P Element Transposition Contributes Substantial New Variation for a Quantitative Trait in Drosophila melanogaster

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ABSTRACT

The P-M system of transposition in Drosophila melanogaster is a powerful mutator for many visible and lethal loci. Experiments using crosses between unrelated P and M stocks to assess the importance of transposition-mediated mutations affecting quantitative loci and reponse to selection have yielded unrepeatable or ambiguous results. In a different approach, we have used a P stock produced by microinjection of the ry<sup>506</sup> M stock. Selection responses were compared between transposition lines that were initiated by crossing M strain females with males from the "co-isogenic" P strain, and ry<sup>506</sup> M control lines. Unlike previous attempts to quantify the effects of P element transposition, there is no possibility of P transposition in the controls. During 10 generations of selection for the quantitative trait abdominal bristle number, none of the four control lines showed any response to selection, indicative of isogenicity for those loci affecting abdominal bristle number. In contrast, three of the four transposition lines showed substantial response, with regression of cumulative response on cumulative selection differential ranging from 15% to 25%. Transposition of P elements has produced new additive genetic variance at a rate which is more than 30 times greater than the rate expected from spontaneous mutation.

Evidence for transposable elements in Drosophila initially came from studies of unstable mutations (Green 1967, 1969). Male recombination and unusual rates of mutations were discovered to be associated with certain wild second chromosomes (MR chromosomes) (Hiraizumi 1971; Green 1977). These chromosomes were later found to carry a subset of a family of transposable elements called P elements (Kidwell, Kidwell and Sved 1977; Engels 1989). The P-M system of transposition in Drosophila melanogaster has demonstrated powerful mutagenic effects for many visible and lethal loci (Simmons et al. 1980; Zusman, Coulter and Gergen 1985; Yukihiro, Harada and Mukai 1985; Ingham et al. 1985; Tsukuba and Schedl 1986; Suh and Mukai 1987).

Initial investigations of the influence of P transposition on quantitative genetic variation compared lines derived from crosses of unrelated P and M strains. Selection lines derived from "nondysgenic" (P<sup>2</sup> × M<sup>2</sup>) crosses were used as negative controls for lines from "dysgenic" (M<sup>2</sup> × P<sup>2</sup>) crosses, in which much more transposition was expected (Morton and Hall 1985; Mackay 1984, 1985; Torkamanzehi, Moran and Nicholas 1988). This type of experiment has two disadvantages. First, it is now realized that transposition actually occurs in lines derived from both dysgenic and nondysgenic crosses, which means that these experiments have lacked a valid control (Mackay 1988; Pignatelli and Mackay 1990; Lai et al. 1987; Sandholt and Mackay 1990; Torkamanzehi 1990). Second, since the P and M strains are unrelated, both types of cross produce lines that are heterozygous at many loci affecting the trait being selected. Even in the absence of transposition, substantial genetic variation is introduced into control and experimental lines.

"Chromosome contamination" experiments, initially used to assess effects of transposition on fitness traits (Yukihiro, Harada and Mukai 1985; Fitzpatrick and Sved 1986; Mackay 1986; Eanes et al. 1988), are an alternative to selection experiments for analysing the effect of transposition on quantitative variation. For example, Lai and Mackay (1990) used compound X chromosomes to construct isogenic X chromosome lines in which the X chromosomes had been exposed to dysgenic, nondysgenic or control crosses. By partitioning of variance components rather than analysis of selection responses, they revealed that even one generation of transposition on one chromosome causes remarkably large increases in genetic variation for abdominal and sternopleural bristle number. A similar effect of transposition in relation to an autosome was reported by Mackay (1987). In providing clear evidence for the effect of transposition on individual chromosomes, these studies complement selection experiments, which provide evidence in relation to the whole genome.

In this paper, we report the results of a selection experiment conducted in such a way as to overcome...
the disadvantages of the early experiments described above. It compares selection responses in an “inbred” M strain with responses in lines derived from a cross between the M strain and its “co-isogenic” P strain derivative. This allows accurate measurement of the effect of transposition. Such experiments are free of any possibility of transposition in the control, and avoid the necessity to distinguish the transposition effect from the large genetic variance introduced by crossing unrelated lines. Furthermore, they assess the effect of transposition on the entire genome.

MATERIALS AND METHODS

Origin of the parental strains: Both the M strain (ry\(^{896}\)) and the P strain (88-4-1) were kindly provided by M. G. Kidwell. The ry\(^{896}\) strain contains a deletion of 3.4 kb of the rosy locus (Cote et al. 1986) induced by \(\gamma\)-radiation (Gelbart et al. 1974). The mutant arose in the ry\(^{896}\) homozygous third chromosome of the Storr’s collection (Cote et al. 1986) and was captured against a chromosome \(B\) balancer (Gelbart et al. 1974). It is isogenic for chromosome \(B\) at least. The P strain was created in 1982 by microinjection of P element DNA into ry\(^{896}\) embryos (Daniels et al. 1987). It has approximately 15 intact, and 40 degenerate, P elements per haploid genome; very similar to the standard P strain Harwich-77 (Daniels et al. 1987). It has developed strong P factor activity in dysgenic crosses, and strong P cytotype (Daniels et al. 1987). As a ry\(^{896}\) P element marker was also cojected with the complete P element DNA, in order to facilitate transformat detection during the process of producing the P stock, the transformed flies (P strain) have wild-type eye color, whereas the untransformed (M strain) flies have the ry\(^{896}\) phenotype. All flies were maintained by mass rearing in bottle cultures with no special attempts to either cause or avoid inbreeding prior to their use in the selection experiment. The cytotype of ry\(^{896}\) and 88-4-1 was tested before their use in the selection experiments, with the former being an M stock and the latter a strong P stock as expected.

Crossing and selection scheme: Transposition and control lines were established as shown in Figure 1. Two replicates of the entire crossing and selection scheme were conducted contemporaneously with 800 flies in total being scored per generation. Due to the physical limitations of scoring bristles in large numbers of flies, P X P controls were not maintained, as this would have increased the size of the experiment by 50%, making it unmanageable. Flies were kept at 20° in ¼-pint glass bottles cultured with standard cornmeal-treacle-yeast medium.

Test of cytotype: The ovarian dysgenesis test of cytotype (Engels 1979) was carried out for all selection lines at each generation up to G\(_{10}\). The test involves crossing flies from the Para Wirra (PW) strain, a strong P stock (Angus and Colgan 1978), with females from the selection lines at high temperature, and measuring the fertility of daughters using the same procedure as Torkamanzehi, Moran and Nicholas (1988), who monitored cytotype in lines derived from crosses of unrelated P and M stocks. High levels of ovarian dysgenesis (infertility) indicate that the female is incapable of suppressing P transposition. Thus the test monitors the ability of females from the transposition selection lines to suppress P transposition, providing an indication of the level of transposition possible within the selection line. For the control selection lines, very high levels of ovarian dysgenesis are expected, as P cytotype is dependent on the presence of P elements; thus the test monitors for accidental contamination by P elements. Dysgenic (Canton-S (CS\(_{10}\) X PW\(_{5}\)) and nondysgenic (PW\(_{5}\) X CS\(_{10}\)) crosses were also made throughout the experiment as positive and negative controls respectively for the ovarian dysgenesis test.

Estimation of quantitative genetic parameters: The most important parameter that can be estimated from this experiment is \(V_\text{s}\), which is the rate of production of new additive genetic variation per generation (Hill 1982). This parameter was estimated from Equation 6 of Hill (1982), using the same approach as adopted in Hill’s (1982) analysis of a range of bristle selection experiments in Drosophila. In this approach, \(V_\text{s}\) is estimated as \(G_C \sigma^2 / [t - 2N (1 - \mu^{2N})]\), where \(C\) is the total response after \(t\) generations of selection, \(\sigma^2 = \text{environmental standard deviation, and } N = \text{effective population size. For the present experiment, } t = 12; \sigma = 2.75\), which is estimated as the square root of the phenotypic variance in the control lines (see footnote to Table 1); and \(N\) is assumed to be 20, because in a selection program like the one described here, effective population size is expected to be very similar to the total number of parents (F. W. Nicholas and J. W. James, manuscript in preparation). The assumptions inherent in this approach have been discussed in detail by Hill (1982). Among other things, Hill’s formula assumes that the contribution of mutational variance is small and constant. As the mutation rates will vary markedly throughout our experiment due to the gain of P cytotype and consequent repression of transposition, the rate of contribution of new mutational variance will not be constant. But Hill’s approach provides an estimate of the average level of \(V_\text{s}\).

In order to provide a broader overview of selection response, the regression of cumulative response on cumulative selection differential was estimated for each selection line. Such regression values are usually interpreted as realized heritability estimates (Falconer 1989) relevant to the base population. As all transposition lines have experienced bursts of transpositional mutation, the regression parameter does not have this simple interpretation in these lines, due to the introduction of new mutations during the course of the experiment. In other words, the changes in the means for the transposition lines are not due entirely to genetic variation present in the base population. The regression coefficients are most conservatively considered as measures of the rate of change in population mean per unit selection differential due to the introduction of transpositional mutations. In common with other reported attempts to quantify the effects of transpositional mutation from selection response (Mackay 1984, 1985, 1988; Torkamanzehi, Moran and Nicholas 1988), the regressions will be interpreted as heritability estimates in order to calculate \(V_\text{s}\) and other variance parameters. For the control lines, the regressions are unambiguously interpretable as realized heritabil-
ities. Regression estimates were obtained from unidirectional (Hill 1972a) rather than divergent (Hill 1972b) response, since apart from the common P element sites acquired from the 88-4-1 parent stock, each transposition line is an independent sample of a unique set of transposition events occurring from G0. In the transposition lines, we are estimating parameters of a population in a state of genetic and mutational flux, which is quite different from the base population from which it was derived.

Even where the regressions could be interpreted as straightforward heritabilities, the standard errors of the realized heritabilities could not be calculated due to the nonhomogeneity of the phenotypic variances across generations (Hill 1972b; Steele and Torrie 1981); instead, the standard errors of the regression coefficients are used as an approximation.

RESULTS

The results of the ovarian dysgenesis test of cytotype, shown in Figure 2, indicate that the transposition selection lines gradually developed P cytotype, i.e., the ability to repress further transposition of P elements, with strong P cytotype being attained by about generation 8 or 9. Substantial transposition would have been possible in the early generations of the transposition lines. The results have been pooled separately within replicate 1 and replicate 2, as both the replicate 1 values tended to be higher each generation than the replicate 2 values, indicating that both replicate 2 transposition lines developed the ability to repress transposition more quickly than replicate 1 lines. Thus, the replicate 2 transposition lines probably experienced lower levels of total transposition. For the control selection lines, the results are pooled across all four lines, which showed very consistent high levels of ovarian dysgenesis. Cytotype in the control selection lines has remained M (high levels of ovarian dysgenesis) throughout the experiment, demonstrating that they were not accidentally contaminated with P elements.

Selection results are shown in Figure 3. Although there was clear sexual dimorphism in bristle number, the trends in male and female data were identical (Torkanmanzehi 1990). The arithmetic means across sexes are presented so that the graphs are not too cluttered. Phenotypic variances were estimated each generation as the error mean square term from analysis of variance of the male and female data from each replicate. The slight difference in the means between the transposition and control lines at G0 is due to the effect of the rosy+ transposon in the transposition lines. Independent comparison of abdominal bristle counts (data not presented) in flies with red and rosy eye color among the F2 progeny of a separate cross of the ry506 and 88-4-1 parents showed that presence of the transposon (red eye color) caused an increase of 1.1 and 0.89 bristles in females and males, respectively, similar to the difference between transposition and control lines at G0 (Torkanmanzehi 1990). It is not known whether this is due to expression of the rosy gene product, xanthine dehydrogenase, or to the site of insertion of the rosy+ transposon. Despite the potential to contribute to the selection response in an observable way in the transposition lines selected for decreased bristle number, these lines retained red eye color.

None of the control lines showed response to either up or down selection, indicative of a lack of genetic variation for abdominal bristle number in the ry506 M control line. Thus the control parent stock is apparently isogenic for all loci affecting bristle number. As the 88-4-1 P parent stock originated from ry506 by transformation (Daniels et al. 1987), the P and M parental strains have identical genetic backgrounds.
and must be co-isogenic with respect to loci affecting bristle number, differing only with respect to the \( P \) transposons which were introduced artificially into the \( P \) strain. Therefore, lines derived from the crosses \( M\delta \times P\delta \) and \( M\delta \times M\delta \) should differ only by the introduced \( P \) elements. However, the cross used to establish the transposition selection lines is dysgenic in order to induce a new round of \( P \) element transposition, because strong repression of transposition had already been established in the transformed stock 88-4-1 (DANIELS et al. 1987).

Three of the four transposition lines showed substantial responses, with the regression of cumulative response on cumulative selection differential being significantly different from zero (Table 1). The average total response across all four transposition lines was 4.8 bristles over 11 generations, which represents an average response of 0.6 bristles per generation. This is very close to the rates of response predicted and observed in outbred domestic animals for a wide range of economically important traits (SMITH 1984).

Preliminary analysis has revealed that about half of the cumulative response in the transposition downline replicate 1, which is the line with the greatest response, is due to a semidominant mutation of large effect mapping to the tip of the \( X \) chromosome. Crosses to a multiply marked \( X \) chromosome stock (our unpublished data) revealed that the locus was approximately 6 cM from the yellow locus (LINDSLEY and GRELL 1968). The mutation causes loss of bristles from many parts of the body in addition to the abdomen and, in all areas except for the abdomen, causes a distinctive and easily noticed empty bristle socket effect, particularly for the scutellar bristles. This phenotype was never observed in the parental stock 88-4-1, and was definitely absent from the flies chosen to set up the initial crosses. Furthermore, the mutation has an adverse effect on female fertility; it has been impossible to establish a homozygous stock, and the mutation was quickly eliminated from the selection line on relaxation of selection (data not shown), so it would be impossible for it to have persisted in the 88-4-1 parental stock. Additional evidence for the origin of this mutation during the course of the experiment is that the phenotypic variance in this line showed a large increase beginning at about \( G_6 \), when the mutation was first noticed, but returned to about average levels by \( G_{12} \) when the mutation had been selected to high frequency (Figure 3). It is therefore certain that the mutation occurred sometime after the cross of \( ry^{106} \) females and 88-4-1 males and not during the first round of transposition following microinjection.

Of particular interest is the extent to which new additive genetic variation has been produced each generation, namely \( V_a \). As described in MATERIALS AND METHODS, estimates of \( V_a \) were obtained from Equation 6 of HILL (1982). The conventional manner of presenting such estimates (see, for example, HILL 1982), is to divide them by \( V_E \). The resultant ratio, namely \( V_a/V_E \), is the rate at which narrow-sense heritability would increase each generation in an initially totally inbred population, due to the contribution of mutation. As shown in Table 1, the estimates of \( V_a/V_E \) range from +0.002 to +0.070 in the transposition lines, and from -0.003 to +0.003 in the control lines; the average \( V_a/V_E \) is +0.033 in the transposition lines and -0.001 in the control lines. Noting that the generally accepted value of \( V_a/V_E \) arising from spontaneous mutations at loci affecting abdominal and

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**TABLE 1**

Regression of cumulative response on cumulative selection differential, and estimation of variance components of the selection lines

<table>
<thead>
<tr>
<th>Component</th>
<th>Transposition lines</th>
<th>Control lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( U_1 )</td>
<td>( U_2 )</td>
</tr>
<tr>
<td>( b^* )</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td>( \pm SE )</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>( V_a/V_E )</td>
<td>****</td>
<td><strong>NS</strong></td>
</tr>
<tr>
<td>( V_a/V_E )</td>
<td>0.027</td>
<td>0.070</td>
</tr>
<tr>
<td>( V_a/V_E )</td>
<td>5.39</td>
<td>6.62</td>
</tr>
<tr>
<td>( V_a/V_E )</td>
<td>0.81</td>
<td>1.66</td>
</tr>
<tr>
<td>( V_a/V_E )</td>
<td>1.83</td>
<td>2.21</td>
</tr>
</tbody>
</table>

**Notes:**
- **NS** = Not significantly different from zero, after allowing for multiple comparisons (COOPER 1968).
- **** = Significantly different from zero \((P < 0.01)\).
- The value for \( V_a \) was taken as the average \( V_a \) of the four control lines, as all variance in the control lines is non-genetic. This assumes that environmental sensitivity is the same in transposition and control lines.
- Phenotypic variances \((V_a)\) are simple arithmetic means across generations and ignore obvious heterogeneity of variances particularly in the transposition lines, where there is a trend for increasing variances in two of the four lines.
- \( V_a \) is estimated from \( \delta V_P \). Where \( b \) is not significantly different from zero, \( V_a \) is set equal to zero.
- \( V_{a1} \) is estimated as \( V_P - V_a - V_E \), taking the average phenotypic variance of the control lines (2.75) as the estimate of \( V_E \).
sternopleural bristle number in *D. melanogaster* is around 0.001 (LANDE 1976; LYNCH 1988), we conclude that transposition of *P* elements has created more than 30 times as much additive genetic variation per generation as expected from spontaneous mutation. However, the rate of mutation in the transposition lines would have varied from very high at the start of the experiment to background levels by the end of the experiment.

As shown in Table 1, an overall estimate of $V_A$ was obtained for each selection line, as the product of average phenotypic variance and the regression of cumulative response on cumulative selection differential. The average $V_A$ in the transposition lines, 0.76, contrasts with the zero estimate from the control lines. As also shown in Table 1, the estimates of average $V_A$ in the transposition lines can be used to estimate the magnitude of nonadditive genetic variance, $V_{NA}$. It is evident that transposition has created substantial nonadditive genetic variance as well as additive genetic variance.

**DISCUSSION**

Both the response graphs and the $V_A/V_E$ estimates make it clear that transposition has had a very substantial effect on response to selection over the short term of this experiment. However, this effect occurred in only three of the four transposition lines, with the effect being substantially greater in the lines from the replicate 1 cross. Why was it not observed in line U2, and why was it much less in line D2? The answers to these questions may lie in the results of the ovarian dysgenesis tests (Figure 2), which show that *P* cytotype was established more rapidly and therefore cumulative transposition was likely to be substantially less in the replicate 2 lines than in the lines from replicate 1. In addition, random sampling of the elements present in 88-4-1 might have influenced both the selection results and the rate of acquisition of *P* cytotype.

How much might spontaneous mutation due to factors other than *P* elements have contributed to variation within the lines? The realizedheritabilities of the control lines show clearly that spontaneous mutation has not contributed to selection response in the controls for this experiment, and therefore is unlikely to have contributed in the transposition lines. The best estimate of the cumulative contribution of non-*P* element spontaneous mutation in this experiment is actually zero, as none of the control lines have heritabilities significantly different from zero.

It is likely that some of the response in the transposition lines can be attributed to genetic variance that accumulated as a result of transposition in the transformed stock between 1982 when it was first produced and 1988 when it was used in this experiment. However, as our main objective was to demonstrate the production of new quantitative genetic variation by transposition, it is not particularly important whether it was created during the first round of transposition, when 88-4-1 was produced and when there was no selection, or during the second round of transposition following the dysgenic cross between 88-4-1 and *ry*$.^96e$. The difference in response between the replicate 1 and replicate 2 lines does suggest that the second round of transposition was more important in this experiment and it is clear that the mutation of large effect in line D1 occurred at this stage.

The advantage of having negative controls which are "co-isogenic" with the experimental lines, enhanced our ability to detect the mutational effects of *P* element transposition on the quantitative trait abdominal bristle number. These results confirm that such an experimental design could be successfully used in detecting the mutagenic effect of *P* elements for other polygenic loci and has since been used to demonstrate the production of genetic variation for susceptibility to intoxication by ethanol fumes (FRANKHAM, TORKAMANZEHI and MORAN 1991). An important prospect for this type of design is the resolution of the controversial questions concerning the role of transposable-element-induced mutation on adaptive and evolutionary changes. While a large part of the genome remains an uncharted mystery, even in a species as well characterized as *D. melanogaster*, there is plenty of scope and great incentive for performing experiments which simply test the overall effect of transposition on the many known and even more unknown loci affecting variation for a quantitative character. For example, both this experiment and the experiment of TORKAMANZEHI, MORAN and NICHOLAS (1988), in which there has been mass transposition of many elements, have generated mutations at loci apparently not described in LINDSLEY and GRELL (1968). Finally, this experiment provides a model for the generation of genetic variation in economically important species of plants and animals.

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**LITERATURE CITED**


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