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Transposable element-induced fitness mutations in *Drosophila melanogaster*

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Summary

P element mutagenesis was used to contaminate M strain second chromosomes with P elements. The contaminated lines were compared to uncontaminated control lines for homozygous and heterozygous fitness and its components. Mean homozygous fitness, viability and fertility of chromosome lines contaminated with P elements is decreased relative to the uncontaminated control lines by, respectively, 55, 28 and 40%. Variance among contaminated homozygous lines of total fitness increases by a factor of 1·5, variance of viability by a factor of 5·9, and variance of fertility by a factor of 1·9, compared to variance of these traits among the population of uncontaminated homozygous chromosomes. Estimates of P-element-induced mutational variance among second chromosome lines for homozygous fitness, viability and fertility are, respectively, 2×10^{-2}, 5×10^{-2} and 2×10^{-2}. This magnitude of mutational effect is equivalent, in terms of incidence of induced recessive lethal chromosomes and D : L ratio, to a dose of approximately $1{\cdot}0$–$2{\cdot}5 \times 10^{-3}$ M EMS. The distributions of fitness traits among M-derived second chromosome homozygous lines contaminated with P elements are remarkably similar in many regards to distributions of fitness and viability of chromosomal homozygotes derived from natural *Drosophila* populations. It is possible that a proportion of the fitness variation previously observed (reviewed by Simmons & Crow, 1977) following homozygosis of wild chromosomes was not present in the natural populations, but was generated by P-element transposition during the chromosome extraction procedure. P-element-induced fitness mutations appear to be completely recessive. Implications for models of evolution of transposable elements are discussed.

1. Introduction

Mobile dispersed DNA sequences are a common feature of eukaryotic genomes, and can comprise a substantial fraction of total genomic DNA. Approximately one-fifth of *Drosophila* DNA represents sequences of *copia*-like, P, I, F and FB transposable elements (Rubin, 1983). What is the evolutionary significance of these transposable elements? Transposition is known to be mutagenic, as insertion of elements may disrupt a structural locus or alter its regulation, and excision may generate deletions and other chromosome rearrangements. Transposable element-induced mutations affect both loci with major morphological effect (indeed, Green (1982) has suggested that most spontaneously occurring visible mutations in *Drosophila* are caused by insertions of transposable elements) and loci controlling quantitative characters (Mackay, 1984, 1985). Such mutations may also be associated with deleterious fitness effects, at least in *Drosophila*, as inferred from the induction of lethal chromosomes with high frequency following mobilization of elements of the P family (Kidwell, Kidwell & Sved, 1977; Mackay, 1985, this report; Yukohira, Harada & Mukai, 1985; Fitzpatrick & Sved, 1986) and also from the well-known reduction of viability and/or fertility of spontaneously arising mutants of large phenotypic effect (many of which may have arisen by insertional mutagenesis). Otherwise, it has been argued that transposable elements cannot be associated with any discernible effect on fitness, since these sequences are inserted at apparently random sites which vary considerably between individuals (Montgomery & Langley, 1983), and are therefore 'parasitic' or 'selfish' DNA (Doolittle & Sapienza, 1980; Orgel & Crick, 1980; Hickey, 1982) with properties of self-replication and regulation of transposition which ensure their own survival.

In order to determine empirically the relationship between the presence of transposable elements and fitness, it is necessary to estimate fitness for a number

of strains of common origin which differ in copy number and/or sites of insertion of transposable elements of a particular family. For most families of element the spontaneous rate of transposition is too low for this to be feasible on an experimental time scale, even with *Drosophila* (Rubin, 1983). However, the P family of transposable elements in *Drosophila* is unusual in that transposition rates are greatly enhanced under certain defined conditions (Bregliano & Kidwell, 1983; Engels, 1983). When males from a strain bearing 30–50 copies of the P element (P males) are crossed to females from a strain lacking these elements (M females), the rate of germline transposition of P (and perhaps other) elements in the F_1 hybrids is accelerated to perhaps 0·82 transpositions/chromosome arm/generation (Bingham, Kidwell & Rubin, 1982). Associated with the transposition of elements is a syndrome of correlated abnormalities collectively termed 'hybrid dysgenesis', which include F_1 hybrid temperature-sensitive sterility, male recombination, and increased frequency of chromosome rearrangements and lethal and visible mutations (Kidwell, Kidwell and Sved, 1977). These phenomena do not occur in the reciprocal non-dysgenic hybrids (P♀♀ × M♂♂) or in intra-P and -M strain crosses. The exceptionally high rate of transposition of P elements following a dysgenic cross means this system is ideal for the study of transposable element-induced mutations. In this paper I report the application of P-element mutagenesis to the problem of assessing the effect of transposable elements on fitness.

The experimental design involves the establishment of a number of second chromosome lines, originally from an M strain but potentially contaminated with P elements following dysgenic and non-dysgenic crosses, and the comparison of homozygous and heterozygous fitness of these contaminated lines with that of a control series of uncontaminated chromosomes. Fitness was measured by the competitive multi-generation technique of Sved & Ayala (1970).

2. Materials and Methods

(i) Drosophila *stocks*

Canton-S (*M*). A long-established laboratory stock of the M cytotype. No intact or defective P elements are present in this strain.

Harwich (*P*). A strong P strain bearing approximately 50 copies of the P element per haploid genome. Samples of both Canton-S and Harwich were kindly provided by M. G. Kidwell.

Cy/Pm (*M*). An M cytotype second chromosome balancer strain bearing the recessive lethal but phenotypically dominant markers *Cy* (curly wings) and *Pm* (plum eyes). Both markers are associated with a series of inversions covering the whole of Chromosome II. For a complete description of this strain see Lindsley & Grell (1968).

Cy/Pm (*P*). A P cytotype second chromosome balancer strain constructed by independently backcrossing males bearing either the *Cy* or *Pm* marker chromosome to Harwich females for seven generations. The *Cy/Pm* combination was reconstituted by reciprocally crossing *Cy*/+ and *Pm*/+ males and females from the seventh backcross generation, and using *Cy/Pm* progeny to establish the *Cy/Pm* (P) strain. It is assumed that the balancing properties of this strain are not altered by the effects of any P element transposition which may have occurred during its construction.

(ii) *Derivation of chromosome lines*

'*M*' *Control lines*. Single Canton-S males were crossed to *Cy/Pm* (M) females, and single F_1 male progeny backcrossed to *Cy/Pm* (M) females. F_2 *Cy*/+ males and females were then crossed *inter se* to produce chromosome lines homozygous for single second chromosomes from the Canton-S strain, balanced against the *Cy* marker. Twenty-three such chromosomes were extracted from Canton-S, and two replicates were maintained of each. Since Canton-S contains no P element, the distribution of a quantitative character among the lines forms the control against which the distribution among Canton-S chromosomes contaminated with P elements may be compared.

'*PM*' *Contaminated lines*. Canton-S second chromosomes were contaminated with P elements by the following procedure. The initial generation was a dysgenic cross of single *Cy/Pm* (P) males to Canton-S females. This was followed by eight generations of backcrossing a single *Cy*/+ male to *Cy/Pm* (P) females, in order to create the P cytotype necessary for (relative) stability of putative insertions, and also to control background variation between the different lines. With the exception of the initial generation, all crosses are therefore non-dysgenic, since the female parents are of P cytotype. In the F_9 *Cy*/+ males and females were mated *inter se* to produce lines homozygous for a single, possibly mutated, Canton-S chromosome II balanced against the *Cy* marker, but otherwise in a Harwich background. Fifty-four such 'PM' contaminated second chromosome homozygous lines were produced, and two replicates of each maintained separately from F_9.

'*MP*' *Contaminated lines*. A parallel series of lines to the 'PM' contaminated lines were constructed, the only difference in procedure being the initial cross. To establish these lines the initial generation was a non-dysgenic cross of single Canton-S males to *Cy/Pm* (P) females, followed by backcrossing single *Cy*/+ sons to *Cy/Pm* (P) females for eight generations. In the F_9 *Cy*/+ males and females were crossed *inter se* to produce lines homozygous for a single Canton-S second chromosome passed through a non-dysgenic cross and subsequently preserved balanced against *Cy*

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in a Harwich background. Thirty-four 'MP' chromosome lines were produced, with two replicates of each maintained separately from F_9.

If transposition occurs only in the germ line of hybrids produced from a dysgenic cross, then the PM and MP lines will differ as a result of a single generation of transposition in the former set of lines. If transposition also occurs following non-dysgenic crosses of P and M strains then both sets of lines will be contaminated, and the phenotypic effects of P-element insertion into chromosomes previously free of P elements may be ascertained by comparison to the M control lines.

(iii) *Estimation of homozygous fitness*

Competitive fitness was estimated for each homozygous line by the multi-generation technique of Sved & Ayala (1970; Sved, 1971, 1975). Each chromosome line contains two chromosomes, the *Cy* balancer and a wild-type chromosome. Because the *Cy*/*Cy* combination is lethal, only *Cy*/+ and +/+ adults survive each generation. When these chromosomes are allowed to compete over several generations, two outcomes are possible. If fitness is overdominant (the +/+ homozygote is less fit than the *Cy*/+ heterozygote), a stable equilibrium frequency of the two chromosomes will be attained, and the fitness of the +/+ homozygote relative to the *Cy*/+ heterozygote is a function of the equilibrium frequency. The function which relates fitness of the homozygote +/+ to the heterozygote *Cy*/+ is $w = (r-h)/r\ (1-h)$, where h is the observed proportion of *Cy*/+ adults in the population at equilibrium, and r is the proportion of *Cy*/+ adults emerging from a cross of two *Cy*/+ heterozygotes (Sved, 1971). r enters into the formulation as a correction because the observations are made on viability-selected adults and not zygotes.

If the wild-type homozygote is more fit than the balancer heterozygote, the balancer chromosome will be eliminated from the population, and the fitness of the balancer heterozygote relative to the +/+ homozygote is a function of the rate of elimination. The function which relates the frequency of the balancer chromosome in one generation (Q_{t+1}) to the frequency in the previous generation (Q_t) is $Q_{t+1} = Q_t\, w/(1+Q_t(f-2)+2Q_t\, w)$, where w is the fitness and f the fertility of the balancer heterozygote relative to the wild-type homozygote. The reciprocal of the regression of $1/Q_{t+1}$ on $1/Q_t$ provides the estimate of fitness in this case (Anderson, 1969). Note that if h is the observed proportion of balancer heterozygotes, $Q = h/2$.

Experimental values of h and r were determined for both replicates of every homozygous line by the following procedure. Twenty *Cy*/+ males and females were collected from the progeny of F_9 parents for all PM and MP contaminated lines, and from the progeny of F_2 parents of the control lines. These animals were placed in culture bottles and allowed to mate and oviposit for four days, then removed and discarded. Thirteen days after the introduction of parents, all adult progeny from the culture were counted and classified according to genotype. The proportion of *Cy* heterozygotes, h, was calculated separately for males and females, then 40 males and 40 females were randomly chosen as parents of the next generation, keeping the value of h for each sex in the selected parents the same as in the total sample for that generation. These parents were introduced to fresh cultures fourteen days after the initiation of the previous generation, thus maintaining all lines as discrete generations with constant population size and generation interval throughout the course of the experiment. Results were recorded for ten generations.

r was estimated in a separate series of experiments at Generation 10, by which time all homozygous lines had attained their equilibrium values of h. Eighty *Cy*/+ males and virgin females were selected from each replicate of all homozygous lines, and four replicate cultures each with 20 males and females were set up and treated according to the regime described above. At day 13 all adult progeny were counted and classified, and the proportion of *Cy*/+ heterozygotes, r, calculated. The choice of only twenty pairs of parents per replicate viability culture, compared to the forty pairs used in the fitness population bottles, means that r is estimated under slightly less stringent competitive conditions than h. This was necessary because the total number of *Cy*/+ animals emerging from a single bottle in any one generation is limited; and since viability determinations are subject to considerable sampling error it was decided to partition the resources into as many replicates as was feasible.

(iv) *Estimation of heterozygous fitness*

It is not possible to measure heterozygous fitness of a particular pair of homozygous lines by the method of competition against a balancer chromosome over several generations, because the heterozygous wild-type genotype recombines and segregates and so is not preserved intact from generation to generation. However, an indication of the average heterozygous fitness of a number of homozygous lines may be obtained by this method if the number of homozygous lines is sufficiently large to ensure that the wild-type genotypes competing against the balancer are mostly heterozygous. Homozygous lines were therefore pooled using a circular mating design in the initial generation so that all progeny in the following generation were heterozygotes for two different homozygous chromosomes. Homozygous lines within each replicate of the M, PM and MP series were labelled 1 to N in the order in which they were extracted, i.e. random with respect to homozygous fitness. A virgin *Cy*/+ female from line 1 ($♀_1$) was then crossed to a *Cy*/+ male of line 2 ($♂_2$), $♀_2 \times ♂_3$, $♀_3 \times ♂_4$ etc., the final cross being of a

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virgin $Cy/+$ female of line N to a $Cy/+$ male from line 1. All N pairs of flies were then placed in a culture bottle and subsequently treated in the same manner as the homozygous fitness lines. A total of 12 heterozygous populations were established (two for each replicate of each of the three series of chromosome lines) and estimates of h recorded over 12 generations. Only one estimate of r was available from each heterozygous population, and was obtained from the proportion of heterozygotes emerging in the first generation.

(v) *Estimation of fitness components*

Viability (v) of $+/+$ relative to $Cy/+$ is easily estimated from the observed proportion of $Cy/+$ heterozygotes r, emerging from a $Cy/+ \times Cy/+$ cross. The expected ratio of heterozygotes to homozygotes is $2r$: $(1-r)v$, from which v, viability, is $2(1-r)/r$. Four replicate viability estimates were obtained for each replicate of all homozygous lines, and from each heterozygous population.

Total fitness may be resolved into the product of the components, viability and fertility. Therefore a single estimate of fertility (f) for each homozygous and heterozygous population was obtained by $f = w/v$, or $(r-h)/[2(1-r)\,(1-h)]$.

(vi) *Culture conditions*

Crosses for the extraction of all chromosome lines were set up in vials with approximately 10 ml cornmeal–agar–molasses medium, at 20 °C (a temperature at which gonadal dysgenesis does not impair the fertility of inter-P and M strain hybrids). Subsequently populations were maintained in $\frac{1}{3}$ pint milk bottles containing approximately 100 ml medium, at 25 °C.

3. Results

Not all of the extracted second chromosomes were homozygous viable. Two of the 23 M chromosomes (8·7%), 11/54 PM chromosomes (20·4%) and 8/34 MP chromosomes (23·5%) were lethal as homozygotes. The two M homozygous lethal chromosomes did not complement, so the Canton-S population has at least one segregating lethal. All 19 lethals from the contaminated chromosomes did complement with the M lethal (data not shown), indicating that they were probably experimentally induced. This is somewhat surprising, as one would have expected 7–8 of the 88 contaminated chromosomes to be copies of the original M lethal.

Fitness and its components were measured on the remaining homozygous viable lines. Fig. 1 depicts the weighted average frequency of $Cy/+$ heterozygotes (h) in successive generations for the M, MP and PM homozygous (Fig. 1*a*) and heterozygous (Fig. 1*b*) lines. Weighted averages were computed separately

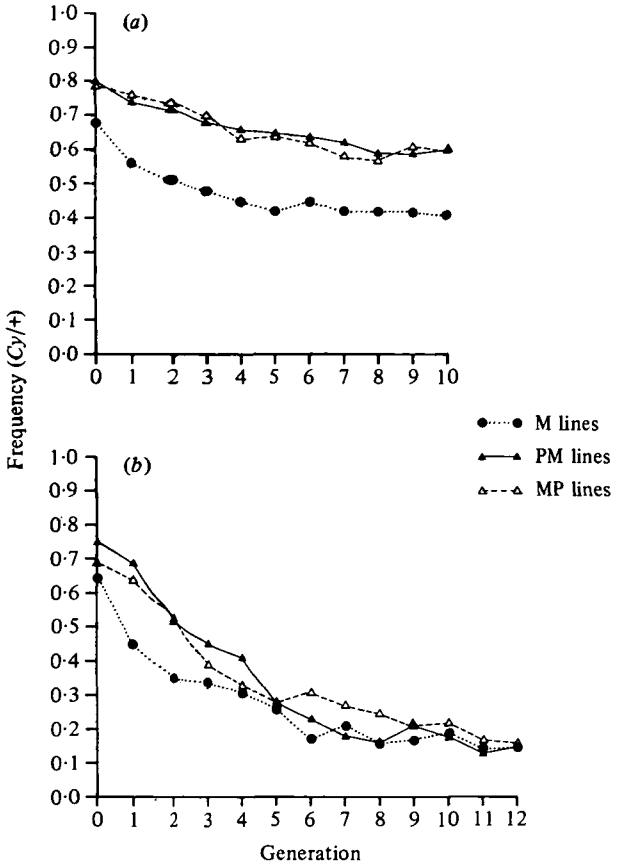

Fig. 1. Frequency of $Cy/+$ each generation averaged over M second chromosome lines (circles and dotted lines), PM-derived second chromosomes (solid triangles and solid lines) and MP-derived second chromosomes (open triangles and dashes). Fig. 1(*a*) Homozygous lines; Fig. 1(*b*) Heterozygous lines.

for the M, MP and PM chromosomes each generation by pooling observed numbers of $Cy/+$ and $+/+$ flies across each of the two replicates of all homozygous viable lines, and across the four replicate heterozygous populations, then recalculating the value of h using these total numbers (Fig. 1 summarizes data from the classification of over 560000 flies). On average, all populations attained equilibrium frequencies of $Cy/+$; by generation 7 for the homozygous populations and generation 9 for the heterozygous populations. The equilibrium frequency (h) for the three homozygous and three heterozygous populations of chromosome lines was estimated by pooling observed numbers of $Cy/+$ and $+/+$ flies over the last four generations, then recalculating h using the total numbers.

Equilibrium frequencies of $Cy/+$ for the M, MP and PM homozygous populations are, respectively, 0·419, 0·591 and 0·601; and estimates of h for the M, MP and PM heterozygous populations are 0·162, 0·189 and 0·166. There are several noteworthy features of these results. First, as is typical for this sort of experiment, homozygous populations of chromosomes are less fit than heterozygous populations (Sved & Ayala, 1970; Sved, 1971, 1975; Tracey & Ayala, 1974;

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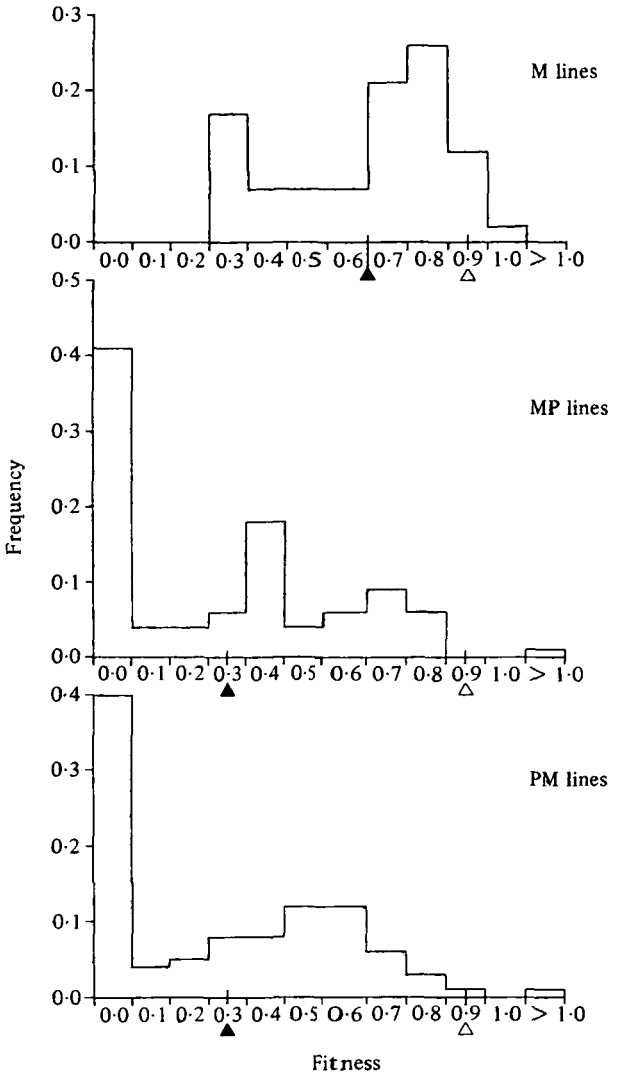
Mackay, 1986*a*). Secondly, there is no apparent difference between the two populations of contaminated chromosomes, both with respect to homozygous and heterozygous fitness and to the proportion of homozygous inviable chromosomes. Finally, contaminated chromosomes suffer an appreciable reduction in fitness as homozygotes, compared to the M chromosome controls, but not as heterozygotes. Therefore it appears that element-induced fitness mutations are on average recessive.

Estimates of relative homozygous fitness and viability and fertility components were calculated separately for each chromosome line. A weighted average value of h was obtained for each replicate of all homozygous lines by pooling the numbers of $Cy/+$ and $+/+$ flies emerging from generations 7–10, inclusive, and using these total numbers to compute h. A weighted average value of r was similarly obtained for each replicate of all lines using the total numbers of flies of each genotype from the four replicate viability cultures. Estimates of fitness, viability and fertility of $+/+$ relative to $Cy/+$ were then calculated according to the procedure outlined above. For several of the contaminated lines, the estimated value of h was greater than that of r, thus giving a negative estimate of fitness. The negative estimates were set to zero; further study of these lines indicated that all were indeed male- and/or female-sterile.

The distribution of fitness among M, MP and PM chromosome homozygote lines is shown in Fig. 2. The distributions of the MP- and PM-derived lines are so similar that they will be considered together in subsequent discussion as contaminated chromosomes. It is apparent from the figures that the distribution of fitness among contaminated chromosomes is dramatically altered compared to the M chromosome control distribution. Mean fitness is reduced by over 50%, and variance in fitness is increased. Approximately 40% of the contaminated chromosomes are either inviable or infertile, which accounts for much of the change in mean and variance, but the remaining non-lethal chromosomes also appear to have accumulated minor deleterious mutations. However, two of the wild-type contaminated chromosomes eliminated the *Cy* marker, and hence have estimated fitnesses greater than one. It is possible that these chromosomes are truly more fit as homozygotes than their uncontaminated counterparts, which would argue for occasional selective advantage accruing from the presence of transposable elements. It is perhaps more likely that these two chromosomes are no longer homozygous, but have polymorphic sites of insertion of elements as a consequence of transposition following the initial chromosome extraction, and hence they have the fitness of wild-type heterozygotes.

There are few published studies of total fitness of chromosomal homozygotes derived from natural populations based on a sufficiently large number of chromosomes to assess the shape of the distribution.



Figs 2–4. Distributions of fitness traits among homozygous second chromosome control (M) and contaminated (MP, PM) lines. The two inviable M chromosomes are excluded from these figures because the results of the complementation tests showed that they did not appear among the contaminated lines. The position of the solid triangle represents the mean of the homozygous population, and the open triangle represents the heterozygous mean.

Fig. 2. Distributions of competitive fitness of $+/+$ homozygotes relative to $Cy/+$ heterozygotes.

However, the fitnesses of 41 third chromosome homozygotes extracted from a Death Valley population by Mackay (1986*a*) formed an inverse J-shaped distribution with a large peak at lethality and all other values equally frequent, and studies based on smaller numbers of chromosomes are consistent with this pattern (Sved, 1971, 1975; Tracey & Ayala, 1974). It is interesting that the distribution of homozygous fitnesses among contaminated chromosome lines is also approximately this shape.

The distributions of the fitness components, viability and fertility, among the three populations of control and contaminated homozygous second chromosomes

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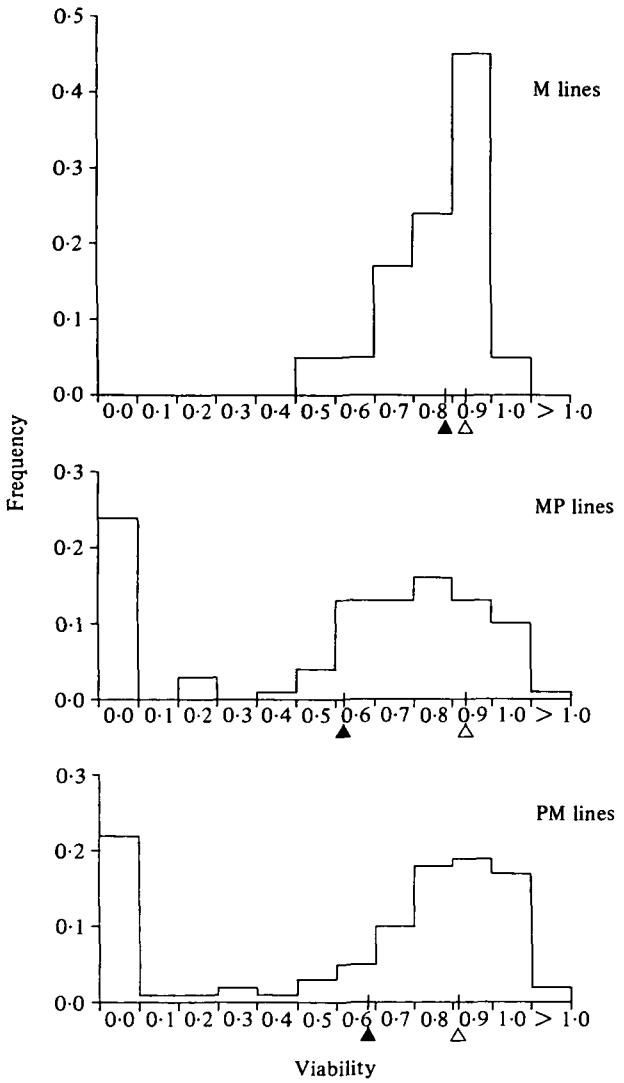

Fig. 3. Distributions of viability of +/+ homozygotes relative to *Cy*/+ heterozygotes.

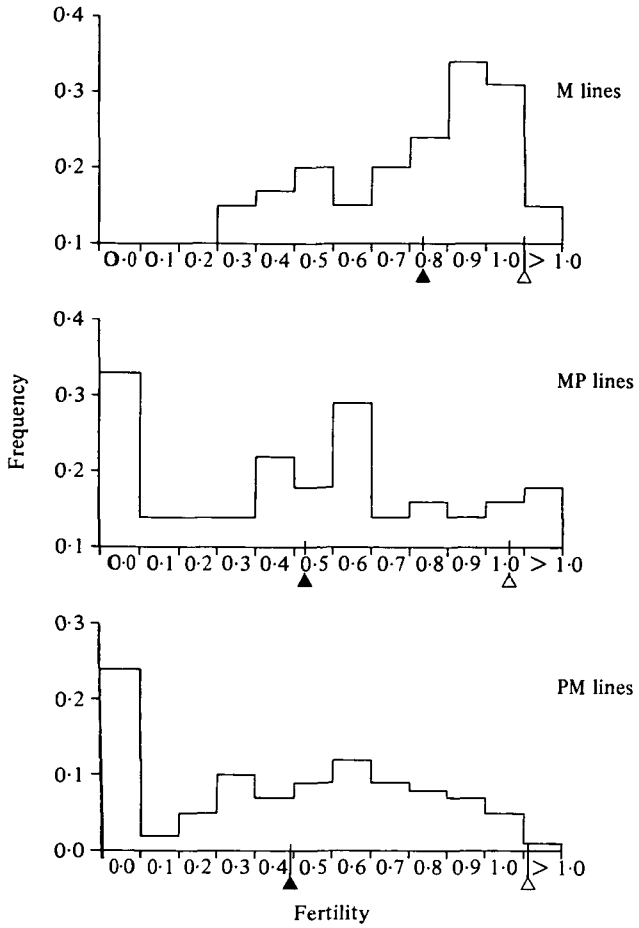

Fig. 4. Distributions of fertility of +/+ homozygotes relative to *Cy*/+ heterozygotes.

follows a similar pattern to that described for total fitness. The average viability and fertility of both populations of contaminated chromosomes decreased and the variance increased, relative to the uncontaminated controls (Figs 3 and 4). The change in the distribution of viabilities as a consequence of transposition is most striking – from a unimodal distribution with low variance to a bimodal distribution with one peak at lethality and another for chromosomes of quasi-normal viability, with only a few chromosomes of intermediate (0·1–0·5) viability, and a small but significant proportion of supervital chromosomes. There is a strong sense of *déjà vu* about the distributions of homozygous viabilities of contaminated chromosomes, which closely resemble the classical distributions of homozygous viabilities of chromosomes extracted from natural populations presented in numerous studies by Dobzhansky and his co-workers (summarized by Lewontin, 1974). It appears therefore that the nature of transposable element-induced mutations for total fitness and its viability component is not quantitatively or qualitatively different from naturally occurring variation. It is tempting to speculate that perhaps fitness variation observed in natural *Drosophila* populations might be caused by insertional mutagenesis.

The effect of putative insertional mutagenesis by P elements on mean fitness and its components is quantified in Table 1, and the effect on variance of these characters is documented in Table 2. All estimates of means and variances include lethal and deleterious chromosomes, as well as those with 'quasi-normal' performance. Mean homozygous fitness of chromosome lines contaminated by P elements is decreased relative to the uncontaminated control lines by 55%; a similar comparison for viability and fertility components gives decreases of 28% and 40% respectively. Mean heterozygous fitness, viability and fertility of contaminated chromosome lines are unaltered compared to the control chromosome heterozygotes. Comparison of the estimates of inbreeding depression given in Table 1 indicates that mutations affecting fitness arising by insertional mutagenesis are similar in character to spontaneously arising and chemically induced fitness mutations (reviewed by Simmons & Crow, 1977) in that their effect on reduction in homozygous total fitness is much greater than the effect on homozygous viability alone. For this class of mutation

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Table 1. *Mean homozygous and heterozygous performance, and inbreeding depression expressed as a proportion of the non-inbred mean, for fitness and its components from contaminated (MP, PM) and uncontaminated (M) second chromosome lines*

Character	Line derivation	Mean of all homozygous lines ($\bar{X}_T$)	Mean of heterozygous lines ($\bar{X}_H$)	Inbreeding depression (%) (100 ($\bar{X}_H - \bar{X}_I$)/$\bar{X}_H$)
Fitness	M	0·6477	0·9155	29·25
	MP	0·2902	0·8972	67·65
	PM	0·2880	0·9141	68·50
Viability	M	0·8252	0·8752	5·71
	MP	0·5695	0·8823	35·45
	PM	0·6258	0·8632	27·50
Fertility	M	0·7819	1·0460	25·25
	MP	0·4747	1·0169	53·32
	PM	0·4380	1·0590	58·64

as well as the previously studied ones, the deleterious effect on fertility exceeds that on viability, either because numerically more mutations occur at loci controlling fertility than viability, or because pleiotropic effects of single mutations are such that fertility is more adversely affected than viability. Further evidence that fitness mutations induced by transposition of P elements are comparable to spontaneously occurring fitness mutations in their effect is that the estimate of average homozygous fitness of contaminated lines relative to fitness of random wild-type contaminated heterozygotes is 0·32, which is coincidentally identical to that obtained for third chromosomes extracted from a natural population by Tracey & Ayala (1974), and of the same order as estimates reported in other studies (Sved & Ayala, 1970; Sved, 1971, 1975; Mackay, 1986*a*).

Variance components from analysis of variance of equilibrium values of h, r, total fitness, viability and fertility among and within control and contaminated homozygous lines are presented in Table 2. From analysis of variance of h, it is clear that equilibrium genotypic proportions of $Cy/+$ have been well established within each line, since the proportion of variance arising from the four estimates of h within each replicate/line is low. However, the variance between replicate/line estimates for this and all other characters is much greater for the two series of contaminated lines than for the control, which can be interpreted to be a consequence of appreciable amounts of transposition-induced mutation arising after replicate lines diverged. All observations were made within ten generations of replicate separation, so it appears that the effects of transposition even in P cytotype and over a short time span are not negligible.

Comparison of variation among control and con-

Table 2. *Variance components ($\times 10^3$) of fitness traits from analysis of variance of contaminated (MP, PM) and uncontaminated (M) second chromosome homozygote lines*

	Variance components (percentage of total variance)				
Character	Line derivation	Among lines	Between replicates, within lines	Within replicates	Total
h	M	26·3 (85·07)	2·6 (8·55)	2·0 (6·38)	30·9
	MP	31·6 (72·20)	8·5 (19·38)	3·7 (8·42)	43·8
	PM	22·3 (55·71)	14·3 (35·72)	3·4 (8·57)	40·0
r	M	0·7 (24·18)	0 (0)	2·0 (75·82)	2·7
	MP	6·9 (70·22)	0 (0)	2·9 (29·78)	9·8
	PM	6·0 (60·28)	2·3 (23·23)	1·6 (16·49)	9·9
Fitness	M	36·5 (81·72)	8·2 (18·28)	—	44·7
	MP	60·3 (70·03)	25·8 (29·97)	—	86·1
	PM	50·8 (63·52)	29·2 (36·48)	—	80·0
Viability	M	8·4 (21·44)	0 (0)	30·8 (78·56)	39·2
	MP	62·4 (61·72)	0 (0)	38·7 (38·28)	101·1
	PM	54·4 (54·48)	20·4 (20·42)	25·0 (25·10)	99·8
Fertility	M	24·8 (50·01)	24·8 (49·99)	—	49·6
	MP	48·8 (38·53)	77·8 (61·47)	—	126·6
	PM	43·5 (38·01)	70·9 (61·99)	—	114·4

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taminated lines illustrates the power of transposition in generating quantitative variation for fitness and its components. Variance components among PM- and MP-derived contaminated lines are not detectably different and so are considered together in the following summary. The effect of P-element contamination is to increase variance among lines of total fitness by a factor of 1·5, variance of viability by a factor of 5·9, and variance of fertility by a factor of 1·9, compared to variance of these traits among uncontaminated chromosomes. These data may be used to estimate the magnitude of P-element-induced mutational variance (σ^2_m) for homozygous fitness, viability and fertility if one assumes that the observed variance components among contaminated lines may be linearly resolved into a component of variance among uncontaminated Canton-S chromosomes plus a component of variance due to mutation. Estimates of mutational variance are then obtained by subtracting observational components of variance among control chromosome lines from observational components of variance among contaminated chromosome lines; these estimates are given in Table 3. Average estimated values of P-element-induced σ^2_M on the second chromosome for fitness, viability and fertility are, respectively, 2×10^{-2}, 5×10^{-2} and 2×10^{-2}. Since the second chromosome of *Drosophila melanogaster* represents approximately $\frac{2}{5}$ of the entire genome, these values could be multiplied by $\frac{5}{2}$ to estimate the total potential P-element-induced mutational variation for fitness traits.

Table 3. *Estimates of P-element-induced mutational variance ($\sigma^2_m \times 10^2$) of fitness traits from second chromosome homozygous lines*

Line derivation	σ^2_m		
	Fitness	Viability	Fertility
MP	2·4	5·4	2·4
PM	1·4	4·6	1·9

4. Discussion

The effect of P-element mutagenesis on homozygous fitness is to decrease the mean and increase the variance of performance for total fitness and its components. Fitzpatrick & Sved (1986) and Yukohiro, Harada & Mukai (1985) report similar levels of transposable element-induced mutations affecting, respectively homozygous fitness and viability of P-strain-derived second chromosomes passed through a dysgenic cross. Indeed, the incidence of recessive lethal chromosomes recovered by Yukohiro, Harada & Mukai (1985) following the exposure of a non-lethal P-bearing chromosome to dysgenic conditions (45%) was twice as high as that following similar exposure of M-strain-derived chromosomes reported here. Chromosomes harbouring P elements are subject to mutations caused by both insertion and imprecise excision of these elements, whereas P-induced mutagenesis of chromosomes previously free of these elements can only be insertional. The difference in frequency of lethals observed after dysgenesis-induced mutation of P- and M-derived chromosomes may therefore be interpreted as evidence that imprecise excision of elements is as effective as insertion of elements as a mechanism of generating mutations.

The distributions of fitness traits among M-derived second chromosome homozygous lines contaminated with P elements bear a remarkable similarity to distributions of fitness and viability of chromosomal homozygotes derived from natural *Drosophila* populations, regarding both mean and variance of effect. The proportion of lethal chromosomes arising from P-element mutagenesis, the magnitude of inbreeding depression for fitness and its components, the relative contribution of the viability and fertility components to total fitness, and indeed the absolute amount of transposable element-induced fitness variation are all comparable to results from past experiments documenting the effects of homozygosis of wild chromosomes (Sved & Ayala, 1970; Sved, 1971, 1975; Lewontin, 1974; Tracey & Ayala, 1974; Mackay, 1986*a*). This may be interpreted as evidence that the nature of P-element-induced fitness mutations is the same as that of spontaneously occurring fitness mutations, which leads to the speculation that spontaneously arising polygenic mutations affecting fitness may be caused by insertion of transposable elements. However, most of the experiments in which fitness variation in natural populations was studied involved crossing wild-caught males, likely to be of P cytotype, with laboratory strains of females carrying the appropriate balancer chromosomes, likely to be of M cytotype. It is thus possible that a proportion of the observed fitness variation was not present in the wild populations, but was generated by P-element transposition during the chromosome extraction procedure. It is unlikely that this explanation accounts entirely for the similarity with respect to fitness traits between chromosomes contaminated with P elements with wild-type chromosomes, because fitness experiments with species of *Drosophila* which do not harbour P elements yielded comparable results (although this does not preclude the existence of other dysgenic systems peculiar to these species).

Comparison of the effects of P-element-induced mutations on fitness with the effect of other mutagens is complicated. There is well-documented information on the effects of EMS on polygenic variation for viability and total fitness (Mukai, 1970; Ohnishi, 1977*b*, *c*; Mitchell, 1977; Mitchell & Simmons, 1977; Simmons, Sheldon & Crow, 1978), but the purpose of this work was specifically to study mutations with minor effects, which involved the analysis of a large number of chromosomes with quasi-normal viability

(i.e. viability greater than 50 or 60% of normal) and the exclusion of any chromosomes with more drastic effects. The number of quasi-normal chromosomes considered in this study is too small to justify such detailed analysis. However, the proportion of experimentally induced recessive lethal chromosomes can be compared for both P- and EMS mutagenesis, from which it appears that the mutational effect of transposition is equivalent to a dose of $1{\cdot}0$–$2{\cdot}5 \times 10^{-3}$ M EMS (Ohnishi, 1977*a*; Simmons, Sheldon & Crow, 1978). The ratio of detrimental (D) to lethal (L) genetic loads (Greenberg & Crow, 1960) is similar for P- and EMS-induced mutations. The D : L ratio for the P-contaminated chromosomes (MP and PM chromosomes pooled) is 0·421, compared to an average value of 0·365 for Ohnishi's (1977*a*) chromosomes treated with $1{\cdot}0 \times 10^{-3}$ and $2{\cdot}5 \times 10^{-3}$ M EMS. The average D : L ratio for *Drosophila melanogaster* second chromosomes extracted from natural populations (1·017, Simmons & Crow, 1977) is much higher than that calculated from P-element and EMS-mutagenized chromosomes. It is not unexpected that lethal chromosomes should contribute proportionately more to the load in populations carrying newly arisen fitness mutations than in natural populations.

Unlike EMS-induced and spontaneously occurring fitness mutations, which are partially dominant (reviewed by Simmons & Crow, 1977), insertional fitness mutations appear to be completely recessive. This statement is based on comparison of mean performance for fitness and its components of heterozygous populations contaminated with P elements to heterozygous control populations with no P element; average heterozygous performance is unaltered by insertional mutagenesis. This conclusion cannot be drawn with much confidence because the experimental design is not competent to address fully the question of heterozygous effect. However, there is an indirect argument which supports the interpretation of recessivity of insertional fitness mutations. When Mitchell (1977) and Simmons, Sheldon & Crow (1978) compared the effects of EMS-induced fitness mutations in homozygotes and heterozygotes, they discovered that the heterozygous effect of the mutations on fitness was equal to their homozygous effect on viability, for chromosomes of quasi-normal viability. Simmons & Crow (1977) argue that this is also true for mildly detrimental spontaneously arising fitness mutations. If this relationship between the effect on homozygous viability and heterozygous fitness is generally valid it can be applied to the case of insertional mutations affecting fitness. One can argue that if insertional mutations are completely recessive and thus have no effect on heterozygous fitness, the homozygous viability of quasi-normal chromosomes contaminated with P elements should be unimpaired, relative to the viability of control chromosomes. The average viability of control chromosomes is 0·825, of MP-derived chromosomes with viability greater than 0·6 is 0·817, and of PM-derived quasi-normal chromosomes is 0·850. Therefore the average viability of both MP and PM quasi-normal contaminated chromosomes (0·83) is equal to the viability of uncontaminated controls, which is consistent with the hypothesis that there is no effect of these mutations on heterozygous fitness. Simmons & Crow (1977) also deduce that the heterozygous effect on total fitness of mutations affecting viability is the same regardless of the severity of the viability reduction, so by extension the more drastic P-element-induced mutations should also be recessive with respect to total fitness.

If transposable element-induced mutations only affect fitness when homozygous, a resolution of the argument concerning the evolutionary consequences of the existence of transposable elements may be proposed. There is considerable evidence that insertion of elements causes harmful mutations (Shapiro, 1983), but also that these elements are successful parasites with no discernible effect on fitness (Doolittle & Sapienza, 1980; Orgel & Crick, 1980). Both views may be accommodated if the elements cause mutations which are deleterious with respect to fitness when homozygous, but neutral when heterozygous. Montgomery & Langley (1983) have shown that sites of insertion of three families of *Drosophila melanogaster* transposable elements in a natural population are individually rare, perhaps even unique (at least in the sample of 20 *X* chromosomes surveyed). It follows that if population size is sufficiently large, individuals will be heterozygous for sites of insertion of elements on the autosomes and hence suffer no loss of fitness resulting from their presence. This argument does not apply to insertions on the *X* chromosome, as any deleterious mutations affecting viability and/or male fertility are exposed to natural selection as hemizygotes in males and would be quickly eliminated, although female-specific fertility mutations could accumulate. Therefore insertions on *X* chromosomes surviving in natural populations must also be neutral with respect to fitness. It will be interesting to compare the phenotypic effects of newly arisen insertional mutations on fitness of *X* chromosomes with the second chromosome effects reported here.

Recessivity of insertional mutations with respect to fitness also has implications for the formulation of models of evolution of copy number of transposable element families. Two broad categories of such models have been proposed. One possible scenario for copy-number regulation is that sites of insertion are neutral, and transposition is self-regulated such that the probability of transposition of an element is a decreasing function of the number of elements in the genome (e.g. Langley, Brookfield & Kaplan, 1983). Alternatively, copy number may be stabilized by a balance between a constant rate of replicative transposition (i.e. independent of the number of elements already present) and natural selection such that fitness is a decreasing function of copy number (e.g. Charles-

worth & Charlesworth, 1983). However, the fitness function of the latter model is based on the assumption that deleterious insertional mutations, like spontaneously arising and chemically induced fitness mutations, are semi-dominant. If this is not true, then this class of model may be inappropriate.

One puzzling aspect of this experiment is the equivalence in all regards of the MP- and PM-derived contaminated chromosomes. It is clear from all previous work on hybrid dysgenesis that dysgenic phenomena are only exhibited by F_1 hybrids of a dysgenic (PM) cross, and not the reciprocal, non-dysgenic (MP) cross, but it is not clear what the relationship is between the phenotypic manifestation of dysgenesis and rates and patterns of transposition. In particular, the amount of transposition in later generations of crosses between P and M strains is unknown, but may be deduced from the indirect evidence of fitness mutations reported here to be equal regardless of which of the parents was the P-bearing strain. This result is not in accord with previous studies of mutations occurring in progeny of dysgenic and non-dysgenic crosses. Fitzpatrick & Sved (1986) studied fitness of a single P-bearing second chromosome passed through both a dysgenic and a non-dysgenic cross, and found substantial differences in fitness and also in the proportion of lethal-bearing chromosomes of the dysgenic chromosomes compared to the non-dysgenic controls. Mackay (1985) reports accelerated response to artificial selection for abdominal bristle number and also a twofold increase in incidence of recessive lethal chromosomes in the progeny of dysgenic, compared to non-dysgenic hybrids. However, if I had relied totally on the differences between the MP- and PM-derived contaminated chromosomes of this experiment to detect transposable element-induced fitness mutations, I would have concluded that no such effect exists. Only by reference to the uncontaminated control population were the dramatic alterations in fitness and its components revealed.

Mackay (1985) and Fitzpatrick & Sved (1986) both observed evidence of transposition in non-dysgenic crosses, but the effect was minor in comparison to the effect of dysgenesis-induced transposition. However, these experimental designs were different from the one reported here. Mackay's (1985) dysgenic and non-dysgenic hybrids were mass-mated (with selection) following the initial strain cross; Fitzpatrick & Sved (1986) isolated a single P chromosome using a Q (neutral) balancer strain, and did not backcross for many generations to the marker strain to homogenize the background genotype; and the contaminated chromosomes described above were studied only after the original M chromosomes were repeatedly exposed to P cytotype. One could therefore hypothesize that regulation of copy number operates at the level of individual chromosomes, and that the imbalance in copy number when chromosomes free of P elements were placed in a strong P background was sufficient to mediate transposition, regardless of the original direction of the interstrain cross. Experiments designed to track transposition events over successive generations following interstrain crosses are in progress.

A long-term objective of the study of P-element-induced mutagenesis of quantitative characters is to utilize insertions of P elements in putative polygenes as 'bait' in the molecular 'fishing' experiment first described by Bingham, Levis & Rubin (1981) in order to characterize polygenic variation at the molecular level, in the manner which has been so successful for *Drosophila* genes of major effect. The control and contaminated second chromosome lines have been analysed further for three additional quantitative characters (female productivity, sternopleural and abdominal bristle number; Mackay, 1986*b*). It is intended to correlate sites of insertion of P elements, as visualized by *in situ* hybridization of biotinylated DNA to polytene chromosomes of contaminated lines, with extreme phenotypic effects of the quantitative characters, as a first step in the identification of relevant polygenic loci. One by-product of the information yielded by the *in situ* analysis of sites of residence of P elements on chromosomes previously free of them will be the establishment of an empirical fitness function, relating numbers of copies of P elements to the homozygous fitness determinations presented above.

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