THE ROLE OF TRANSPOSABLE ELEMENTS IN GENERATING QUANTITATIVE GENETIC VARIATION.

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SUMMARY

The experimental procedures for assessing the contribution of contemporaneous bursts of transposition to short-term selection response in the *Drosophila*/P element model system are reviewed. Using the only satisfactory procedure, involving inbred M stocks and co-isogenic P stock transformants, substantial selection responses attributable to transpositional mutation were obtained for abdominal bristle number and inebriation resistance. V_M/V_E ratios for abdominal bristle number averaged 0.045 (range 0.0025-0.105) for the transposition lines, 45 times greater than the usual rate of 10^{-3} for spontaneous mutation (Lynch, 1988). Realised heritability due to transposition in these inbred lines averaged 14% (range 0-25%). For inebriation resistance/susceptibility, transposition significantly affected selection for increased time to inebriation (regression of deviation from control means on generation of 0.266±0.089, 0.245±0.109), whereas no significant response was obtained for decreased time to inebriation.

These results, obtained using stocks of flies transformed by microinjection, are of immediate relevance to domestic animal species, some of which have transposable elements which could be perturbed in a similar way. Furthermore any quantitative trait loci producing selection responses as a result of transposition are, *ipse facto*, identifiable and clonable.

INTRODUCTION

The mutagenic potential of transposable elements has been amply demonstrated in a wide range of organisms (Berg and Howe, 1989), from prokaryotes to higher plants and animals, and the significance of this endogenous source of genetic variation is now becoming more widely recognised. In *Drosophila*, the majority of spontaneous visible mutations that have been analysed at the DNA level have been found to contain inserted transposable elements (Rubin, 1983). Even in model organisms more closely related to domestic animals, there is increasing evidence for the importance of transposition. In mice, two spontaneous visible mutations, the *dilute* coat colour mutation (Jenkins et al, 1981) and the *hairless* mutation (Stoye et al, 1988) have been proven to be the result of insertion of Murine Leukemia viruses. Also there are numerous examples of visible and lethal mutations in mice induced by artificial mutagenesis with retroviral elements (reviewed in Gridley et al, 1987). Indeed there is even one economically important gene which appears to be the result of a retroviral insertion. The dominant, Z-linked slow feathering gene (K) used for sexing day old chickens is associated with the insertion of an endogenous avian leukosis virus (Bacon et al, 1988). This case highlights a potential problem, particularly where retroviruses are used for mutagenesis, since the endogenous avian leukosis virus, putatively inserted into the k locus, has a detrimental effect on the egg laying capacity of the daughters of slow feathering mothers.

However such genes of large effect are not the main source of animal improvement and no insertion mutants affecting quantitative traits in a simple manner have been recognised in domestic species, which in part is attributable to the difficulty of recognising quantitative trait loci. Thus it is necessary to use a model system to measure the contribution of enhanced levels of transposition to new quantitative genetic variation. Such studies complement and extend the analyses of the contribution of spontaneous low frequency mutation to quantitative variation (reviewed by Enfield, 1986), where with the exception of unequal crossing over in the rRNA tandon (Frankham et al, 1980), the molecular nature of the mutations is unknown. Further they permit assessment of the possible contribution of bursts of transposition to adaptive evolution in general and its potential contribution to selective improvement of domestic species.

To do this effectively an easily manipulable model system is required. The P transposable elements of *Drosophila* have been well characterised at both the biological and the molecular level (Engels, 1989). Thus they can provide a useful model for assessing the contribution of transposition to quantitative genetic variation, selection response and ultimately adaptive evolution for all higher organisms. However, even for this very well characterised system, the regulation of transposition is imperfectly understood. As a result, most of the early attempts to determine the importance of *de novo* generation of novel genetic variation by transposition have used an inappropriate experimental design. In this paper, the range of experimental procedures using short term selection that have been applied to the P element system will be reviewed and the strengths and weaknesses of the different approaches will be highlighted.

MEASURING THE EFFECT OF TRANSPOSITION

Reciprocal Hybridisation Experiments

The P element system was discovered because of the syndrome of effects, including male recombination, temperature sensitive sterility and elevated mutation rate, collectively called "hybrid dysgenesis" (Kidwell et al, 1979), which occurs in the germline of the F1 hybrid progeny of lines containing P elements (P stocks) and lines lacking P elements (M stocks). These effects are caused by the transposition of P elements (Bingham et al, 1982; Rubin et al, 1982) and occur in the germline of M♀x$P_{♂}$ hybrids, which are termed dysgenic, but not in the germline of the reciprocal P♀x$M_{♂}$ hybrids, which are called non-dysgenic. This nonreciprocity is determined by an as yet uncharacterised, cytoplasmically transmitted regulatory factor, which has been called cytotype (Engels, 1979a,b).

While it has long been known that lines established from a dysgenic cross eventually develop P cytotype, that is the ability to repress transposition, after several generations (Engels, 1983), it has generally been assumed that lines established from non-dysgenic crosses would continue to successfully repress transposition in all subsequent generations. Thus in order to assess the effect of transposition on selection response, comparisons have been made between lines established from reciprocal crosses of P and M stocks, with the "dysgenic" line representing the experimental line and the "non-dysgenic" line being the negative control (Mackay, 1984, 1985).

Despite the initial and spectacular success of this approach in Mackay's

lab, where she was able to observe consistent and large effects of transposition in her dysgenic lines selected for abdominal bristle number (Mackay, 1984, 1985), the results have not been repeatable in three independent experiments for abdominal bristle selection (Torkamanzehi, Moran and Nicholas, 1988a,b; Moran and Torkamanzehi, 1990; Torkamanzehi, 1990) nor for selection on photactic (Woolf, pers comm) or geotactic (R. Phillis, cited in Engels, 1989) behaviour. Also Mackay (1988) and Pignatelli and Mackay (1989) have failed to corroborate the enormously enhanced responses previously found in dysgenic lines when selecting for scutellar or even abdominal bristles. Torkamanzehi (1990) monitored the cytotype in his "non-dysgenic" control lines and found that they transiently lose P cytotype before gradually regaining the ability to repress transposition at a very similar rate to the dysgenic lines. *In situ* hybridisation studies using P element probes also have provided clear evidence of high levels of transposition in non-dysgenic lines (Mackay, 1988; Moran and Torkamanzehi, 1990). Thus "non-dysgenic" lines are totally unsuitable as transposition-negative control lines, and the reciprocal hybridisation experimental design is invalid, as there is no base line against which the effects of transposition can be gauged. Estimates of mutational variance obtained in this way must be disregarded.

Use of modified P elements (Δ2-3)

It is possible to modify the molecular structure of cloned P elements and then return them to their host. Since the intron between exons 2 and 3 determines the germline specificity of P transposition (Laski et al, 1986), clones have been constructed in which this intron has been deleted, and such elements mediate somatic transposition as well. One such element, which has inserted at location 99B, has apparently damaged a terminal repeat during transposition and is incapable of further transposition itself (Robertson et al, 1988). However it continues to produce large amounts of transposase in both somatic and germline cells and thus has the potential for producing high levels of transposition indefinitely. Since this Δ2-3(99B) stock was produced by microinjection into an inbred ry^{506} M stock, it is possible to devise an efficient experimental design for assessing the effect of transposition. The Δ2-3 stock is crossed with an "ammunition" stock, containing defective P elements which are mobilised by the Δ2-3 element. Since the Δ2-3 transposon is marked with a $rosy^{+}$ gene and the ammunition stock is homozygous for the ry^{506} mutation, it is possible to maintain the element in subsequent generations and ensure continuous production of transposase and continuous transposition. The transposition-negative control is initiated by crossing the ry^{506} M stock, from which the Δ2-3 stock was originally derived, with the same ammunition stock. Thus it is possible to initiate selection experiments in identical genetic backgrounds in which transposition will either be occurring at high frequency or absent.

Although this design is effective in the sense that the controls and experimental lines will undoubtedly perform as required, it suffers from the problem that transposition is so high in the experimental lines at the usual temperatures at which *Drosophila* are maintained that lethality due to somatic transposition (Engels et al, 1987) and sterility due to germline transposition cause extinction of the lines. To overcome these problems, it is necessary to maintain flies at 16°C, which is unfavourable for the long term survival of stocks and greatly retards development rates.

One experiment performed at the unfavourable temperature of 20°C produced evidence of substantial enhancement of phenotypic variance for

bristle number in the transposition lines over the very short period for which it was run (Torkamanzehi, 1990). Although the phenotypic variances across lines are clearly heterogeneous, an attempt has been made to partition the phenotypic variance in the F2 transposition line, using estimates of variance components obtained from other lines (See Table 1.). Approximately 27% of the variance is environmental, 10% is due to F2 segregation following the crossing of the Δ2-3 and ammunition stocks, 50% is due to somatic transposition and thus is non-heritable variation and 13% is heritable variation due to germline transposition.

Table 1. Phenotypic variances averaged across replicates for transposition-positive and control lines (range in parentheses), where V_E is environmental variance, V_S is variance attributable to F2 segregation, V_{ST} is variance attributable to somatic transposition and V_{GT} is variance attributable to germline transposition which is expressed in the F2.

	Transposition Lines	Control Lines
F1	8.6 (7.5-9.6) $V_E + V_{ST}$	3.0 (2.3-3.7) V_E alone
F2	11.2 (5.9-15.2) $V_E+V_{ST}+V_{GT}+V_S$	4.1 (2.4-5.8) V_E+V_S

The advantage of using elements for which transposition is restricted to the germline is very clear as the somatic transposition has simply added another component to environmental variance. However an important general message for animal breeders is that it is possible to modify the regulatory mechanisms governing transposition or other properties of transposable elements, although in this case the overly high levels of transposition coupled with the detrimental somatic effects have actually been disadvantageous.

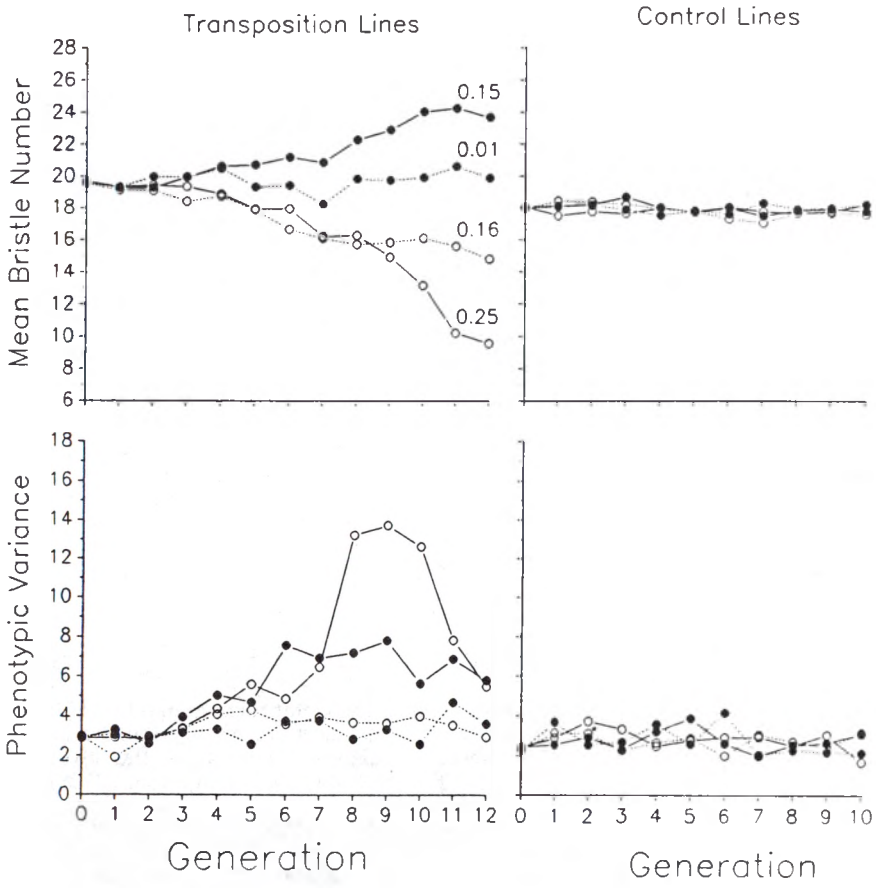
Use of transformed lines with untransformed co-isogenic controls.

Since P element transposition is being promoted here as a model for the effects of transposition in economically important species, it is important to assess the impact of introduction of intact transposable elements to the germline by microinjection, as this is the most likely mechanism for perturbing transposition rates in these other species. Even if the reciprocal hybridisation approach had been a valid method for assessing the effect of transposition, it would not provide a practical model for application in economically relevant species, since there are few other systems analogous to hybrid dysgenesis. An exception is the use of hybrids between SWR/J mice, which lack ecotropic murine leukemia proviruses, and RF/J mice, which possess three ecotropic viruses, to generate new germline insertions of retroviruses (Jenkins and Copeland, 1985). Another difficulty is that hybridisation between highly selected lines is generally unacceptable to those responsible for maintaining and improving lines of domestic animals.

Fortunately the most effective experimental design for assessing the impact of transposition on selection response is also the most relevant to this potential application in practical animal breeding. Since the production of transgenic domestic and experimental animals by microinjection of DNA is becoming increasingly common, a technique for introducing or perturbing transposable DNA is already available. Insertional mutagenesis of mice is now being routinely obtained by introduction of retroviral DNA

via microinjection and other techniques (Gridley et al, 1987). Experimental transformation of the germline of domestic chickens with avian leukosis virus has already been achieved (Crittenden and Salter, 1988) and it is likely that an effective system of insertional mutagenesis of chickens will develop from this.

Figure 1. Selection responses and phenotypic variances for abdominal bristle number in transposition and control lines. Lines were selected for increased (●) or decreased (o) bristle number, with replicate 1 (——) and replicate 2 (·····) being established from independent crosses. The slight difference in the means of transposition and control lines at generation 0 is due to the *rosy* gene, with the wild type allele expressed in the transposition lines conferring a small positive effect relative to ry^{506}. For each transposition line, realised heritability is written beside the response plot.



Following the pioneering work of Spradling and Rubin (1982), many lines of *Drosophila* have been experimentally transformed with microinjected P elements. Our model system in *Drosophila* utilised a ry^{506} M strain transformed by microinjection of complete P elements, along with a rosy^{+} marker P transposon (Daniels et al, 1987). The ry^{506} strain is inbred and the transformed P stock is co-isogenic with it, apart from the approximately 15 intact and 40 defective P elements per haploid genome. Since it was not possible to select the transformed stock during its nascent P stage, a new round of transposition was induced by crossing it dysgenically with the ry^{506} stock to establish the experimental lines. The negative control consisted of the co-isogenic ry^{506} stock. This experimental approach meets two important criteria for success, neither of which is satisfied by the reciprocal hybridisation design. These are:

i) Transposition is restricted to the experimental lines, with no possibility of transposition in the negative controls.

ii) Since there is no hybridisation between unrelated lines, the contribution of insertional mutation to selection response can be assessed independently from the effect of the substantial background genetic variance generated by hybridisation. Thus the sensitivity of detection of transposition effects is likely to be enhanced.

The results of this experimental approach have demonstrated an unequivocal and substantial enhancement of response to selection for abdominal bristle number (Figure 1; Moran and Torkamanzehi, 1990; Torkamanzehi, 1990; Torkamanzehi, Moran and Nicholas, in preparation) which is attributable to transposition. As expected, none of the inbred transposition-negative controls responded to selection, and all control h^2 estimates were close to and not significantly different from zero. By contrast, three of the four transposition-positive lines produced substantial responses to selection with highly significant realised h^2 ranging from 15% to 25%. Phenotypic variances were also substantially increased from an average of 2.75 in the controls (essentially an estimate of V_E) to an average of 4.67 across the four transposition lines. Non-additive genetic variation accounts for some of the increase in phenotypic variation (33-34% of V_P for replicate 1 lines and 4-5% for replicate 2 lines).

Application of the same experimental design and stocks to a selection experiment on inebriation susceptibility/resistance following exposure to ethanol fumes, using an automatic selection apparatus or inebriometer (Weber, 1988), has confirmed the effect of transposition on quantitative variation, although significantly enhanced response was obtained only for increased resistance (Frankham, Torkamanzehi and Moran, in preparation; Frankham, this conference).

DISCUSSION

Bursts of transposition, unlike infrequent spontaneous mutation (Hill, 1982), can affect selection response in the short term, by generating additive genetic variances within inbred populations identical to the levels found in outbred natural populations. For example, Sheridan et al (1968), using diallel techniques, and Frankham et al (1968), using selection response, estimated the h^2 for single segment abdominal bristle number in the Canberra outbred population of *Drosophila* to be 15-16%, very similar to the average h^2 of 14% attributable to transposition. The standard parameter for assessing the contribution of mutational variance to selection

response is the V_M/V_E ratio. This parameter, often called the mutational heritability, is implicitly suitable for quantifying the effect of low level spontaneous mutation in inbred populations in which V_E is large relative to V_M, but is less suitable when V_M is large, particularly if the "heritability" terminology is to be retained. In the abdominal bristle selection experiment reported here, V_M was estimated using formula (6) of Hill (1982), while V_E was estimated directly from the co-isogenic controls. The average value of V_M/V_E of 0.045 estimated from 12 generation of selection should not be taken too literally as the mutation rates are likely to vary enormously over this period, with transposition being very frequent in the early generations, but being gradually repressed to insignificant levels by about generation five, when the cytotype has become P. Thus the mutational heritability or the contribution of new mutations per generation is likely to decrease from very high early values to essentially background levels by the end of the experiment.

In situations of lack of genetic variance or depletion of variance due to prolonged selection, the artificial introduction of transposable elements as mutagens or the manipulation of transposition rates may generate sufficient variation to allow selective improvement to continue. Of course there are some actual and potential disadvantages. Transposition, whether excision or insertion, is capable of generating mutations with adverse fitness effects, just as artificial mutagenesis with radiation or chemicals does. Indeed the frequency of recessive lethals on chromosome 2 in the transposition selection lines discussed above is 32% (15/47), compared with 8% (3/36) in the transposition-negative controls (Torkamanzehi, 1990). It is not clear how much of a problem the generation of these recessive lethals may be for the population in which they arise. With the exception of a dominant mutation of large effect on bristle number in one of the transposition lines, which had severely reduced homozygous fitness, there is no strong evidence for adverse fitness effects associated with the mutations providing the selection response. The other potential disadvantage for transposable element mutagenesis as a means of generating genetic variance is that single locus mutation experiments have indicated that some loci are very refractory to such mutagenesis. Thus if there is only a small number of loci which are capable of producing variation for a particular quantitative trait and all or most of them are refractory to transposable element mutagenesis, no variation will be generated. The significance of this problem awaits extension of studies to further quantitative traits, although the two traits so far studied indicate every reason for optimism.

Of course, hybridisation or microinjection are not the only possibilities for manipulating transposition rates. Junakovic et al (1986) have shown that heat shock can be used to perturb the transposition rate of copia-like elements. McClintock (1978) has speculated on the existence of a feedback mechanism which couples transposition rates to levels of "genomic stress", which would ensure that the evolutionary potential for change would be generated to meet the requirements of a changing environment.

Finally the very nature of insertion mutation means that the loci of interest are tagged with a molecular marker which makes them amenable to cloning. Transposon tagging is probably the most important method of cloning genes of large effect in *Drosophila* and is also being applied in mice (Rinchik et al, 1986; Stoye, pers comm). There is considerable interest in extending this technique to genes influencing quantitative trait loci (Mackay, 1985; Soller and Beckman, 1987). Again the *Drosophila* model is informative. Mackay (1988) has obtained a mutant at the *smooth*

locus in one of her selection lines which contains a P insert at the appropriate cytological location of 56E and therefore would be clonable. In our laboratory, a mutation which causes a large reduction in abdominal bristle number, which has been provisionally called *tonock*, appeared in a selection line (Torkamanzehi et al, 1988a). *In situ* mapping of a P site at 2A–C (Moran and Torkamanzehi, 1990) is consistent with the position of the gene at 2B5 as determined by deletion mapping (Marsh, 1989). Further, the mutation is unstable in dysgenic conditions, which confirms that it is due to a P insert. The deletion mapping has in fact allowed us to locate the mutant locus within a region of about 60kb, which has been extensively characterised and cloned by Chao and Guild (1986), so cloning of the gene should be very straightforward. If transposon mutagenesis ever contributes to the tools of trade of animal breeders, then any genes of interest which appear in the selection lines will be amenable to similar scrutiny and possible application in transgenic programmes.

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