisms of gene expression have led to expectations of the types of molecular change that would constitute mutation. However, the profile and distribution of mutational types that appear in natural populations, and their phenotypic effects, remain unclear. Furthermore, recent observations in molecular genetics, such as the rediscovery of transposable elements, challenge us to re-examine our views of mutation.

For many years *Drosophila* geneticists had observed occasional bursts of mutation, lines with so-called mutator activity (Demerec, 1937; Neel, 1942), as well as unique alleles of recessive mutations which were dramatically unstable, reverting to wild-type phenotypes and mutant derivatives at high frequencies (Bowman, 1965; Green, 1967). These observations were reminiscent of the 'controlling' elements described earlier in maize (McClintock, 1956*a*, *b*). It was through early attempts to clone genes in *Drosophila* that the first molecular evidence for the presence of mobile genetic elements or transposons appeared (Strobel, Dunsmuir & Rubin, 1979; Young, 1979). It is now clear that these elements constitute a significant component of the genome in *Drosophila*.

While much is being described at the molecular level (see Shapiro, 1983), the relative contribution of transposable elements to the genetic variation in fitness in natural populations is not known. There is increasing evidence from the detailed examination of mutant alleles at cloned loci that a significant proportion of classical Mendelian mutations in *Drosophila* is the result of transposon insertion (Kidd, Lockett & Young, 1983; Zachar & Bingham, 1982; Bender *et al.* 1983; Scott *et al.* 1983; Coté *et al.* 1986). However, the contribution of element transposition to the detrimental load or to polygenic variability in general is unknown. Many of the unique features associated with transposons, such as their induced movement under hybridization (as in the P–M and I–R systems), both the presence and absence of site specificity for insertion, as well as their potential ability to disrupt gene function from a distance, make them targets of continuing speculation. For instance, it has been proposed that increases in the deleterious load over a number of years in some populations of

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Drosophila melanogaster coincide with the invasion of the *P* element (Mukai *et al.* 1985; Yukuhiro & Mukai, 1986; Choo & Lee, 1986).

There are two ways that fitness mutations may be examined. Effects could be detected directly via the accumulation of transposition events on a replicated stem chromosome, as used by Mukai (1964) to measure polygenic mutation rates. Using this approach recent studies have attempted to characterize the mutational effects on either viability or total fitness that arise as the consequence of hybrid dysgenesis (Mukai & Yukuhiro, 1983; Yukuhiro, Harada & Mukai, 1985; Fitzpatrick & Sved, 1986; Mackay, 1986). These studies have not actually characterized the extent of *P*-element insertion on the mutagenized chromosomes. Alternatively, the impact on total fitness may be measured indirectly by contrasting observed element distributions with null distributions expected under competing theoretical population genetic models, such as those presented by Charlesworth & Charlesworth (1983) and Montgomery, Charlesworth & Langley (1987).

In this report we estimate the average effect on male hemizygous viability of single *P*-element insertions which were generated *de novo* by transposition into the *X* chromosome of *Drosophila melanogaster*. We demonstrate that these insertions represent a type of mutational event like that measured by Mukai (1964) as polygenic viability mutation, and have designed the experiment with an assumed average effect of about three percent drop in viability per insertion. In contrast to the studies mentioned above, we follow the number of *P* elements acquired on the test chromosomes by *in situ* hybridization. To detect the possibility that we may be inducing co-transposition (so-called 'bursts') of other elements as claimed by Gerasimova, Mizrokhi & Georgiev (1984), we screen a subset of these chromosomes for the movement of five other elements, *copia*, *I*, *gypsy*, *B104*, and *412*. Finally, we indirectly examine the fitness consequences of transposition by contrasting the *P* element densities on the *X* chromosome with those observed for the autosomal arms sampled from thirty haploid genomes recovered from a natural population, and examine these under the model of detrimental selection-transposition balance proposed by Montgomery, Charlesworth & Langley (1987).

2. Methods and materials

(i) *Strains and wild genomes*

The stem chromosome used in these studies contains no *P* elements and is marked with the *X*-linked visibles z^a w^{ch}. The stock carrying this chromosome has an M cytotype, and also contains the *FM6* balancer chromosome. This stock was made isochromosomal for a single z^a w^{ch} *X* chromosome one generation prior to the start of the experiment. We used the π_2 line as a P strain (Engels, 1979). Two P cytotype strains, *FM6*/N^{264-84} and *C(1)DX y f*, were used in the genetic manipulation of chromosomes carrying *de novo* insertions. These lines originally possessed M cytotype but were backcrossed for five generations into the π_2 line to convert their cytotypes to P. The lines were tested for gonadal sterility to confirm the acquisition of P cytotype. The thirty isofemale lines collected in 1985 in Botswana, Africa were provided by Dr Martin Kreitman.

(ii) *Viability estimation*

The general design for examining the effects on viability of *P* element insertions is illustrated in Fig. 1. This involved passing copies of an *X* chromosome (from z^a w^{ch}) from an M-strain through a P–M dysgenic cross, where they are expected to acquire varying numbers of *P* elements via transposition. The resulting dysgenesis is arrested in the next generation by crossing the male bearers of this chromosome with P cytotype females, thereby avoiding future P–M dysgenesis. Each *X* chromosome was derived from a different dysgenic male in the first generation. This avoids the repeated sampling of identical insertions recovered as premeiotic germ line events (Simmons & Lim, 1980; Engels & Preston, 1984), and thereby ensures independence of observations.

We first recovered a large number of chromosomes to estimate the lethal mutation rate. The relative viability of a random sample of non-lethal chromosomes was then estimated. Finally, a subset of these chromosomes was examined to determine the number of *P* elements acquired. A total of 592 chromosomes reached the third generation where they were sheltered against the *FM6* balancer chromosome. The absence of z^a w^{ch} males in the third generation indicated the acquisition of a lethal mutation. A sample of 114 non-lethal chromosomes (all derived from the same female) was selected for viability estimation. In the third generation, male third instar larvae from 49 of the 114 chromosomes were screened for *P*-element insertions by *in situ* hybridization to their polytene chromosomes. All *X* chromosomes with acquired lethals were crossed with z^a w^{ch} males from the original M strain and the female larvae screened for *P*-element insertions.

Hemizygous male viability was measured by crossing males from each line against females from a *C(1)DX* line and counting the number of male z^a w^{ch} and *C(1)DX* females emerging in the next generation, with a Mendelian expectation of 1:1. For each chromosome line, 25 males were mass crossed with 30 *C(1)DX* females and allowed to mate for 24 h. Each mass cross was subsequently subdivided into three replicate sets of 5 males and 10 females each. Each replicate cross was placed in an eight-dram vial with food. On days 4 and 8, the adults were transferred to fresh media in a new vial. All adults were discarded on day 12. Thus for each line there are three replicate

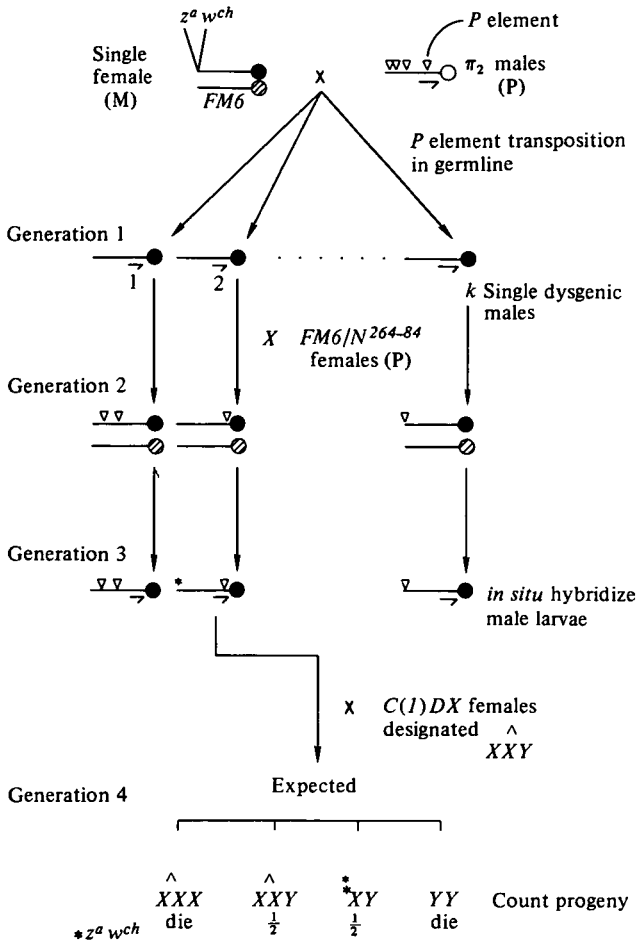

Fig. 1. Experimental design used to generate *X* chromosomes with *de novo P*-element insertions.

crosses, each with three transfers. Emerging adult progeny were counted for each vial on days 12, 16, and 20, and the vials were then discarded. For a given vial, the viability index was computed as the ratio of the number of males to females. We assume segregation distortion with the *Y* chromosome is not a significant contributor to the viability measure, and that other sources of variation, such as the viability variation of *C(1)DX* females, are independent of the number of *P* elements on the *X* chromosomes in males. There was no statistically significant covariance between the average number of *C(1)DX* females emerging in a test cross and the number of *P*-element insertions on the test chromosome in that cross.

Using pilot data on the variances associated with estimating viability using the ratio of marked males to *C(1)DX* females, and earlier data on the number of elements acquired by $z^a w^{ch}$ chromosomes (J. W. Ajioka & W. F. Eanes, unpublished data), we designed our experiments with sufficient statistical power to detect average viability effects as large as those found by Mukai (1964). Mukai estimated the average drop in viability due to a polygenic mutation as 2·7% or less. Accepting a Type 1 error of $\alpha < 0{\cdot}05$, we expect to detect an effect of this magnitude with probability greater than 99% using 50 chromosomes in our design.

(iii) *Chromosome preparation and* in situ *hybridization*

The salivary gland chromosomes were prepared as described in Pardue & Gall (1975), with several exceptions. *In situ* hybridizations were carried out using biotinylated probes (Langer, Waldrop & Ward, 1981), and sites of hybridization were identified with a streptavidin-peroxidase complex, as described by Montgomery, Charlesworth & Langley (1987). The following elements and probes were used: *P* element, pπ 25·1 (O'Hare & Rubin, 1983); *gypsy*, sc^{3B} 19a (Campuzano *et al.* 1985); *I*, p1407 (Bucheton *et al.* 1984); *412*, cDm 2042; *copia* (cloned from w^a by Z. Zachar); and *B104* (cloned from the *white* locus by Z. Zachar). Nick translations were carried out on DNAs using the BRL biotin-11-dUTP from Bethesda Research Laboratories. Approximately, 30–50 ng of probe was applied to each slide in 20–30 μl, and hybridizations were carried out overnight at 37 °C.

(iv) *Scoring of element counts*

Single polytene squashes were made of most lines. Counts for lines in the male viability analysis were made on male larvae. Homology of the pπ 25·1 probe to its genomic origin precluded recognition of insertions at chromosome site 17C. Analysis of copy numbers in wild African genomes was made in female larvae heterozygous for a single wild genome and the genome from the $z^a w^{ch}$ stock. Since this stock contains no *P* elements, we are effectively examining only the wild genome. Examining females removes the potential bias resulting from contrasting element counts in males where the density of Giemsa staining of polytene DNA differed between the *X* chromosome and autosomes.

3. Results

(i) *Viability effects of* P *element insertion*

The analysis of variance for relative viability between chromosome lines is provided in Table 1. We partitioned the total variance in the relative viability for individual vials ($n = 1011$) into its environmental, replicate chromosome cross, and individual chromosome components. The genetic variance among the 114 chromosome lines is 0·0046, and is highly statistically significant ($P < 0{\cdot}0001$). This represents about 8% of the total variance among vials. The replicate chromosome cross component is not statistically significant. Clearly, individual chromosomes have acquired *de novo* mutations. In the initial cross shown in Fig. 1, the dysgenic males recovered were all derived from a single $FM6/z^a w^{ch}$ female, thereby making all $z^a w^{ch}$ chromosomes in the generations to

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Table 1. *Analysis of variance (ANOVA) for relative viability among 114 dysgenically derived* X *chromosomes*

Source	D.F.	SS	MS	*F*
Chromosomes	114	11·465	0·101	1·92****
Replicate crosses	223	13·449	0·060	1·14
Error	674	35·674	0·052	—

**** $P < 0{\cdot}0001$.

follow identical-by-descent relative to that generation. The input of spontaneous polygenic mutations is expected to contribute to the genetic variance among chromosomes in the first and second generations, and to the replicate variance in generations three and four. Since the chromosomes were sheltered over the *FM6* balancer, there is no bias in the selection of chromosomes for viability measurement.

In our study, the average number of elements acquired per *X* chromosome in the third generation was 3·26 with variance 8·52 for $n = 49$ chromosomes. The fact that the variance is substantially larger than the mean suggests that there is heterogeneity in the degree of dysgenesis in different germ lines. It is also possible that some residual dysgenesis persisted following back crossing into the *FM6* balancer line in the second generation. This is consistent with the fact that we found about three times the overall rate of transposition observed in other studies (Engels, 1983). However, the inheritance of cytotype is complex (Engels, 1983), and it is likely that lines were segregating differentially for factors determining cytotype in subsequent generations. We also examined over 100 chromosomes cytologically, and no rearrangements were seen. The absence of rearrangements, which are commonly associated with the dysgenic mobilization of elements in place (Berg, Engels & Kreber, 1980), suggests that little dysgenesis continued into the second and third generations.

Out of a total of 592 chromosomes recovered, nine acquired at least one lethal mutation for a rate of 9/592 = 0·0152 recessive lethal chromosomes per generation. Using the average number of elements acquired per chromosome, and adjusting for the background spontaneous lethal rate (Simmons & Crow, 1977), we estimate that the lethal rate per *P*-element insertion is 0·004.

The average effect of a *P*-element insertion can be estimated by the regression of relative viability on the number of elements acquired for the 49 chromosomes where *in situ* hybridizations are available. This is shown in Fig. 2. The linear regression is estimated as $Y = 1{\cdot}302 - 0{\cdot}014X$, where Y is the relative viability and X the number of elements found. The slope of $-0{\cdot}014$ ($\pm 0{\cdot}006$ S.E.) is statistically significant ($t_s = -2{\cdot}39$; $P < 0{\cdot}02$), and explains eleven percent of the

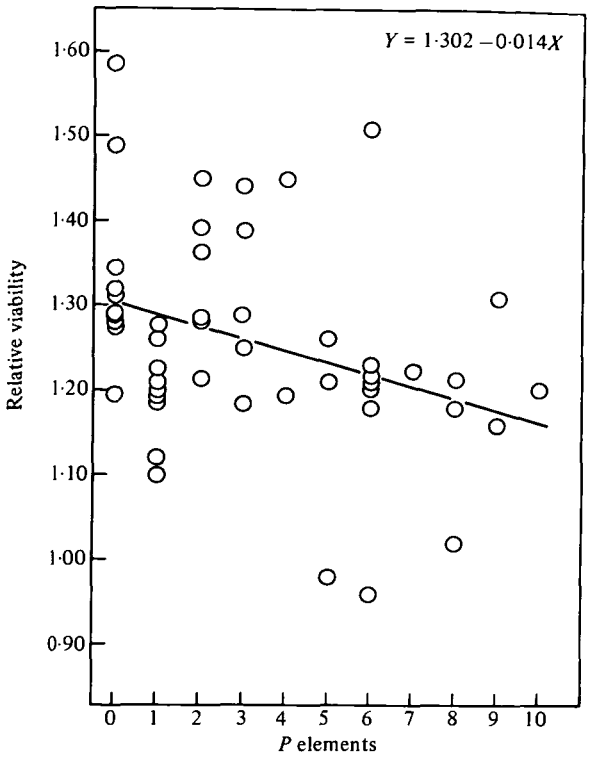

Fig. 2. The relationship between relative hemizygous viability and number of *de novo* *P*-element insertions for 49 independent *X* chromosomes. The regression is statistically significant ($P < 0{\cdot}02$).

variance in chromosome means. If we divide all the raw data by the mean for the zero insertion class, this slope translates to a drop of about one percent in relative viability per *P*-element insertion. There is no statistically significant variation in viability among chromosomes in the zero class.

There are two potential sources of error in estimating absolute *P* element numbers across chromosome lines, which our results share with other studies such as the widely cited estimate of Bingham, Kidwell & Rubin (1982). The first is that we may have failed to detect all insertions. *P* elements are known to exist as partially deleted copies (O'Hare & Rubin, 1983), which may be too small for the *in situ* procedure to detect. However, we have been able to detect partially deleted copies as small as 500 bp, as revertants at the *G6pd* locus (unpublished observations). In addition, the pπ 25·1 probe contains 1·8 kb of homology to the *hdp* locus at 17C, and serves as a hybridization control. Slides were not used unless that site exhibited a strong signal with our biotinylated protocol. This error will have no effect on the regression in Fig. 2, because we expect this error to be random with respect to copy number classes. Failure to observe very small insertions will cause us to overestimate the probability that a single insertion will generate a lethal mutation.

The second potential source of error is residual dysgenesis-associated movement of elements in the germline of generation 2 females (see Fig. 1). Again,

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such movement would be random with respect to the viability of chromosomes and so have no effect on the regression in Fig. 2. However, residual movement would cause us to underestimate the probability that insertions cause lethal mutations. This is because chromosomes are scored as lethal on the basis of mutational events occurring in generation 1 only, while the element counts were made after a second generation of potential transposition. Our expectation is that transposition in generation 2 was taking place at a much lower rate than in generation 1.

(ii) *Co-transposition of other elements*

In this study we assume that there is no mobilization of other elements during P–M dysgenesis. Transposition of other elements is probably continuing at low levels. However, this transposition will only contribute to the estimate of average *P* element effects if it co-varies with *P* element mobilization. Otherwise, co-transposition simply adds to the background mutation rate, and therefore the unexplained genetic variance. To partly evaluate this assumption we compared the distribution of five other elements on the stem chromosome with that on ten of the chromosomes from the viability study. These chromosomes were selected to span the range of copy number observed for the *P* element. We observed the following sites for these five elements on the stem chromosome: *copia* (none), *gypsy* (1F, 3B, 4A), *B104* or 'roo' (1B, 2F, 3D, 4C, 7B, 9A, 9B, 10B, 12A, 12C, 12D, 12E, 14B, 17B), *1* (8B, 9A, 15F, 17C), and *412* (1F, 8C, 13C). None of the ten test chromosomes showed any gain or loss of any of these elements.

Very recently, studies by Yannopoulos *et al.* (1987) and Blackman *et al.* (1987) have implicated the *hobo* element in an additional type of hybrid dysgenesis, similar to, but independent of, the P–M system. We cannot rule out *hobo*-related dysgenesis in our crosses, so it could be a contributor to the increased genetic variance in viability. This would only tend to obscure the observed relationship between *P* insertions and viability.

(iii) *Distribution of* P *elements in wild genomes*

For the thirty haploid genomes examined from our African isofemale lines, the average genome carried 23 *P* elements. Table 2 presents the distribution of these elements across the *X* chromosome and the four autosomal arms. Once again, the failure to detect all *P* elements is a random source of error. We expect the distribution of the number of elements per arm to follow a binomial distribution, and if the average number of elements per arm is much less than the number of occupiable sites, then a Poisson distribution is also appropriate. In general the variances tend to be less than the mean for several arms, although none is statistically significantly different from the Poisson

Table 2. *The total number of* P *elements observed on the* X *chromosome and the four autosomal arms in 30 haploid genomes*

	X	2L	2R	3L	3R
Totals	153	138	156	100	143
Mean/Arm	5·10	4·60	5·20	3·33	4·76
Variance	2·56	3·28	3·20	3·61	4·53
	$\chi^2_{[0\cdot175]} = 10{\cdot}44^{***}$				
	$\chi^2_{[0\cdot115]} = 77{\cdot}24^{***}$				

*** [H_0 with 1 D.F.], $P < 0{\cdot}005$.

expectation. The tendency towards low variance in copy number should not be construed as evidence for stabilizing selection on copy number, but rather as evidence for heterogeneity in occupancy frequency across sites on that arm (see Charlesworth, 1985). The null hypothesis of equal numbers of elements on all arms is not rejected ($\chi = 14{\cdot}62$, n.s. with 5 D.F.). We may further test the counts against the competing hypotheses proposed by Montgomery, Charlesworth & Langley (1987). Both a general model of detrimental selection against *P* elements, which predicts 11·5% of all elements will reside on the *X* chromosome ($\chi^2 = 77{\cdot}24$, $P < 0{\cdot}005$), and the null model of no selection, which predicts 17·5% on the *X* chromosome ($\chi^2 = 110{\cdot}44$, $P < 0{\cdot}001$), are clearly rejected. Thus, it appears that the *X* chromosome has too many elements for either model.

4. Discussion

(i) *Expectations*

We expect *P* elements to contribute to mutation and to genetic variance in fitness through two different types of mutational events. The simplest is insertion *per se*. Any insertion has some probability of directly entering a region essential for gene expression, thereby altering function, with the ensuing fitness consequences depending on the nature of the gene. However, a second, and probably more important, mutational phenomenon occurs when *P* elements already in place undergo rearrangement and local deletion, usually with drastic fitness consequences. These events are known to occur at enormously elevated rates in dysgenic crosses (Engels, 1979; Berg, Engels & Kreber, 1980; Simmons & Lim, 1980; Simmons *et al.* 1984*a*, *b*). Existing elements become the foci of localized mutational lesions generating site (or locus) specific effects. Even an element inserted into a selectively benign site can, in turn, generate drastic mutations upon mobilization. Thus, the results of any study of *P*-element fitness effects will depend on the particular chromosome used. For instance in a study of a third chromosome with a significantly elevated lethal mutation rate, Kidwell, Kidwell & Nei

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(1973) noted an enormous level of lethal allelism, indicating site specific effects. No previous studies have attempted to separate the effects of the two kinds of mutations. In this study we have focused on the effects of insertion, by using a stem chromosome initially free of *P* elements, and monitoring the actual numbers of elements acquired by the chromosome under a single generation of defined dysgenesis.

(ii) *Expected background mutation*

The chromosomes recovered from our P–M dysgenic cross accumulated significant genetic variation for viability. Some of the variation between chromosomes was contributed by spontaneous mutation not associated with *P* elements. The expected rate of spontaneous mutation to lethal *X* chromosomes is 1 in 500 (Simmons & Crow, 1977). If we assume a background level of polygenic mutation of 0·07 per *X* chromosome accumulating over the first two generations, each with an average effect of $a \leqslant 0{\cdot}027$ as estimated by Mukai (1964) and Mukai *et al.* (1972), then we predict an accumulated genetic variance of $V_g \leqslant 4pqa^2 \leqslant 1{\cdot}74 \times 10^{-4}$ contributed by spontaneous mutation (Falconer, 1981). Our observed genetic variance of $4{\cdot}6 \times 10^{-3}$ reflects substantially larger direct mutational input by *P*-element insertion. From Mukai's data, we predict that each chromosome in this study would have acquired 0·2 mutations by spontaneous mutation, whereas the average chromosome acquired three *P* elements, as determined by our direct counts. The I–R dysgenic system and four other transposable elements do not seem to be contributing to this variance in a significant fashion.

Our estimate of the among-chromosome line variance component could be inflated due to our mass mating of males and females within a line prior to separation into replicate vials. Any common paternity among replicates could generate unwanted covariance between replicate vials. However, *D. melanogaster* females invariably remate within 24 h (Gromko, Gilbert & Richmond, 1984), and the duration of each of the three successive replicate transfers was 4 days. If common paternity were a signficant factor we would expect the between-replicate variance to increase with time. No such increase was detected.

(iii) *Average effects on viability*

Our experiment to measure the average effect of *de novo P*-element insertions on relative viability estimated the average reduction in viability to be approximately 1% per insertion. The lower 95% confidence limit for the slope is near zero, and the upper limit is about 2%. Assuming that element insertion contributes negligibly to the non-genetic variances, *P*-element insertion explains about one-third of the genetic variance in viability. Since we expect individual insertions to show a large variance in effect, from benign to lethal, these results are consistent with the hypothesis that most of the induced genetic variance in viability in our experiment is the consequence of *P*-element insertion. We also estimate that roughly one in 250 (0·004) insertions results in a lethal mutation.

We cannot dismiss *X*–*Y* segregation distortion associated with dysgenesis, or the presence of autosomal loci affecting sex ratio, as contributors to the viability variation seen between chromosome lines. Segregation distortion on the second chromosome has been associated with dysgenesis as male recombination (Hiraizumi, 1979). It has been a potential source of viability variation in most other studies. Autosomal loci affecting sex ratio, if segregating in these lines, should be distributed at random with respect to the regression in Fig. 2, contributing only to unexplained line variance.

On the supposition that some element insertions disrupt gene function, we expect element number and fitness (in this case viability) to be negatively related. In designing this experiment, we supposed that the average effect of a non-lethal *P*-element insertion might be comparable with that inferred by Mukai (1964; Mukai *et al.* 1972) in his studies of the accumulation of mutations affecting relative viability, where the upper limit on the average reduction in viability per mutation was 2·7%. In the event, our results are remarkably similar to Mukai's. The major difference between our studies is that we are recognizing mutational events as *P*-element insertions, while Mukai could only recognize events when they affected viability. If only a third of our *P*-element insertions had any effect on viability our estimate of the average effect of a mutation on viability becomes essentially identical to that of Mukai. Our measure of 0·004 lethals per insertion is not substantially different from the Simmons *et al.* (1985) estimate of 0·01, considering that their rate was based on an assumed insertion rate of 0·82 insertions per chromosome in dysgenic crosses, based on the study by Bingham, Kidwell & Rubin (1982) and unpublished data reported in Engels (1983).

The rate of induction of lethal mutations depends on parameters such as the density of vital loci and the randomness of *P*-element insertion. If elements insert effectively at random (there is a weak consensus target sequence (O'Hare & Rubin, 1983)) then the proportion of insertions which induce lethals will reflect the proportion of the genome which is vital. The total DNA content of the *D. melanogaster* genome is estimated at $1{\cdot}4 \times 10^8$ bp (Lewin, 1980). The *X* chromosome constitutes one fifth of the total genome and it is estimated that about two thirds is genetically active euchromatin. Therefore our estimate of the total euchromatic DNA on the *X* chromosome is $(0{\cdot}20)(0{\cdot}66)(1{\cdot}4 \times 10^8) = 1{\cdot}9 \times 10^7$ bp. Using our lethal rate, and assuming insertion is random, we estimate that $(0{\cdot}004)(1{\cdot}9 \times 10^7) = 7{\cdot}6 \times 10^4$ bp are lethal-

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mutable if disrupted by the insertion of a *P* element. The coding portion of an average sized gene, which codes for a protein of 40000 Da (Lewin, 1980), is approximately 1 kb. Assuming only insertions into the protein coding region are sufficient to disrupt gene expression, we estimate that there are only $(7{\cdot}6 \times 10^4)/1000 = 76$ vital loci on the *X* chromosome (186 if we use the Simmons *et al.* (1985) estimate). Assuming a larger vital region per locus susceptible to disruption reduces these estimates. Both of these estimates are substantially less than Lefevre & Watkins' (1986) estimate of about 614 lethal-mutable loci on the *X* chromosome. These results suggest that vital loci are not as susceptible to *P*-element insertion as would be expected if they were simply random targets. Perhaps potential *P*-element target sequences are poorly represented in coding regions.

(iv) *Comparisons with other studies*

The lethal rate associated with most P–M dysgenic crosses has been estimated for chromosomes bearing *P* elements. One study by Simmons *et al.* (1984*b*) gave estimates of 1·36 and 3·04% for the ν_6 and π_2 chromosomes respectively. We have passed a sample of 100 wild *X* chromosomes from Lincoln, Massachusetts through a single generation of P–M dysgenesis in males and examined the rate to newly acquired, independent lethals. The adjusted lethal rate was 16/625, or 2·6% (Eanes *et al.* unpublished results). We do not know the number of new insertions acquired by the *X* chromosomes in any of these studies, but if it is on the order of one per chromosome, this implies that most of the lethals are due to element mobilization and not insertion.

Several other studies have attempted to examine the impact of putative transposable element movement on fitness or its components. These studies are notably different from ours in design. Yukuhiro, Harada & Mukai (1985) examined the accumulation of lethals and milder viability mutations on a *P*-element-bearing stem chromosome that had been passed through a P–M dysgenic cross for nine generations. They estimated the recessive lethal rate per generation at 0·067, which is ten times the standard rate and twice the rate we observed under insertion for the $z^a\ w^{ch}$ *X* chromosome, once adjusted for chromosome size. The average effect per non-lethal mutation 'event' was estimated as 0·64, which is many times greater than our estimate under simple insertion. We feel these markedly different results clearly demonstrate the added mutational consequences associated with element mobilization, which accumulated over the nine successive dysgenic generations. Rearrangements, and especially deletions, are expected to produce drastic fitness reductions. Unfortunately the allelism of lethals or major detrimental mutations was not examined by Yukuhiro, Harada & Mukai (1985).

Two studies have examined the impact of P–M dysgenesis on the total fitness of second chromosomes. Fitzpatrick & Sved (1986) passed a Harwich-derived second chromosome through a single generation of P–M dysgenesis and examined the average reduction in total fitness using the population cage approach of Sved (1971). Because the Harwich chromosome carried *P* elements, mutational damage could arise from the joint effects of new insertions and localized effects of elements in place. The dysgenic chromosomes showed a 10–20% drop in total fitness relative to the non-dysgenic controls.

Mackay (1986) measured fitness of a set of Canton S second chromosomes, which lack *P* elements, after they were passed through a single generation of dysgenesis. She reported no differences in relative fitness between chromosomes run through a dysgenic cross *vs.* a non-dysgenic cross with a *P* strain, which is consistent with the small viability effects we observed due to direct insertion. Yet, when she compared these chromosomes to a second set of chromosomes, which were not crossed with a *P* line, she concluded that the total fitness, viability, and fertility of the *P*-crossed chromosomes had declined 55, 28, and 40% respectively. Her results are difficult to interpret, since the second control set has a completely different genetic and experimental background. The movement of *P* elements was not monitored in either of these studies.

(v) *Fitness effects and the distribution of elements on the chromosome arms*

Alleles on the *X* chromosome are subject to different evolutionary forces than those on autosomes because they are exposed to selection in a hemizygous state one third of the time, and because the effective population size of *X* chromosomes is only $\frac{3}{4}$ that of the autosomes. This is demonstrated by a number of empirical studies showing a decreased mutational load associated with the *X* chromosome in *Drosophila melanogaster* (see Eanes, Hey & Houle, 1985). The relative frequencies of mutants on the two types of chromosomes will vary with the homozygous or hemizygous phenotype and degree of dominance of the mutations. Thus, the relative frequencies of *P* elements on the *X* chromosome and the autosomes should reflect the average effect of an element on fitness. Recently, Montgomery, Charlesworth & Langley (1987) mathematically modelled these predictions for both the case of no selection (the null model) and the case of additive detrimental mutation. If insertions are neutral, the prediction is that the *X* chromosome will carry 17% of the elements in the genome. That percentage drops as selection intensity increases or the average dominance decreases. Their data for three transposons, *B104*, *412*, and *297*, gave variable results. Two elements (*B104* and *297*) possessed distributions that led to rejection of the selection model, but not the null model, while *412*'s distribution led to rejection of the null model, but not the model of deleterious

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selection. Our data for the *P* element are inconsistent with both models, because elements are too frequent on the *X* chromosome.

Why, given the observation of a significant average effect of insertion on viability, do we not see the predicted differential densities on the chromosomes? We can think of two possible reasons. First, the actual counts will be very sensitive to hot spots of element retention. For instance, we have shown that the tip of the *X* chromosome is unique in its high frequency of *P* elements (J. W. Ajioka & W. F. Eanes, unpublished results). This particular site effectively accounts for twenty percent of all *X* chromosomal elements, and adds nearly one element to the average for the chromosome. Furthermore, the high variance to mean ratios for element counts on the chromosomal arms suggests heterogeneity in site occupation frequencies within arms. The presence of such heterogeneity will tend to obscure the action of selection–insertion balance. For instance, without the high-frequency tip site on the *X* chromosome, the neutral model would not be rejected with the data reported in this paper ($\chi^2 = 0{\cdot}725$, n.s.) although the selection model would still clearly be rejected ($\chi^2 = 34{\cdot}260$, $P < 0{\cdot}005$). The second reason concerns the heterozygous expression of the mutations generated by element mobilization. If most transposon generated mutations are dominant, the selective properties of mutation on the two types of chromosomes converge and the null model is expected (Montgomery, Charlesworth & Langley, 1987). Many of the non-insertional mutational events associated with *P*-element mobilization, especially deletions, may have dominant phenotypes. In a study of segmental aneuploidy in the *Drosophila* genome, Lindsley *et al.* (1972) concluded that about half the deletions spanning as little as 2·5% of an autosome behaved as dominant lethals. At this time there are no estimates of the rate at which dominant lethal mutations are generated by heterologous exchange under P–M dysgenesis, but there is evidence that the rates could be substantial (see Charlesworth & Langley, 1986).

These issues are central to considerations of the forces that determine transposon copy number in wild genomes. One important question is the degree to which autoregulation of copy number, or a balance between transposition on the one hand and excision and selection on the other determine steady-state copy numbers. Theoretical models by Charlesworth (1985) predict that stable copy number can be maintained by selection–transposition balance alone, if mean fitness decreases faster than multiplicatively with copy number. For instance, stable copy numbers are expected when mean fitness is described by the function, $\exp -sn^2/2$ (where s is the selection coefficient, and n is the number of elements per genome). Such nonlinear relationships have been detected by Mukai (1969) for polygenic viability mutations. One might also expect mean fitness to drop faster than multiplicatively with *P* element copy number if unequal exchange between elements, as occurs in dysgenesis, were an important component in the generation of dysgenesis-associated mutation in natural populations. This was proposed by Montgomery, Charlesworth & Langley (1987) as a possible reason for the failure of elements *B104* and *297* to fit the additive selection model, and may also serve as a suitable explanation for our observations on the interchromosomal distribution of *P* elements.

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