The Influence of P Element Transposition on Quantitative Genetic Characters of Drosophila melanogaster

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Acknowledgments

In a piece of work like this it is hard to determine where all the ideas have come from, and therefore to acknowledge the individual influences on the final work is an impossible task indeed. Some individuals have, however, had a particular influence. Dr. A. Leigh-Brown and the people in his group provided facilities, guidance and supplies which were greatly appreciated. Other members of the Institute of Animal Genetics, Edinburgh, have also provided support and stimulating discussion, and I would particularly like to thank James MacPherson and Chaoquiang Lai. Simon Montador deserves a special thank you for his assistance with the flies in my time of need, as do David Barton and Professor M.A. Ferguson-Smith of the East Anglian Regional Genetics Service, whose tolerance and support permitted me to complete the work reported in Chapter 5. Finally, I'd like to thank April, for all her help, support and tolerance of my erratic commas.
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Abstract

Experiments designed to quantify the effects of P transposable elements mobilised in dysgenic crosses on quantitative phenotypic variation in the fruit fly Drosophila melanogaster are reported.

Comparison of previously isogenic IIind chromosomes exposed to P elements in dysgenic (female parent lacking elements) or non-dysgenic (female parent carrying elements) hybrid flies indicated that there was significantly more variation among the former. Estimates of induced mutational variance $V_M/V_E = 0.06$ and 0.37 were obtained for the abdominal and sternopleural bristle scores among the dysgenic chromosomes, which also showed a significant 25% reduction in viability relative to the controls.

In a second series of experiments, IIind and IIIrd chromosome pairs, isolated from dysgenic $F_1$ flies, were compared to originally isogenic chromosomes which had been isolated using balancer strains lacking P elements. Three independent comparisons of the variation in these groups were made, and a combined estimate of $V_M/V_E = 0.124 \pm 0.03$ calculated for the abdominal bristle score. On average, a line was found to contain 3.5 elements, giving an estimated $V_M/V_E = 0.035 \pm 0.006$ for single homozygous inserts. Regression of squared deviations of line means from the population mean gave estimates of the effect of a homozygous insert of approximately 2% of the environmental variance.

One quarter of the dysgenic derived chromosomes were recessive lethal when isolated, 0.1 appeared near lethal, and a significant reduction of 10% viability was found among a sample of the remainder, compared to uncontaminated controls. These effects were found to be correlated with the number of elements in the lines, and estimates of a 3% $\pm$ 2.4% drop in viability and 0.06 lethals per insert were made.

None of the monitored lines converted to P cytotype during the 12 generations for which they were observed, although several harboured active P elements.
Chapter 1.
A Brief History

1. Introduction

"I have hitherto sometimes spoken as if variations were due to chance. This is, of course, a wholly incorrect expression, but it serves to acknowledge plainly our ignorance of the cause of variation" (Darwin, 1859).

Quantitative genetics, the study of characters that show a non-discrete distribution of phenotypes, is one of the most and at the same time one of the least understood branches of modern genetics. Since the dawn of agriculture, man has been tailoring the physiology and anatomy of his domestic animals and plants to suit his needs, by artificially enhancing or inhibiting the survival and reproduction of individuals. One hundred years have passed since Galton (1889) laid the foundation of what was to become modern quantitative genetics. In this time elegant biometrical theories have been produced that accurately predict the behaviour of such traits in populations exposed to the selective pressures of the environment as well as the activity of man. However, we still know virtually nothing about the nature and function of the specific genes that determine the genetic component of observable variation.
It is generally assumed that the non-discrete variability found in quantitative traits, such as height and weight, results from the action of many genes, each of tiny individual effect (polygenes), in combination with the truly continuous effects of a variable environment (Wright, 1968). The power of traditional genetic analysis, and the molecular approaches that have led to our understanding of how some major genes operate, critically depend on the ability to delimit unambiguous phenotypic groups into which individuals can be classified. Once made, such a subdivision allows the identification of the common genetic entities shared within each group and their study at whatever level is required from the simple gross phenotypic description, through genetic localisation to molecular cloning and the identification of the gene product or enzyme pathway affected. For polygenic systems the problem of identifying individual classes is, at least in part, one of terminology, for if the definition of the polygenes as of tiny and equal effect (Mather, 1941) is strictly adhered to, then any allele whose effect is large enough to detect is, ipso facto, not part of the polygenic system. It seems unlikely that this definition was ever meant to be more than a gross simplification, and if some polygenic loci are allowed to harbour alleles of moderately large effect, even if
only rarely, then examination of the system becomes merely a problem of detecting these "effective factors" (Mather and Jinks, 1971; in line with these authors this term will be used in the following discussion wherever the effect isolated could equally well be determined by n linked loci of effect xσ/n as it could by a single locus of effect xσ) and then studying the variants that are more normally resident at the revealed loci.

There is some evidence that mutations that result in alleles of large effect occur at a lower frequency than those with small effects (Gregory, 1965) and theory suggests that such large alleles are also removed quickly from populations as a result of pleiotropic fitness effects (Lande, 1983). It therefore seems likely that, although several hundred loci could perhaps influence a given trait either directly or pleiotropically, and that many, if not all, could harbour alleles of drastic effects, no given population will harbour more than a handful of effective factors at any one point in time. This means that any attempt to examine the nature of polygenic loci using variation present in a population may well be severely limited in the number of loci that could be identified.

The alternative approach, using mutagens to generate effective factors de novo, has the advantage that it is not limited to detecting those loci that have alleles
of large effect segregating in any particular population. However, the alleles, once created, have still to be detected using the same laborious methods that are applicable to the study of variation already present in a population, and thus have not represented that large an advantage. However, the recent 'discovery' of transposable elements (TEs) and their mutagenic effects promises to open the field of quantitative genetics to the detailed study that has so far been reserved for loci with major allelic effects. The single largest advantage of transposable elements is that, when a mutation is created by the insertion of an element, that element remains at the site of the lesion acting as a beacon for the detection and isolation of the region of the chromosome that has been damaged.

As a prelude to the work of this thesis, the next section will briefly review the state of our knowledge to date (1989). This will be followed, in Section 1.3, by an overview of the evidence that suggests the importance of TEs as mutagens not only in the laboratory, but also in nature. Section 1.4 presents a description of, and arguments for the use of, the P element as a tool for the study of quantitative genetics, and Section 1.5 reviews the work that has so far been done with this system.
Three of the questions that have bedevilled the study of quantitative genetics are:

1. How many loci are (or can be) involved in the determination of population variation?
2. How are the loci arranged on the chromosomes?
3. What is the shape of the distribution of effects between different alleles within a locus and between separate loci and how is the observable effect of allele substitution related to the activity of the genes?

Although the earlier work in this field was mainly concerned with plants, much of the recent material deals specifically with one organism, *Drosophila melanogaster*; for this reason most of the following discussion will centre on this particular animal.

**Number of loci**

When considering how many loci are involved in the control of variation, a distinction must be drawn between the number that *could* be involved and the number actually involved in any particular population. The former will be much the larger of the two and, from the point of view of determining the rate at which new variation is added to the population, a much more important one (since animal and plant development is in
effect a series of interacting genic cascades, it may be that all loci could affect all traits to some extent). The latter, although smaller and possibly less important in the long term because it will be a population specific value dependent on the previous history of the population studied, is, in principle at least, a measurable characteristic. Lande (1981) has summarised the results of experiments in which inbred lines were hybridized and the variation in the $F_1$, $F_2$ and backcross compared to that expected from biometric models (Wright, 1968) to estimate the number of genes involved in any differences between the two original populations. The results suggest figures of around 5-20 factors being involved in the difference between populations for characters such as fish eye diameter and maize kernel oil content, although values in excess of 90 have been reported (Dudley, 1977).

It seems likely that these values are severe underestimates, since the method assumes that both lines are fixed only for factors acting in the direction of the difference between them, that the loci affecting the trait are unlinked and that all loci are additive in effect. All these assumptions are likely to be violated under the experimental conditions used, resulting in underestimates of the number of factors involved (Serebrovsky, 1928; Dempster and Snyder, 1950; Wright, 1968). Since the method is only capable of
estimating a maximum of $nr$ factors (where $n =$ the number of linkage groups and $r =$ the number of recombination events per chromosome per generation; Darlington, 1937) it would seem that the estimates are certainly consistent with a large number of segregating factors, although this is not conclusive.

In systems where recessive marker genes exist, it is possible to link the effect of particular factors to single chromosomes or to particular regions of the chromosome (Thoday, 1979). The largest limitation on this method is the availability of suitable marker alleles that do not interfere with the trait examined and are close enough together to prevent the factors from dissociating from them due to double crossovers. This means that this method has almost exclusively been confined to the study of *D. melanogaster*, and in particular to the study of bristle traits.

In a series of experiments, Thoday and co-workers (see Thoday and Thompson, 1976) assigned the difference in sternopleural bristle score between pairs of laboratory lines to about 9 major factors. However, similar studies have indicated that much larger numbers of factors may well be involved with this particular trait; Davies (1971) and Louw (1966) both found at least 8 factors on the IIIrd chromosome alone (generally considered to be about 40% of the genome) and Piper (1972) identified at least 5 in a short 18 cM
region of this chromosome between the genes *sepia* and *scarlet*. In what is perhaps the most detailed study of this type, Shrimpton and Robertson (1988a,b) found a total of at least 18 factors, ranging in effect between the detection threshold of 0.4σ and 2σ, in the same short region studied by Piper (1972), with many other factors distributed along the rest of this chromosome.

The small population sizes and intense selection pressures used to create the divergent lines in the earlier experiments are conditions that favour the fixation of relatively few genes of large effect (Lande, 1983) and this may be the simplest reason for the smaller estimates from these studies. In contrast Shrimpton and Robertson (1988a) analysed a high chromosome selected from a hybrid population that was itself derived from two lines that had independently undergone more than 20 generations of selection. Not only should this procedure have maximised the value of this chromosome, and the probability of all factors on it acting in the same direction (although even in this study, at least one factor with the opposite effect appears to have become fixed), but it should also have maximised the number of loci responsible for the chromosomes' effect.

As with the biometric analyses above, the estimates from this approach, roughly 10-200, are consistent with very large numbers of alleles potentially being
involved in population variation. The development of marker systems based on the phenotypically neutral restriction enzyme fragment length polymorphisms (RFLPs) (Botstein et al, 1980) promises to open other species to study, including those of economic importance e.g. tomatoes (Paterson et al, 1988). However, as with the biometrical approach, chromosomal mapping by this method will always be limited to characters where lines differing by several standard errors exist, and can only identify a small fraction of the possible effective factors resulting in a severe underestimate of the number of factors that could influence a trait.

Distribution of loci.

There is little evidence on the overall pattern of the loci controlling quantitative traits. What does exist suggests that they are usually spread widely throughout the genome (Sax, 1923; Breese and Mather, 1957; Paterson et al, 1988; Thompson and Thoday, 1979; Falconer, 1981), although the distribution by no means appears random. Instead, the factors that have been identified tend to form clusters that appear to be relatively closely linked on small regions of the chromosomes (Shrimpton and Robertson, 1988a; Spickett, 1963; Davies, 1971). Why this is the case is unclear, but various molecular studies have also shown
that genes apparently controlling the same or similar systems tend to occur in closely linked regions: for example; major thoracic bristle effects associated with the achaete-scute region (Campuzano et al, 1985), segment pattern genes associated with the bithorax region (Bender et al, 1983) and muscle development genes associated with the mdx region of the mouse X chromosome (Heilig et al, 1987).

Distribution of effects.

Virtually nothing is known about the distribution of effects either within a locus or between loci - this despite the importance of this parameter in many of the current theoretical models (see for example Keightley and Hill, 1987; Turelli, 1984; Hill, 1982b).

Shrimpton and Robertson (1988b) present a distribution of effects that looks very leptokurtic, although they were unable to detect effects accurately below about 0.5σ and, as stated, this type of work tends to exaggerate the numbers of large effects due to the selection procedures used. As to the symmetry of the distribution about zero, this obviously depends on the trait concerned; on the one hand, most mutants have a negative effect on the fitness of a stock, while on the other, mutation does not appear to alter the mean of a population for bristle traits and most selection work for these traits appears symmetrical overall.
suggesting that the mutations for these traits are more or less symmetrically distributed about zero. This is in direct contrast to the pattern shown for major effects where, for example in a survey of major mutants on the IIInd, 36 were found that reduce bristle score but only 13 that increase it (Mackay personal communication, based on a survey of Lindsley and Grell, 1968).

Mutations

In the above discussion, only methods that take advantage of the variation already present in the population were considered. These methods will miss all those major alleles that have either never existed or have been lost or fixed in the population prior to the commencement of the experiment. The study of de novo variation has the advantage that this variation is as amenable to study as that already in the population; it is also of intrinsic interest, since it may well be the single most important force in the maintenance of observable variation, as has been emphasised in recent theoretical treatments of natural (Lande, 1976; Barton, 1986; Lynch and Hill, 1986; Cockerham and Tachida, 1987) and artificial selection (Hill, 1982b, Keightley and Hill, 1983,1987: Hill and Rasbash, 1986).
Since the number of loci that could contribute to a trait is an unknown quantity, estimates of per locus mutation rate cannot be used in a meaningful way. An alternative is to consider the trait in terms of the number of new mutations generated per chromosome or genome per generation (the gametic mutation rate) (Mukai, 1964; Bateman, 1959; Mukai et al, 1972) or in terms of the amount of new variation \(V_N\) that is accumulating in each generation relative to the amount of environmental or background variation (Clayton and Robertson, 1955).

Estimates of gametic mutation rate are rare, but those that have been performed indicate that around \(10^{-2}\) is not an unusual number (Hoi Sen, 1972; Russell et al, 1963; Mukai, 1964; Mukai et al, 1972). This at first sight seems somewhat high given the estimates of effective factors outlined above and mutation rates of \(10^{-7}\) to \(10^{-5}\) for individual loci (Auerbach, 1976; Mukai, 1970; Tobari and Kojima, 1972). It is possible that the high gametic mutation rates are actually an artifact of the experimental technique, since it makes several untenable assumptions about the nature of the loci, including poisson distribution along the chromosome. It seems more likely that for some traits they are in actual fact only slight overestimates. This is because the limitations of the biometrical and linkage analyses mean that many
more than 100 loci might be responsible for standing variation, that these probably represent only a small fraction of the loci that can mutate in any one generation and that only 1,000 loci mutating at a rate of $10^{-5}$ are required to explain the estimates. For viability 1,000 mutable loci is probably a reasonable estimate, since there are estimates for *Drosophila* of around 5,000 lethal mutable genes and a total of around 30,000 genes, many of which are presumably able to harbour viability affecting alleles (quoted in Turelli, 1984), but for other traits, such as bristle score, this may be somewhat less tenable. Another alternative is that per locus mutation rates to polygenic alleles are much higher than to protein mutations. This might be the case if it is assumed that polygenes are often the result of control mutations that could occur in the 'junk DNA' surrounding the structural locus as well as within the locus itself, while the mutations causing major effects and affecting the electrophoretic mobility of the protein can only occur in the smaller region of the structural locus itself (for discussion see Mukai and Cockerham, 1977).

Estimates of mutation rates based on the increment of variation added to the population each generation ($V_m$), while providing less information about the genic changes underlying the variation, are experimentally
simpler to measure and are of greater importance in the
determination of the balance between selection, drift
and the maintenance of population variance.

Estimates of $V_m$ can be obtained from the response to
selection of a population that has been severely
inbred, and can be assumed to be nearly totally
homozygous. Using such a system, estimates of around
$3 \times 10^{-4}$ to $10^{-3}$ times the variance resulting from the
environment have been obtained for abdominal and
sternopleural bristle score in *D. melanogaster* (Mather
and Wigan, 1942; Clayton and Robertson, 1955, 1964;

Alternative approaches, using balancer chromosomes
to extract chromosomes and accumulate mutations over
several generations while protecting the mutated
chromosomes from recombination and natural selection,
have been used to obtain estimates for bristle traits
(Durrant and Mather, 1954; Paxman, 1957) and viability
(Mukai, 1964; Mukai et al, 1972; Ohnishi, 1977; Mukai
and Yamazaki, 1968; and Cardellino and Mukai, 1975).
The results are variable, but similar to those obtained
for selection experiments giving estimates of $10^{-3}$ for
non viability traits and around $10^{-2} - 2 \times 10^{-1}$ for the
viability estimates (for a full review see Lynch
(1988)).

Given the potentially leptokurtic distribution of
effects, factors that are large enough to detect and
isolate may in fact be relatively rare events. One way of increasing the yield of 'useful' mutations is to increase the mutation rate. This approach has been applied using ionising radiation, but the results appear to be somewhat contradictory.

Scossiroli and Scossiroli (1959) selected sternopleural bristle scores with concurrent X-irradiation, and found vastly increased rates in those lines irradiated each generation, but most of this could have been explained as the result of rare mutants of large effect and the lack of a control population makes interpretation very difficult. Yamada and Kitigawa (1961) and Kitigawa (1967) carried out selection for abdominal bristle and found relatively similar but somewhat lower results (around $2 \times 10^{-4}$/Roentegen) as did Hollingdale and Barker (1971) from an inbred and Jones (1967) selecting from a non-inbred base population. Using a chromosome contamination method, Tobari and Nei (1964) obtained estimates that were around ten times as high. Clayton and Robertson (1955, 1964) found lower results (roughly $6$ and $2 \times 10^{-5}$ per Roentegen per generation), but since they irradiated prior to selection, a process that allows natural selection to remove any detrimental alleles, this is hardly surprising. The estimates obtained by Hollingdale and Barker (1971) and Kitigawa
(1967) when combined give a rough estimate of $V_M/V_e \approx 3 \times 10^{-3}$ per generation per 1000 Roentegens.

Unfortunately, both X-rays and EMS induce considerable levels of lethal and near lethal mutation at relatively low dose rates (Simmons and Crow, 1977) and, while this has been used to study the genetics of the trait viability, this means that the creation of high levels of mutation in other traits is limited to doses at which the organism can survive. Also the mutations once created must be identified and isolated as if they had arisen spontaneously. Recently observations have been made that suggest that about 10-15% of the eukaryotic genome is composed of sequences that appear to be mobile and that these appear to be involved in the generation of spontaneous mutations (Rubin, 1983). In fact these may represent a powerful biological mutagen, as is suggested by the data summarised in the next section, which may be useful in the generation of high levels of new mutation. Further, a mutation created by the insertion of an element retains the element at the site of damage making identification and isolation relatively easy (Bingham et al, 1981).
1.3. Element-induced mutations.

This section presents a survey of some of the evidence that exists for the importance of transposable elements as mutagens. The table below is intended to give some indication of the range of phenotypes that can be induced by transposable elements. It is a rather eclectic survey and is by no means a complete list either of loci or of mutants at any particular locus. Very few P induced mutations created by dysgenesis are included, not because of a lack of information, but because to do so would have greatly increased the size of the table, and a detailed survey is given by Kidwell (1987).

Table 1. Element related mutation in Eukaryotic species

<table>
<thead>
<tr>
<th>Allele</th>
<th>Insert/element</th>
<th>notes</th>
<th>reference</th>
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<tr>
<td>Drosophila melanogaster</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>step</td>
<td>5.2kb</td>
<td>'spotted' phenotype, inserted in an intron</td>
<td>1</td>
</tr>
<tr>
<td>st1</td>
<td>7.6kb</td>
<td>disrupts RNA transcript</td>
<td>1</td>
</tr>
<tr>
<td>wa</td>
<td>Copia</td>
<td>inserted 2nd intron-</td>
<td>2</td>
</tr>
<tr>
<td>wc</td>
<td>Copia</td>
<td>reduces all RNA transcripts</td>
<td>2</td>
</tr>
<tr>
<td>wsp1</td>
<td>B104/Roo</td>
<td>inserted 1kb 5' to gene</td>
<td>3, 2</td>
</tr>
<tr>
<td>wsp3</td>
<td>Copia + 500bp</td>
<td>deletion of previous insert</td>
<td>2</td>
</tr>
<tr>
<td>wsp55</td>
<td>5.8kb</td>
<td>plus new small insert</td>
<td>4</td>
</tr>
</tbody>
</table>
$w^{DZL}$

14kb(DZL) a composite element with two FB elements and some unique DNA between-reverts at very high frequency 4

$wbf\text{1}$

$B104/Roo$ unstable leaky insert in 4th intron

$wbl$

blood inserted 2nd intron - reduces RNA transcript to 25% 2,5

$wc$

FB 6

$wa$

Copia 4

$wa^4$

BEL 4,7

$w^1$

pogo 4

$w1R2$

I revertant of $w^1$ due to insert of I element 10

$wh$

$B104/Roo$ revertant of $w^1$ 8

$w2N$

7.5kb(S18) unstable mutant 3,4

$waRM$

2.3kb reversion of $w^a$ inserted into 5' Long terminal repeat of Copia 9

$waR84e19(13)$

6kb revertant of $w^a$ 9

$waR79127$

4.5kb revertant of $w^a$ 9

$waR84h$

83bp revertant of $w^a$ homology to I element 9

$w^i$

F 3

$bx^1$

412 11

$bx^3$

gypsy + Doc only gypsy seems involved in the mutant expression 11

$bx^{3+4e}$

gypsy 12,11

$bx^9$

gypsy 13,11

$bx^{AV}$

gypsy 13,11

$bx^6$

gypsy 13,11

$bx^{83kb}$

gypsy 13

$bx^{d1}$

gypsy 11

$bx^{55i}$

gypsy 11

$bx^{51j}$

gypsy 11

$bx^dK$

3.4kb + gypsy 11

$bx^d^9$

? 11

23
bxdsR

Ubxs

cmph2PN10

cmph2

scI

gypsy

scD1

gypsy + ? gypsy similar position to

scD2

gypsy

scL3

gypsy + ? gypsy similar position to

sc3B

gypsy + gypsy LTR

ac phenotype due to insert

of gypsy in region of RNA

transcript 6, at sc locus there is

a 0.5kb insert with homology to

LTR, probably site of reversion

from original insert

sc1744

? inserted into non transcribed DNA

scA

? inserted into non transcribed DNA

v1

412

v2

412

vk

412

v36f

B104/Roo

AntNS

B104/Roo

f1

gypsy

CP3

HMS Beagle

inserted into 5' promoter

region

yIR3

I truncated I element cause reversion

of y1

yIR4

I truncated I element cause reversion

of y1

y2

gypsy

inserted 700bp 5' to RNA cap site-

effect due to gypsy sequences
interfering with normal transcription 17,16,25

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Notes</th>
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<tbody>
<tr>
<td>y^{2PR1}</td>
<td>Jockey</td>
<td>partial revertant of ( y^2 ) inserted into \textit{gypsy} 16</td>
</tr>
<tr>
<td>y^{2PR2}</td>
<td>Hobo</td>
<td>partial reversion due to insert in \textit{gypsy} 16</td>
</tr>
<tr>
<td>( y^3d )</td>
<td>7kb</td>
<td>inserted 5' to the start of transcription 25</td>
</tr>
<tr>
<td>( y^{2S} )</td>
<td>4.5kb</td>
<td>inserted 5' to the start of transcription 25</td>
</tr>
<tr>
<td>( y^{2D} )</td>
<td>3.5kb</td>
<td>inserted 5' to the start of transcription 25</td>
</tr>
<tr>
<td>( y^{td} )</td>
<td>7kb</td>
<td>inserted 5' to the start of transcription 25</td>
</tr>
<tr>
<td>( y^{V2} )</td>
<td>?</td>
<td>complex mutant, insert near start of transcript 25</td>
</tr>
</tbody>
</table>

\( y \) type 1 (14) \( P \) dysgenic induced mutants - 2 inserted 5', 8 inserted in the transcribed but not translated region and 3 within the coding region, 1 is a 4kb deletion. 25

\( \textit{Hsp}^{28st1} \) \( P(\text{induced}) \) inserted in 5' region between TATA box and RNA cap site alters developmental expression 19

\textit{Drosophila mauritiana} \( \textit{wpch} \) 1.3kb white eyed reverts to wild type at sex specific rates 28

\textit{Drosophila ananassae} \( \textit{sn9g} \) \( \text{tom} \) 29

\( \textit{OM} \) \( \text{tom} \) series of optical morphology mutants at 20 sites 29,30

\textit{Antirrhinium majus} \( \textit{nivea} \) \( \text{Tam 1} \) inserted 5' in promoter region unstable 34

\( \textit{chs} \) \( \text{Tam 3} \) inserted into first exon/ intron
<table>
<thead>
<tr>
<th><strong>Species</strong></th>
<th><strong>Gene</strong></th>
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<tbody>
<tr>
<td><em>pallidarecurrans</em></td>
<td>Tam 3</td>
<td>inserted just upstream to the transcription start site- unstable</td>
<td>35</td>
</tr>
<tr>
<td><em>Gallus domesticus</em></td>
<td>U1 CR1</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td><em>Caenorhabditis elegans</em></td>
<td>10 x unc-54 Tc1</td>
<td>identified in a screen for spontaneous mutations</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2 x unc-86 TC3</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>2 x unc-22 TC3</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td><em>Mus musculus</em></td>
<td>pun</td>
<td>pink eyed dilution mutant-unstable, ≈ 6% somatic reversion</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>W4?J</td>
<td>White spotted, variable coverage, rest of coat grey - shows somatic reversion to wild type (≈11.4% in some backgrounds)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>W2?J</td>
<td>stable in normal background - 8% somatic reversion when outcrossed</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>pe</td>
<td>unstable coat colour, somatic reversion (≈6% when outcrossed from original background where stable)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Va</td>
<td>46% somatic reversion in heterozygote mice</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Miwh</td>
<td>5% somatic reversion to wild type</td>
<td>23, 22</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>DBA ectopic Bal VN(HP-9) associated with the gene- revertants lose the hybridisation site</td>
<td>32</td>
</tr>
</tbody>
</table>

**Key to sources:**

1. Tearle *et al.*, 1989
2. Pirrotta & Brockl, 1989
3. O'Hare *et al.*, 1984
4. Zacher & Bingham, 1982
5. Bingham & Chapman, 1986
7. Goldberg, Sheen & Gehring, 1983
9. Mount, Green & Rubin, 1988
11. Bender et al, 1983
13. Peifer & Bender, 1986
15. Finnegan et al, 1987
17. Searles & Voelker, 1986
19. Eissenberg & Elgin, 1987
21. Melvold, 1971
23. Schaible, 1969
25. Chia et al, 1986
27. Eide & Anderson, 1986
29. Shrimpton, Montgomery & Langley, 1986
31. Snyder et al, 1982
33. Herman & Shaw, 1987
35. Upadhyaga et al, 1985

6. Collins & Rubin, 1982
8. O'Hare, 1986
10. Fawcett et al, 1986
12. Bender & Meselson, 1983
20. Parkhurst and Corces, 1985
22. Witney and Lamoreux, 1982
24. Campuzano et al, 1985
30. Hinton, 1984
34. Bonas et al, 1984
36. Coen et al, 1986

For details of the named elements see Finnegan and Fawcett, 1986, for *D. melanogaster* and the named references for the others.

Some of the spontaneous mutants in the table were studied because of their strange phenotype, for example *w*<sup>bl</sup> and *w*<sup>DZL</sup>, and therefore represent a non random sample of the mutants at that locus. However, if we concentrate our attention on those studies where the mutants were screened at random, we obtain figures of 4/5 spontaneous revertants of the *w*<sup>a</sup> allele (Mount, Green and Rubin, 1988), 7/10 spontaneous type 2 mutants
at the yellow locus (Chia et al, 1986), 13/13 cytologically normal mutants at the bithorax complex (Bender et al, 1983), 7/11 point mutations at the achaete-scute complex (Campuzano et al, 1985) and 10/12 cytologically normal mutants at the white locus (Zacher and Bingham, 1982) appear to result from the insertion of transposable elements. These are comparable to the estimates of 5/7 spontaneous mutants at the yeast ADR locus (Williamson, Young and Ciriacy, 1981) and 10/18 spontaneous mutants in the myosin heavy chain (unc-54) of the Bergerac strain of Caenorhabditis elegans (Eide and Anderson, 1988) having arisen from genetic insertions. Should these figures prove to be representative of other loci and organisms then the influence of TEs on rates of spontaneous mutations may be very great indeed.

From those loci where the exact position and effect of the insert has been studied, it appears that TEs generate most of their effect, not by the simple action of their insertion, but rather by a more complicated interaction of their internal sequences, particularly transcription start sites, with the normal expression of the genes (eg. Tearle et al, 1989). Changes in developmental timing of expression (Eisenberg and Elgin, 1987; Kelley et al, 1987), the level of transcript produced and its tissue specificity (Eisenberg and Elgin, 1987; Chia et al, 1986; Tsubota
and Schedl, 1986; Daniels et al, 1985) and the nature of the interactions between loci (Harrison et al, 1989) have all been recorded as a result of element mutations. The phenotype of individual mutants and their interaction with other loci depends on the type of element inserted (Biessman, 1985; Chia et al, 1986; O'Hare et al 1984), its size and position (Tsubota et al, 1985; Tsubota and Schedl, 1986) and can be modified by the excision of part of the element (Voelker et al, 1984; Tearle et al, 1989) or the insertion of other elements within or alongside the mutation-causing element (Rubin et al, 1984; Engels, 1988; Mount Green and Rubin, 1988), or at an unlinked site in the genome (Modolell, Bender and Meselson, 1983; Harrison et al, 1989).

Among both spontaneous and P induced mutations, there tends to be a predominance of elements inserted into the 5' control or transcribed but untranslated regions of the genes. This may be a bias derived from the fact that mutations in these sites could be more likely to survive or to be detected, but it is a possibility that these sites, which are close to the start of transcription, may be more vulnerable to insertion of the elements as a result of the secondary structure of the DNA.

P element mutations appear to be very similar to those associated with other elements suggesting that
they represent a good model for the type of effect that TEs have in general. Among the spontaneous mutants outlined above, P inserts appear to be rare. Although this may merely be an artifact, it seems more likely that this is evidence for the recent invasion by this element family of the *D. melanogaster* population; at some point after the period when most populations that have been studied in enough detail to identify mutants were isolated from the wild.

1.4. Biology of the P element

In this section an outline of the P family of elements will be given (this will be brief and the reader is referred to Engels, 1988 for a more detailed coverage) along with some of the reasons for its choice as an experimental system.

So far the TEs described in *melanogaster* appear to fall into four major classes; retroposons (including I,F and Doc elements), Copia-like (including copia, blood and gypsy elements), Elements with long inverted terminal repeats (including FB and BS elements) and those with short inverted terminal repeats (including P and hobo elements) (for full description of this classification and the elements see Finnegan and Fawcett, 1986).
Although many of these families are mobile, or have been so in the recent past, as for example indicated by variable site occupancy (Strobel et al, 1979; Rubin, 1983; Ajioka and Eanes, 1986; Montgomery and Langley, 1983; Leigh-Brown and Moss, 1987; Montgomery, Charlesworth and Langley, 1987), they are normally mobilised at rates that are too slow to be utilised over the course of a normal mutagenesis experiment. The P family of element (which may be recent invaders of the D. melanogaster genome, as indicated by their absence from the sibling species and old laboratory strains (Kidwell, 1983; Brookfield et al, 1984)) has been found to mobilise at particularly high rates under conditions that are now fairly well understood (Engels, 1988).

This family consists of a group of elements that range in size from 0.5 kb to 2.9kb and share considerable homology to a 2.9kb P factor, from which the smaller elements may have been derived by internal deletions (O'Hare and Rubin, 1983; Voelker et al, 1984). This element was first recognised by its effect on the F₁ hybrids from certain crosses of wild caught strains with long established laboratory ones (eg. Slatko and Hiraizumi, 1973; Kidwell, Kidwell and Nei, 1973; Green, 1977; Sved, 1976). In these crosses the offspring shared a syndrome of traits including temperature dependent sterility and male
recombination in the F1, major chromosome abnormalities and high mutation rates at particular loci in the F1 and subsequent generations. Although recorded earlier, it was Kidwell, Kidwell and Sved (1977) who finally suggested these phenomena were part of a single system and pointed out that they were only encountered in flies derived from crosses in which the wild caught strain was the male parent, and not in the reciprocal crosses. Hence Paternally contributed factors were to be distinguished from Maternally contributed factors. Genetic characterisation of the strains indicates that hybrid dysgenesis results from elements which map to multiple sites on the chromosomes of the P strain flies (Engels, 1979). These sites also appear to be hotspots for chromosomal rearrangements (Simmons and Lim, 1980; Engels and Preston, 1981; Berg, Engels and Kreber, 1980) and mutations (Simmons et al, 1984 a,b; Tsubota and Schedl, 1986). The cloning of the DNA insert responsible for a dysgenic-induced white mutation (Bingham, Levis and Rubin, 1981) identified this element as a member of a dispersed, repetitive family found in up to 50 copies per haploid genome in P strain flies, but lacking from M strain flies (Bingham, Kidwell and Rubin, 1982).

Far from being separated into merely two classes, strains of melanogaster have been found with many gradations of ability to induce and control hybrid
dysgenesis in inter-strain crosses. Strains can be genetically categorised by crossing them to well-characterised tester strains; two separate protocols exist.

1. GD tests score the proportion of dysgenic ovaries among $F_1$ females from two crosses. To test the $P$ activity levels of the unknown strain, males are crossed to females from a standard $M$ strain such as Canton-S. $P$ cytotype is tested for using crosses of the unknown females to males from a standard $P$ strain such as Harwich.

2. The $sn^w$ assay utilises a hyper-mutable allele of the $singed$ locus (Engels, 1979b). This mutant arose in a dysgenic individual, and appears to be the result of two inserted $P$ elements which produce flies with twisted or kinked thoracic chaetae. Under dysgenic conditions, excision of one of these can occur, resulting in either a more extreme ($sn^e$) or a phenotypically wild type ($sn^+$) allele. The rate at which excision occurs is indicative of the strength of the unknown $P$ strain (when used as a male parent crossed to $(M)sn^w$ females), or its ability to repress $P$ activity (when used as a female parent crossed to $(P)sn^w$ males).

The results from either of these two sets of tests can be combined, plotted and used to determine the
classification of the unknown strain, as shown in Figure 1.

Figure 1.1 The phenotypic relationships between P, Q and M strains in the hybrid dysgenesis tests.

The classification of a line is dependent on the complement of elements that it carries. The strength of a P strain is related to the total number of elements and the ratio of complete, 2907bp elements (the P factor which is capable of producing the 87kD protein required for transposition) to the smaller deletion derivatives which retain the 31bp repeats
necessary for transposition but lack the ability to produce an active transposase (Simmons and Bucholz, 1985; Bingham, Kidwell and Rubin, 1982). Q strains contain very low numbers of P factors, but high numbers of deletion derivatives, and fully repress P activity (Kidwell, 1985; Engels and Preston, 1981). M' strains usually harbour no P factors and a variable number of the smaller elements (Simmons and Bucholtz, 1985; Bingham et al, 1982), although some appear to contain internal restriction fragments normally associated with the intact factor (Black et al, 1987; Jackson, 1988).

This raises the question of what mechanisms control P element transposition. Even in dysgenic flies, activity of the elements is restricted to the germ line cells, apparently as a result of differential splicing of the RNA transcripts, which correctly removes the 2-3 intron only in this tissue (Laski, Rio and Rubin, 1983). In flies with the P cytotype, it appears that even mobility in this tissue is repressed. It has been suggested that P cytotype, which is maternally inherited, results from the production of repressor molecules, either from the P factor itself, or from the deleted elements. To account for the maternal nature of the inheritance pattern, extra-chromosomal cytoplasmic elements or positive regulatory feedback have been proposed (Simmons and Bucholz, 1985; O'Hare
and Rubin, 1983). However, there is at this time no conclusive proof of the exact mechanism by which repression is achieved (see Engels, 1988 for review), but there is evidence that the repression is not complete and that P elements do mobilise in some lines with the P cytotype, albeit at a fraction of the rate found in dysgenic flies (Preston and Engels, 1984).

In some European and Asian M' and Q strains, repression appears to be chromosomally inherited, one example being sexi (Kidwell, 1985). In these strains, one particular deletion derivative has been found at high copy number (Jackson, 1988). This element has been cloned, and it has been suggested that it is responsible for the repression found in these strains (Black et al, 1987; Jackson, 1988). The exact action of this element is unknown, but it is unlikely that it produces a protein capable of competitively excluding the functional transposase from the 31bp terminal repeats, since it lacks the sequences thought to produce the DNA binding domains (Rio et al, 1986; Black et al, 1987).

Regardless of the exact nature of the repression under normal conditions, the simplicity with which it can be removed in dysgenic flies and the resultant high levels of mobilisation constitute the main advantages of the P family for mutagenic purposes. However, to be suitable for the creation of quantitative variation,
the elements must cause damage more or less at random throughout the genome. This does appear to be the case, although, in common with other transposable elements, P elements do show some preference for particular insertion sites. At the level of the DNA, an 8bp sequence GGCCAGAC appears to be a favoured site for insertion; in some cases, however, the affinity seems weak (O'Hare and Rubin, 1983). The secondary structure may also play a part, since detailed analysis has shown that insertions cluster in DNAase hypersensitive sites (Tsubota et al, 1985).

It would appear that around 50% of all genes contain sites which are amenable to P insertion damage (Kidwell, 1986; Searles et al, 1982) and, for lethals at least, these appear to be distributed at random along the chromosome (Eeken et al, 1985; Cooley, Kelley and Spradling, 1988). Some loci are particularly sensitive to P elements, for example, claret (Kidwell, 1986) and singed (Green, 1977), while others seem totally refractory, such as the Adh locus (Engels, 1988). On average, a rate of $10^{-4}$ mutations per generation per locus seems applicable (Simmons et al, 1984; Kidwell, 1986). This suggests that the rate of $V_M$ should be increased by a minimum factor of $100/2 = 50$ above the spontaneous rate.
1.5. P elements as polygenic mutagens.

In this section, I outline the evidence so far gathered as to the efficacy of the P family as a mutagen of quantitative traits.

Viability and recessive lethals

Balancer chromosomes have been used to accumulate mutations in a similar manner to that of Mukai (1964) for spontaneous, and Ohnishi (1977) for EMS induced mutations. Yukuhiro, Harada and Mukai (1985) extracted 100 P strain IInd chromosomes (from the π2 stock) using an (N) Cy/Pm balancer stock. These chromosomes were back-crossed to an M strain for 9 generations to homogenise the background, then made homozygous. During the back-crossing, 47 lines were lost. Among the remaining 53 chromosomes, they estimated 0.067 recessive lethal mutations per generation, and a reduction of 0.64 in viability per 'mutational' event. Since secondary mutagenesis may have occurred, leading to chromosomal rearrangements, deletions, and loss of previously inserted elements, it is impossible to say much about the relationship between this induced variation and insertion of the elements.

Fitzpatrick and Sved (1986) similarly passed P (Harwich) IInd chromosomes through a single generation
of dysgenesis, extracted them using a Q balancer strain, then measured the total fitness of the resultant mutated chromosomes using the cage tests of Sved (1971). Here again, a substantial decrease in fitness (10-20% per generation) was observed when compared to similar chromosomes passed through the reciprocal cross with a high rate (9%) of the chromosomes harbouring lethals. Unfortunately, secondary mutagenesis of the elements resident on this chromosome cannot be ruled out. Simmons et al (1980) detected 3% lethals as having been generated on \( \pi_x \) chromosomes similarly passed through a dysgenic generation.

Mackay (1986) removed some of the error due to secondary mutagenesis by passing M strain (Canton-S) IInd chromosomes through dysgenic and non-dysgenic crosses, then isolating the mutated chromosomes by back-crossing to a (P) Cy/Pm stock for 9 generations. Total fitness, viability and female productivity were found to be similar in both these populations but were considerably reduced by comparison with the original M chromosomes in an independent M background. Since the control population differed in genetic background from both the contaminated populations, it is difficult to assess the accuracy of the reduction in viability observed (28% for the chromosomes exposed to dysgenesis). Also, the back-crossing may have
permitted more mutation than assumed since, as already
discussed, P elements are mobile in the P cytotype. 
However, since the control chromosomes harboured no
recessive lethal alleles the large number found in both
sets of contaminated lines (around 25% of lines
contained such genes) indicates that the elements are
indeed creating a large decrease in the fitness of the
flies. Similarly, Simmons et al (1985) obtained
estimates of 0.67% of their M derived X chromosomes
passed through dysgenesis as having gained new
recessive lethals.

The most recent, and the only experiment in which
the phenotypic effects are related to the actual number
of new insertion events, passed marked X chromosomes
from an isochromosomal M strain through a single
generation of dysgenesis (Eanes et al, 1988). Of the
592 chromosomes examined, 9 were found to harbour
lethals, giving an estimate of 0.015 lethals per
generation. 114 of the non-lethal chromosomes were
selected at random and assayed for viability, and the
number of new inserts was determined for a sub-sample
of 49 lines. Significant differences in viability
were found, and a regression of viability on number of
inserts gave an estimate of 1% drop in viability per
insertion, with an average 3.26 ± 5.8 inserts per
generation.
While comparisons are difficult to make between such widely divergent experimental procedures, particularly given the different chromosomes used and the existence of strain-specific differences in mutability (Simmons et al., 1980, 1984b; Exley and Eggleston, 1989), the general conclusions are that P elements decrease the mean and increase the variance of viability and fitness. The higher estimates obtained when chromosomes carrying P elements are exposed to dysgenesis suggest that secondary mutagenesis is a more powerful reducer of fitness than insertional mutagenesis. This is not unexpected, since even an element inserted in a benign site can generate a viability-reducing mutation in an adjacent gene when undergoing imprecise excision. It is also noteworthy from the above, that those experiments in which estimates of induced lethals were obtained from the autosomes (Yukuhiro et al., 1985; Mackay, 1986; Fitzpatrick and Sved, 1986) give considerably higher estimates than those obtained from mutation on the X (Eanes et al., 1988; Simmons et al., 1980; Simmons et al., 1985), and this will be taken up again in Chapter 5.

Other traits

The study of P-induced variation in traits other than fitness and its components has produced somewhat variable results. Assuming that an F1 population
will contain more variation if derived from the dysgenic cross than the non-dysgenic cross, it might be expected that selection from the former population should lead to a greater early response. Mackay (1984, 1985) studied two replicates selected for high and low abdominal bristle score and found higher response in both directions in the dysgenic derived lines. However, Torkamanzehi, Moran and Nicholas (1988) and Pignatelli and Mackay (1989), selecting for abdominal bristle score, and Pignatelli and Mackay (1989), selecting for sternopleural bristle score, found considerably less clear-cut results with higher response rates in the 'controls' as often as in the dysgenics. Taking these experiments together, it seems that approximately 1/3 of all the replicate populations showed higher response rates than might be expected and that, in general, these took the form of rapid responses over a few generations. Such a pattern is consistent with the occurrence of rare alleles of major effect, and indeed Mackay (1988) found element-induced mutants of smooth, which had apparently occurred independently, in 3 out of 4 low lines from her 1985 experimental lines. It is expected that cytotype repression, which is ultimately determined by the chromosomal complement of elements, might lessen in the F₂ and later generations of the non-dysgenic lines as a consequence of segregation of elements. Under
these conditions, it is hardly surprising that elevated responses are also detected in the 'controls', particularly since element movement is detectable albeit at low levels in even strong P cytotype strains (Preston and Engels, 1984). It then becomes almost impossible to interpret these results, other than to say that they represent some evidence of P elements' involvement in quantitative variation.

Better estimates of the elements' effects have been obtained using chromosome contamination and extraction procedures which allow more than one trait to be examined at once. This approach was used by Mackay (1986, 1987), and the results are summarised for the two bristle traits studied, in Table 1.2.
Table 1.2. Results of previous analyses of P element mutagenesis

<table>
<thead>
<tr>
<th>Cross</th>
<th>method</th>
<th>estimate of $V_M/V_e$ (Abdominal)</th>
<th>estimate of $V_M/V_e$ (sternopleural)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>M contam</td>
<td>0.067</td>
<td>0.186</td>
<td>Mackay, 1987</td>
</tr>
<tr>
<td>MP</td>
<td>M contam</td>
<td>0.151</td>
<td>0.310</td>
<td>&quot;</td>
</tr>
<tr>
<td>PM</td>
<td>M contam</td>
<td>0.014</td>
<td>0.0</td>
<td>Lai &amp; Mackay 1989</td>
</tr>
<tr>
<td>PM</td>
<td>P contam</td>
<td>0.109</td>
<td>0.298</td>
<td>&quot;</td>
</tr>
<tr>
<td>MP</td>
<td>M contam</td>
<td>0.137</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>MP</td>
<td>P contam</td>
<td>0.314</td>
<td>0.629</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Key

MP = dysgenic cross  PM = nondysgenic cross  M contam means contaminated chromosomes M at start, P contam means chromosomes harbouring P elements were passed through the cross.

The results (from Mackay, 1987) given above show considerable extra input of variation from the activity of the elements but the differences between the dysgenic and the reciprocal cross were not significant. These values were estimated from (M) strain IIInd chromosomes passed through a single generation of dysgenesis or the reciprocal cross followed by 9 generations of back-crossing to a P strain to homogenise the background. This procedure, as already mentioned, may have allowed several generations of element movement and this may have
masked any original difference between the reciprocal crosses, while elevating the final estimate of induced mutation. Also, the contaminated and the M strain control population had a different genetic background, making interpretation even more difficult.

Lai and Mackay (1989) have investigated variation on inbred M strain and P strain X chromosomes passed through dysgenic and non-dysgenic flies, compared to M strain X chromosomes without contamination in the same background. The results for abdominal bristles show a clear and dramatic increase in variance, but the results for sternopleural score among M strain chromosomes are somewhat surprising. It would appear that P chromosomes are, on the whole, more susceptible to P mutagenesis than M strain chromosomes, and that dysgenic crosses result in about 3 times as much variation as the non-dysgenic crosses. Again, as with the selection results, the variation, particularly among the P chromosome derived lines, appears to be the result of a small proportion of lines with extreme phenotypes; in this case, they all appear to have low bristle score, and could therefore be accounted for by mutation at a single locus or a small number of loci which contained P elements in the original stock or are particularly susceptible to P element insertion.
Aims

From Table 1.2, it is apparent that the P element can create high levels of variation. However, the exact scale of the insertion effects is uncertain, being only estimable from the work of Lai and Mackay (1989), which was not available at the outset of this thesis. The initial experimental work of this thesis, reported in Chapter 2, extends the work of Mackay. It is primarily an attempt to identify whether or not there is a difference in the level of variation induced by the elements in dysgenic and reciprocal cross $F_1$ flies. The chromosome extraction protocol of Mackay (1986) was modified by removing the potentially confounding generations of backcrossing. Although ostensibly an improvement on the earlier experiment this design generates its own problems and does not allow a precise estimate of the amount of new mutational variation induced.

The second set of experiments largely overcame the problems by extracting 80% of the total genome as homozygous contaminated chromosomes using two specially constructed multiply-inverted-chromosome balancer stocks. These results are reported in chapters 3 and 4, and provide good estimates for the amount of extra variation induced on the $M$ chromosomes in dysgenic flies over and above that created by spontaneous processes.
The problem of attaching estimates of error to the calculated variance values was overcome by designing the experiments in such a way that several independent estimates of the variance could be made and compared to give empirical standard errors. This involved following the lines for several generations, during which the activity of the elements within the lines was monitored by genetic crosses. A molecular investigation, reported in Chapter 5, examined the relationship between the observed phenotypic changes and the element numbers present in a sample of the lines.

Chapter 6 provides an overview of the results and discusses them in relation to the position of Transposable Elements in evolution and in the future study of the molecular nature of quantitative variation.
CHAPTER 2.

Dysgenesis vs Non-Dysgenesis

2.1. Introduction

Elements have been found to be closely associated with many spontaneous major morphological mutants (see, for example, Kidd et al, 1983; Zachar and Bingham, 1982; Bender et al, 1983; Scoet et al, 1983; Coté et al, 1986), and P-induced mutagenesis may represent one route by which some insight into the biology of polygenes can be gained.

Although it is now clear that P elements can create considerable reductions in fitness when mobilised in dysgenic crosses, uncertainty exists over their influence on other traits (see Chapter 1). Indeed, even for fitness characteristics there is variation in the size of the detected effect, which appears to be related to the number of generations used in the isolation of mutations for study (see Chapter 1).

The estimates of induced mutation rates from selection experiments (eg. Mackay, 1985a; Torkamanzehi, Moran and Nicholas, 1988) are unlikely to be valid since the experiments do not allow for the segregation of chromosomes after the initial generations and the resultant induction of element mobility that probably occurs at similar rates in both populations.

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The chromosome extraction procedure used by Mackay (1986, 1987) to study sternopleural and abdominal bristle scores and fitness and its components, detected no difference between a set of Intro chromosomes extracted from dysgenic $F_1$ flies and a similar population extracted from the reciprocal cross, although both populations differed considerably from the original chromosomes extracted into a different $M$ strain background (e.g. 0.25 of the dysgenic lines harboured lethal genes). As discussed in Chapter 1, it seems possible that movement of elements within both sets of contaminated lines during the backcross generations may have masked any difference in the original cross, and may also have affected the estimates of induced $V_M$.

The experiment reported in this chapter set out to establish whether there actually is a detectable difference in the level of new variation induced by $P$ elements when introduced to hybrid $F_1$ flies on the maternal or paternal chromosomes. It was decided to replicate the experiment of Mackay (1986), but to restrict the number of generations after the initial cross to the minimum required to establish homozygous lines. To reduce background variation, a long inbred $M$ strain was used as the donor of the chromosome to be contaminated.
A large decrease in relative viability and an increased level of variation in both abdominal and sternopleural bristle score was detected in the dysgenic lines relative to the non dysgenic lines. However, the values for $V_M$ obtained could be subject to confounding effects of environmental variation, as outlined in the discussion.

2.2. Methods and Materials.

Stocks

**M strains**

**SAM;** An inbred stock derived from a long established Samarkand laboratory population, which had previously been inbred for more than 75 generations followed by an unknown number of generations of random mating in bottle culture, by approximately twenty generations of full sib mating. This population is of the M cytotype and lacks both intact and deleted $P$ elements, as shown by Southern transfer experiments and single fly squash blots.

**SAM $\overset{\wedge}{XX};** An attached-X strain (C(1)DX, Lindsley and Grell, 1968) marked with the recessive alleles *forked* ($f$), *white* ($w$) and *yellow* ($y$). The autosomes of the original strain were replaced by chromosomes isogenic with the inbred SAM population described above by...
backcrossing M $\overset{\text{X}}{\text{X}}$ females to male full sibs from the SAM population for around 14 generations.

**P strains**

P Cy/Pm; This strain was created from a standard M strain balancer stock (Lindsley and Grell, 1968) by independent backcrossing of Cy/+ and Pm/+ $\varnothing\varnothing$ s to strong P strain (Harwich) $\varnothing\varnothing$ for seven generations (see Mackay, 1986). The P Cy/Pm stock was then constructed by crossing these two strains. This stock had been established for eighteen generations prior to the commencement of the experiment, and had a strong P cytotype.

**Cytotype assays**

In the two generations prior to the initiation of the experiment the presumed cytotypes of the above stocks were checked as outlined below.

**sn$^w$ test - P activity**

To test for the presence of fully functional P factors, males of the strain under test were crossed to M strain females carrying the highly mutable sn$^w$ allele (Engels 1979a, 1981a). This allele results from the insertion of two partially deleted P elements into the singed bristle locus (Karess and Rubin, 1984) creating a gene that has a weak singed phenotype.
This allele is stable when in the M cytotype background, as in this strain. However, when males from a P strain are crossed to M strain females carrying it, the resultant permissive (dysgenic) condition in the F₁ offspring can lead to mobilisation of one or other of the resident elements resulting in the reversion of the gene to an allele with the normal phenotype, or one that is more extreme. The frequency of such events is high (up to 60% of F₁ chromosomes can be revertants) and varies with the "strength" of the male strain so that it can be used to characterise that strain with regards to P factor activity (Engels, 1984; Karess and Rubin, 1984; Spradling and Rubin, 1982). To establish the character of a stock, small batches (between 5 and 10 males) were crossed to similar numbers of the M snʷ females. The male progeny from this cross were then mated to females from an attached X strain and the frequency of the extreme singed (sn") and wild type (sn⁺) chromosomes scored in the males of the next generation.

snʷ test - P cytotype

Since the highly mutable snʷ allele is also stable when in the P cytotype, it is possible to test for the ability to control P element mobility by crossing males from a P strain with the allele to females from the strain under test (Engels, 1979a). Small batches of
the progeny from such a cross were allowed to mate inter-se and the frequency of the \( sn^e \) chromosomes determined, relative to \( sn^w \) in the next generation. This test characterises the unknown females' ability to repress P element transposition (P cytotype).

Southern Blotting

Since it is now known that a large number of strains that test as M actually contain P DNA (these are designated as M' or pseudo-M strains (Bingham, Kidwell and Rubin, 1982), and may even harbour complete P factors (Black et al. 1987), whole genomic DNA was extracted from these strains, restricted, separated by horizontal gel electrophoresis, transferred to nylon filters (Hybond, Amersham international) and probed with \( P^{32} \) labelled DNA (nick translation according to the protocol of Rigby et al, 1977; for detailed protocol see Beech and Leigh-Brown, 1989).

Generation of the experimental lines

The initial crosses, both dysgenic (M\( \varphi \times P\delta \)) and non-dysgenic (P\( \varphi \times M\delta \)), consisted of two mass matings of twenty pairs of 2-7 day old virgin flies. From each cross three consecutive egg collections were obtained. Each collection period lasted for two to three days, depending on the density of eggs laid; at this stage, and in all subsequent stages, attempts were
made to establish equal larval densities in all vials and bottles.

All male $F_1$s appearing during the first three days of eclosion were collected from each vial and pooled for the setting up of the next generation eight days from the date of first eclosion, to give a generation interval of 26-28 days. By selecting at random from those males which had been eclosed for between zero and eight days, it was hoped that any effect of male age on mutation rate would be spread evenly over all lines. Each male selected from the pool was individually mated to six $(P)$ Cy/Pm females (a non-dysgenic cross) to found an $F_1$ line.

From each line two egg collections of four days' duration were made, and all Cy/+ individuals collected during the first four days of eclosion from each vial. The progeny from these two vials were then mixed on day 26, 27 or 28, and two replicates, each of six males and six females crossed inter se, established on day 28.

From each of these replicate sets of parents two consecutive egg collections of around four days were made and the progeny collected and recorded separately from each of the vials.

The analysis treated the effect of the egg collection period (vials) as a fixed effect nested within the random effect of replicate, which was itself nested within the random effect of line. A suitable
ANOVA was then performed on the data, and the total observed variation partitioned into the individual components assumed to arise from each of these sources.

Figure 2.1. Crossing scheme to generate contaminated IInd chromosome lines following a single dysgenic cross. Only the IInd chromosomes are shown, see also text.

<table>
<thead>
<tr>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dysgenic</strong></td>
</tr>
<tr>
<td>$G_0$</td>
</tr>
<tr>
<td>$G_1$</td>
</tr>
<tr>
<td>$G_2$</td>
</tr>
<tr>
<td>$G_3$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Non-dysgenic</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_0$</td>
</tr>
<tr>
<td>$G_1$</td>
</tr>
<tr>
<td>$G_2$</td>
</tr>
<tr>
<td>$G_3$</td>
</tr>
</tbody>
</table>

Key: Cy/Pm a balancer stock contaminated with P elements by T. Mackay. The $XX(SAM)$ stock is a standard attached X stock whose autosomes are isogenic with a long inbred M stock. Mutated chromosomes are marked *. For more details of stocks refer to the text.
Culture Conditions

All experimental crosses were performed in 3" x 1" vials containing approximately 10 ml of a standard sucrose/ killed yeast medium seeded with 4-5 grains of bakers' yeast (J.S. Sainsbury's dried yeast). To minimise gonadal dysgenesis the crosses were all performed at 20°C ± 1°C in a temperature controlled incubator. All sn" assays were carried out at 22 ± 0.5°C in a similar incubator.

Characters scored

From each line at this third generation, the following data were recorded:

- The proportion of wild types relative to the total number of individuals from the first eight days of eclosion from all vials in which at least 20 eclosed during this period.

- The number of bristles on the posterior abdominal sternite in 5 male and 5 female Cy/+ and wild type individuals, in those lines with enough flies in all four vials.

- The combined number of bristles on the left and right sternopleural plates from the same 80 flies.

Estimates of Vg in the base population

The Cy/Pm strain used in this experiment had been maintained as an outbred bottle culture, and it was
thought relevant to establish the amount of additive genetic variance within it, to gain some indication of its contribution to the overall variation. This estimate was obtained from the regression of mean offspring score on mean parental score.

210 pairs of individuals were selected at random from the base population, scored for both bristle traits and randomly assigned to food vials, one pair per vial. On day 19 after setting up, two males and two females were selected from each of the 185 vials with live progeny. These individuals were scored for both bristle traits and the family mean estimated.

2.3. Results

Estimation of $V_g(p)$ in the Cy/Pm

The regressions of mean offspring score on mid parental score were $b=0.224$ for abdominal bristle and $b=0.141$ for sternopleural bristle score and, as shown in Table 2.1, these were significantly different from 0. An estimate of the $V_g(p)$ (additive genetic variance) in the stock can be obtained from the covariance of offspring on midparent (Falconer, 1981). Using values of $V_{abdominal} = 1.54$ and $V_{sternopleural} = 0.58$ from the parental generation, estimates of additive genetic variation present in this line were obtained of $0.344$ for the variation in abdominal
bristles and 0.082 for the sternopleurals. These values are low, but may contribute to the background variation. No estimate of $V_A$ from the covariance of sibs was obtained, because common environmental variance due to all members of a sibship having been raised in the same vials would have a confounding influence, leading to an elevation of the estimate above the true value for the segregating variance.

Table 2.1 Analysis of variance (ANOVA) for the regression of offspring mean on mid parent score.

<table>
<thead>
<tr>
<th>STERNOPLEURAL</th>
<th>Source</th>
<th>df</th>
<th>SS.</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>regression</td>
<td>1</td>
<td>24.488</td>
<td>24.488</td>
<td>41.8 ***</td>
</tr>
<tr>
<td></td>
<td>unexplained</td>
<td>183</td>
<td>107.082</td>
<td>0.5851</td>
<td></td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>184</td>
<td>131.570</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ABDOMINAL</th>
<th>Source</th>
<th>df</th>
<th>SS.</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>regression</td>
<td>1</td>
<td>37.319</td>
<td>37.319</td>
<td>16.8 ***</td>
</tr>
<tr>
<td></td>
<td>unexplained</td>
<td>183</td>
<td>405.719</td>
<td>2.217</td>
<td></td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>184</td>
<td>443.038</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(***P<0.01)
Viability

Of the 149 dysgenic and 103 non dysgenic lines set up at G₃, only 90 and 72 respectively provided sufficient flies (a minimum of 20 Cy/+ flies from each of the four vials that comprised the line) for estimation of viabilities. The distribution of line means are shown in Figure 2.2. This data set is based on a mean number of 278 flies scored per line.

Since the Cy chromosome carries recessive lethals, the Cy/+ x Cy/+ cross is expected to form non lethal +/+ and Cy/+ zygotes in the proportion 1:2. Observed alterations to this ratio in the newly eclosed flies can therefore be interpreted as due to differences in the egg to adult survival of the two genotypes, and the expected ratio can be written as; (1-r)v : 2r, where r is the proportion of Cy/+ flies eclosing and v the viability of homozygotes relative to the heterozygote. Assuming the mean viability effect of the Cy chromosome to be relatively similar in all lines, (an assumption based on the observation of a higher degree of recessiveness among more extreme fitness modifiers, (Simmons and Crow, 1977)), the observed proportion of wild type to Cy/+ flies can be used to estimate the homozygous viability of that line's chromosome relative to a 'standard' genotype. The relationship is:

$$v = \frac{2(1-r)}{r}$$
Figure 2.2 Histogram of viabilities (line means) estimated from the average number of flies hatching from two vials per replicate, as outlined in the text.
As Figure 2.2 and line one of Table 2.2 show, there is a large, significant difference in the mean viability between the two populations ($x_1 - x_2 = 0.26$, $P<0.001$, Mann-Whitney rank test for unpaired samples). These distributions lack the high frequency of lethal lines and show a higher number of lines with slightly reduced viability (quasinormal lines) than have been found associated with P activity in other studies (Yukuhiro et al 1985, and Mackay, 1986 for instance). This will be considered in more detail in the discussion. In fact, one of the dysgenic lines may have been lethal, but since very few flies eclosed from this line (a total of 50 Cy/+ individuals from the four vials combined) it is impossible to rule out the possibility that it was actually a viable but weak line.
Table 2.2 Overall means and standard deviations, pooled over replicates, for the dysgenic and non dysgenic populations.

<table>
<thead>
<tr>
<th>TRAIT</th>
<th>dysgenic n=90</th>
<th>non-dysgenic n=90</th>
</tr>
</thead>
<tbody>
<tr>
<td>viability</td>
<td><strong>64% (34%)</strong></td>
<td><strong>89% (30%)</strong></td>
</tr>
<tr>
<td>abdominal bristle score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>20.2 (2.62)</td>
<td>20.2 (2.54)</td>
</tr>
<tr>
<td>Cy/+</td>
<td>18.6 (2.51)*</td>
<td>19.1 (2.36)*</td>
</tr>
<tr>
<td>sternopleural bristle score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>21.4 (1.81)*</td>
<td>21.0 (1.63)*</td>
</tr>
<tr>
<td>Cy/+</td>
<td>20.4 (1.68)</td>
<td>20.3 (1.59)</td>
</tr>
</tbody>
</table>

* indicates that a test of $\bar{x}_{\text{dysgenic}} - \bar{x}_{\text{non dysgenic}}$ was significantly different from that expected by chance at the P<0.05 level; ** = significant at the P<0.001 level. Standard deviations were estimated as the square root of the variance between line means as calculated by the ANOVAs described in the text.

Table 2.3 indicates that the amount of variation in viability between the lines is similar in the two populations, although slightly higher among dysgenic lines. The between vial mean square is much larger in the dysgenic population than in the non dysgenic, and this appears to result from the second vial in each replicate in many of the dysgenic lines having a lower
viability than the first (a fall from 0.71 to 0.56), although again this may have arisen by chance.

Table 2.3 Results of an Analysis of the variation in viability between and within lines of the dysgenic and non-dysgenic derived lines

<table>
<thead>
<tr>
<th>Source</th>
<th>DYSGENIC df</th>
<th>MS</th>
<th>significance</th>
<th>NON-DYSGENIC df</th>
<th>MS</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between lines</td>
<td>88</td>
<td>1168.86</td>
<td>***</td>
<td>70</td>
<td>905.98</td>
<td>***</td>
</tr>
<tr>
<td>Between reps</td>
<td>89</td>
<td>457.45</td>
<td>ns</td>
<td>71</td>
<td>223.97</td>
<td>ns</td>
</tr>
<tr>
<td>Between vials</td>
<td>1</td>
<td>17824.05</td>
<td>***</td>
<td>1</td>
<td>1816.22</td>
<td>*</td>
</tr>
<tr>
<td>Error</td>
<td>177</td>
<td>578.29</td>
<td></td>
<td>141</td>
<td>310.30</td>
<td></td>
</tr>
</tbody>
</table>

The model used for this analysis fitted lines and replicates as random effects and vials (egg collection period) as a fixed effect.

Bristle scores

Table 2.2 indicates that the overall means of the two populations appear to be similar for both bristle traits. However, Figures 2.3 and 2.4 suggest that there are some differences between the two populations of wild type flies for both abdominal and sternopleural score, but that these are not statistically significant.
Figure 23. Frequency plot of line means for abdominal score, split by genotype.

**Dysgenic lines**

**Non Dysgenic lines**

<table>
<thead>
<tr>
<th>Bristle score</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy/+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4. Frequency plot of line means for sternopleural bristle score, split by genotype.
Estimation of Mutational Variance (\(V_M\));

By applying the theory of Lynche and Hill (1986) it is theoretically possible to estimate the mutational effects in both the dysgenic and non dysgenic lines from the within and between line components of variance. However, apart from chromosome II the genetic background of the lines consists of a mixture of sequences derived from either the (P)Cy/Pm or the (M)SAM base stocks. Variation in these genes will contribute to the observed variation.

Variation will arise from the proportion and positions of the P and M regions in the flies (segregational variation) and from variation present within each region in the original stocks (residual genetic variation). It is more or less impossible to derive a satisfactory description for the expected variation from these sources, since the effect of the resident P elements on the frequency and distribution of crossover events is unknown, but thought to be non-trivial (Lim, 1979; Berg Engels and Kreber, 1980; Engels and Preston, 1981a; Goldberg et al, 1983).

However, since the distribution of elements should be similar in both the dysgenic and non dysgenic lines, subtraction of the variation between non dysgenic lines from that between dysgenic lines should remove most of
this confounding effect from that level of the analysis.

The IIInd chromosome

The crossing scheme means that this chromosome (approximately 0.4 of the genome) is entirely inbred SAM in origin and therefore contributes nothing to the background variance between lines.

Effect of mutations

It is assumed that any mutation arising in the P stock will have been removed by subtraction of the control line values from the dysgenic mean squares. New mutations arising on the IIIrd and IVth chromosomes of the $F_1$ flies may well contribute to the response, but since they will mainly be segregating within lines, their contribution to the $V_M$ estimates is assumed to be negligible. All the males within a line share half of the mutations generated on the $F_1$ male's X and such mutations may therefore contribute a small component to the between line variance. This source of error will be treated in more detail in the discussion.

As can be seen from the crossing scheme, any mutations arising on the M derived IIInd chromosome will become homozygous in the analysis generation. They will therefore contribute a component of $0.4(V_M)$ to the
between line variance, where \( V_M \) is the amount of new variation from spontaneous and P-induced mutation.

Any mutations occurring in the \( G_2 \) flies will be distributed at random between replicates within lines and may contribute to the component between replicates if parents do not contribute offspring equally to the next generation.

Table 2.4 Summary Analysis of Variance (ANOVA) Table for the bristle score results. Vials were nested within replicates within lines (all random) and sex was removed as a fixed effect.

**ABDOMINAL bristle score**

### Dysgenic

<table>
<thead>
<tr>
<th>Level</th>
<th>df</th>
<th>MS</th>
<th>Component(SE)</th>
<th>df</th>
<th>MS</th>
<th>Component(SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet line</td>
<td>89</td>
<td>5.56</td>
<td>0.03 (0.03)</td>
<td>14.81</td>
<td>0.20</td>
<td>(0.06)</td>
</tr>
<tr>
<td>B rep</td>
<td>90</td>
<td>4.46</td>
<td>0.08 (1.36)</td>
<td>6.78</td>
<td>0.14</td>
<td>(0.10)</td>
</tr>
<tr>
<td>B vial</td>
<td>180</td>
<td>2.85</td>
<td>0.01 (2.72)</td>
<td>4.08</td>
<td>0.04</td>
<td>(0.21)</td>
</tr>
<tr>
<td>Error</td>
<td>3239</td>
<td>2.73</td>
<td>2.73 (0.07)</td>
<td>3.68</td>
<td>3.68</td>
<td>(0.09)</td>
</tr>
</tbody>
</table>

### Non Dysgenic

<table>
<thead>
<tr>
<th>Level</th>
<th>df</th>
<th>MS</th>
<th>Component(SE)</th>
<th>df</th>
<th>MS</th>
<th>Component(SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet line</td>
<td>68</td>
<td>5.18</td>
<td>0.05 (0.04)</td>
<td>10.39</td>
<td>0.17</td>
<td>(0.07)</td>
</tr>
<tr>
<td>B rep</td>
<td>69</td>
<td>3.25</td>
<td>0.0[-] (na)</td>
<td>3.54</td>
<td>0.0[-] (na)</td>
<td></td>
</tr>
<tr>
<td>B vial</td>
<td>138</td>
<td>3.49</td>
<td>0.06 (0.56)</td>
<td>5.34</td>
<td>0.20</td>
<td>(2.45)</td>
</tr>
<tr>
<td>Error</td>
<td>2383</td>
<td>2.91</td>
<td>2.91 (0.09)</td>
<td>3.34</td>
<td>3.34</td>
<td>(0.10)</td>
</tr>
</tbody>
</table>
### STERNOPLEURAL bristle score

#### Dysgenic

<table>
<thead>
<tr>
<th>Level</th>
<th>df</th>
<th>MS</th>
<th>Component(SE)</th>
<th>df</th>
<th>MS</th>
<th>Component(SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet line</td>
<td>89</td>
<td>8.06</td>
<td>0.11 (0.04)</td>
<td>1375</td>
<td>0.25 (0.05)</td>
<td></td>
</tr>
<tr>
<td>B rep</td>
<td>90</td>
<td>3.80</td>
<td>0.02 (0.14)</td>
<td>3.89</td>
<td>0.02 (0.26)</td>
<td></td>
</tr>
<tr>
<td>B vial</td>
<td>180</td>
<td>3.48</td>
<td>0.09 (0.27)</td>
<td>3.57</td>
<td>0.06 (0.52)</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>3239</td>
<td>2.57</td>
<td>2.57 (0.06)</td>
<td>2.96</td>
<td>2.96 (0.07)</td>
<td></td>
</tr>
</tbody>
</table>

#### Non Dysgenic

<table>
<thead>
<tr>
<th>Level</th>
<th>df</th>
<th>MS</th>
<th>Component(SE)</th>
<th>df</th>
<th>MS</th>
<th>Component(SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet line</td>
<td>68</td>
<td>2.90</td>
<td>0.01 (0.04)</td>
<td>5.36</td>
<td>0.04 (0.13)</td>
<td></td>
</tr>
<tr>
<td>B rep</td>
<td>69</td>
<td>2.67</td>
<td>0.0[-] (na)</td>
<td>3.36</td>
<td>0.0[-] (na)</td>
<td></td>
</tr>
<tr>
<td>B vial</td>
<td>138</td>
<td>2.89</td>
<td>0.07 (1.77)</td>
<td>4.50</td>
<td>0.21 (0.67)</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>2383</td>
<td>2.21</td>
<td>2.21 (0.07)</td>
<td>2.44</td>
<td>2.44 (0.08)</td>
<td></td>
</tr>
</tbody>
</table>

A direct estimate of the extra variation caused by P activity in the dysgenic flies can be obtained by subtracting the components of variation between non dysgenic lines (Table 2.4) from those between the dysgenics. This gives an estimate of $0.4V_{M_{dy}}$.

This estimate is directly comparable with those obtained in the previous studies that have used non dysgenic controls, and gives a good estimate for the variation produced in this study. Estimates of $V_M$ (calculated as 2.5 times the difference between the two
sets of components) and $V_{M}/V_{e}$ for the dysgenic population are shown in Table 2.5.

Table 2.5 Estimates of $V_{M}$ and $V_{M}/V_{e}$ from the data in table 2.4, calculated as outlined in text.

<table>
<thead>
<tr>
<th></th>
<th>ABDOMINAL</th>
<th>STERNOPLEURAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{M}$</td>
<td>0.187 (0.097)</td>
<td>0.518 (0.067)</td>
</tr>
<tr>
<td>$V_{M}/V_{e}$</td>
<td>0.062 (0.097)</td>
<td>0.370 (0.067)</td>
</tr>
</tbody>
</table>

$V_{M}$s were estimated as outlined in the text. $V_{e}$s were obtained from the original inbred M strain under similar conditions (sternopleural=1.4, abdominal=3; Mackay personal communication). Values in brackets represent one SE assuming random normal variances, calculated from

$$\sqrt{\frac{\text{Var } MS_{dys} + \text{Var } MS_{non dysg}}{2(n^2)}}$$

2.4. Discussion

Viability

The apparent lack of lethal chromosomes among the 252 extracted here is in contrast to the high rates that have been reported in some previous experiments outlined in Chapter 1. One feature of the previous work is that the per generation rates based on single
generation experiments appear to give lower estimates than those obtained from multiple generation experiments e.g. Eanes et al (1988) reported 1.5% lethal chromosomes generated on X chromosomes exposed to dysgenesis and assayed the next generation compared to the 25% detected by Mackay (1986) on IIInd chromosomes isolated by nine generations of backcossing after the contaminating cross. However, in general, the short term work has concentrated on the X chromosome and it is possible that there are differences between the concentration of viability mutable genes on the autosomes and X chromosome.

Assuming that the density of lethal mutable genes was the same, then at least 2-4% of the M chromosomes extracted here could have been expected to harbour a lethal mutation as a result of passing through the dysgenic cross (based on the lower estimates of approximately 1% per chromosome arm (Simmons et al, 1985; Eanes et al, 1988)). The total lack of lethal chromosomes among the dysgenic lines therefore appears puzzling. However, the estimate of 0/90 (or 1/149 if all dysgenic lines with some progeny are included) is not statistically different (by a $\chi^2$ test with 1 df) from these estimates.

The design of this experiment assumes that most of the P activity occurs early in the development of the $F_1$ flies (Engels and Preston, 1979), giving rise to a
large proportion of the analysed offspring within a line having the same mutations. Although there is evidence of clustering of major mutations in such offspring (Engels, 1981a; Green, 1977; Golubovsky, 1977), few of these clusters are found to be large; Green, for example, in a 1977 study of X-linked visible mutations, found several clusters with between 2 and 22 individuals in each, but the smaller clusters were by far the more numerous.

Lines in which only a proportion of $G_2$ flies carry the same lethal (that is those experimental lines derived from a male in whose gonads a lethal occurred late in development) will produce live wild type progeny, but in lower numbers than predicted, thus giving the appearance of a line harbouring an allele of intermediate viability. The distribution shown in Figure 2.2 may therefore represent several dysgenic lines, each carrying a small number of lethal events, rather than the alternative hypothesis that the $P$ elements have created polygenic viability mutations in these lines. This may also explain why the difference in overall mean viability (Table 2.3 and Figure 2.2) does not occur in a small number of lines, leading to an increase in the between line component, but rather is the result of small reductions in viability in nearly all dysgenic lines. It appears that not all vials within a line share the same viability, resulting
in the observed tenfold increase in the within replicate variance without a further gain in the between line component (Table 2.3).

The results suggest that the element activity was higher in the dysgenic flies than in the nondysgenic individuals, as the difference in mean viability is significant and in the direction expected as a result of mutagenesis. This is consistent with the work of Fitzpatrick and Sved (1986) who found chromosomes passed through dysgenesis to have reduced total fitness (decreased by 10–20% relative to non dysgenic controls in a cage test of total chromosomal fitness) and viability relative to those from the reciprocal cross. The lack of a detected difference between dysgenic and non dysgenic lines in Mackay's 1986 experiments may have resulted from the backcrossing used in her experiment allowing mutation in both sets of lines to mask any original differences between the populations.

Bristle traits

From the tables and figures above, it seems that the flies from the dysgenic cross do indeed harbour more variation in bristle score than those from the non dysgenic cross. This is true at all levels of the analysis apart from between vials where environmental variation is likely to be a more important source of variance than genetic causes.
The estimation of no variation between replicates in the nondysgenic population probably indicates that environmental variation is confounding the results in some unknown way. For example, it was assumed that the variation between food batches would be no greater than that between vials within a food batch, and therefore that the between vial within batch component would be equal to the between vial between food batch component. Subsequent analysis (see Chapter 4) has shown this assumption to be false, with the between batch component being much the larger of the two; this might have led to more variation being detected between vials than between replicates and hence to the observed negative variances.

The estimates of the contribution of mutational variation in the $F_1$ flies to total variance may well underestimate the contribution of the elements, because only those mutations that occur early in the germ line, and are thus shared by a substantial proportion of the flies at $G_2$, will be homozygous when analysed. Those mutations that are carried by only a few flies will elevate the within line variation, further reducing the sensitivity of the analysis. Thus, the consistently larger within line variation for both traits in the dysgenic lines can be construed as further evidence for a higher level of element activity within these lines. Similarly, the larger between
line and between replicate variation for both traits may be taken as evidence for higher element induced variation in these lines. While none of these components is significantly higher in the dysgenic than the non dysgenic lines, the probability of as many comparisons being in one direction by chance is 0.07 for the sternopleural bristle trait (exact binomial probability of 7/8 observations being of the same sign. Calculated by comparison of the estimated mean squares at all levels shown in Table 2.4). This suggests that for this trait at least there was considerably more variation induced by the dysgenic cross.

Although assumed to contribute an equivalent amount of variation to both sets of lines, mutations arising on the X chromosomes may have elevated the estimated $V_M$ somewhat. Within a line, males have 50% of their X chromosome in common, while females share only 25% of their sequences. Given that X regulatory activity in male Drosophila melanogaster leads to the same level of gene activity as in females (Baker and Belote, 1983), the effect of X linked mutants is twice as large in males as it is in females (James, 1973; Frankham, 1977), and we can thus predict that the between line component includes $0.2 \left( V_{MX} + 0.25 V_{MX} \right) = 0.25 V_{MX}$ from mutations on the X chromosome. These X chromosomes originate from the P stock, and are therefore inherently more mutable than the M.
chromosomes (Lai and Mackay, 1989), as a result of secondary mutagenesis (Tsubota and Schedl, 1986; Kidwell, 1986). The estimated $V_M$ may therefore contain a component due to the X linked mutations, but it is difficult to quantify this contribution and to determine whether it influences the results.

Another source of confounding variation may have originated with the elements themselves. It has been assumed that the extra variation in bristle score among dysgenic lines results from the alterations in the DNA structure induced by the elements. It is known that larval density can influence the bristle score of the flies, and under the relatively dense conditions of crowding used here, any factors that reduce the density of the larvae in a vial are likely to elevate the bristle score of that line. As predicted by this hypothesis, plots of average bristle score against relative viability indicate a significant negative regression for both bristle traits among the dysgenic but not the non dysgenic lines (Figures 2.5 & 2.6). Since these regressions account for a higher proportion of the between line variation among the dysgenic lines ($r_{abd}^2 = 0.273$ and $r_{stern}^2 = 0.106$ for the dysgenic lines as against $r_{abd}^2 = 0.002$ and $r_{ster}^2 = 0.012$ among non dysgenic lines), it is possible that the increase in between line bristle score results from
Figure 15. A graph of viability score (line mean) against average abdominal score.

\[ y = 0.16x + 20.93 \]
\[ r = 0.044 \]
\[ y = -2.26x + 22.72 \]
\[ r = -0.523 \]
Figure 26. A graph of sternopleural bristle score (line mean) against average viability score.

$y = 0.372x + 19.95$

$r = 0.109$

$y = -1.37x + 21.04$

$r = -0.326$

- - - - - non-dysgenic

--- dysgenic

Average viability
the decrease in larval competition resulting from element induced viability mutations alone. Alternatively, the regression could merely indicate that those lines with large decreases in fitness caused by the elements also contain mutations that increase the bristle score of the flies.

It is impossible to determine without further characterisation of all the mutational events which of these two hypothesis is closer to the truth, and indeed it is uncertain to what degree direct element mutagenesis is responsible for any of the results. However, it seems clear that the elements do indeed have a different effect in hybrid flies when carried on the paternal or maternal chromosomes.

2.5. Conclusion

A comparison was made between the variation present among 2Ind chromosomes extracted from dysgenic and non dysgenic hybrid flies from a cross between a long inbred M strain and a P Cy/Pm balancer stock.

The average viability of +/+ homozygotes relative to their Cy/+ heterozygotes was found to be 25% lower in the dysgenic lines than in the non dysgenic lines, and this was associated with an apparent increase in the variability between replicate measurements within the dysgenic lines.
Analysis of bristle score data suggests that dysgenesis is associated with higher levels of variability for both abdominal and sternopleural characters. Although some doubt exists as to the precise contribution of element induced mutations, estimates of $V_w/V_e = 0.06$ and 0.37 were obtained for the abdominal and sternopleural traits respectively.
Chapter 3
Viability and Dysgenesis

3.1. Introduction

The genetic modifications in the fine-tuning of developmental patterns resulting from Transposable elements (see Chapter 1) are exactly the sort of changes that might be expected to result in the continuous variability of a complex phenotypic trait such as height or weight. These elements are potentially an elegant tool for investigating the relationship between observable polygenic variation and the nature of the underlying molecular changes.

The adaptation of Mackay's methodology used in the experiment described in the previous chapter indicated that there is indeed a difference between the dysgenic and the reciprocal cross; however, the level of mutagenesis in the non dysgenic lines was unknown. The experiment described in this chapter was designed to quantify the amount of variation created by a single generation of dysgenesis in relation to the level of spontaneous background mutation, rather than in comparison to that in the reciprocal cross.

To obtain this estimate, the protocol discussed in the last chapter was further adapted. To maximise the probability of detecting an effect, single pairs of
IIind and IIIrd chromosomes (together almost 0.8 of the genome) were extracted and made homozygous using balancer stocks. To ensure that only one set of $F_1$ mutations were present in each analysed line, a second generation of backcrossing to the balancer was used in which only one $F_2$ male was selected per $F_1$ line. Both M and P strain balancer strains were created so that a set of chromosomes initially isogenic with those used in the experimental crosses could be passed through a similar, contemporaneous set of crosses, but using flies lacking P elements.

A comparison of the variation between the lines within these two populations gives a good indication of the rate at which new variation enters the populations, and can be used to evaluate the effect of the elements. This chapter reports the initial setting up of the lines and records the influence of the elements on the relative viability of the chromosomes. Some data on the sternopleural bristle score in the first homozygous generation are also reported.
3.2. Methods and materials

Stocks

**M Strains**

**CANTON S**: A standard M strain, as defined by Kidwell (1983), obtained from Dr. A. Leigh-Brown, July 1988.

**SAM ]** [C(I)DX; y, w, f]: Identical to the stock outlined in Chapter 2, Section 2 above, backcrossed to inbred SAM for a further 18 generations prior to the commencement of this experiment.

**In/1/3/16** [C(I)DX; y, w, f]: A stock containing standard, multiply-inverted IIInd chromosome (In(2LR)SM1), marked with the phenotypically dominant, recessive lethal Cy allele. The IIIrd chromosome balancer (TM6(B); Tb), marked with a second phenotypically dominant, recessive lethal allele, Tb. These two chromosomes are balanced against the compound IIInd and IIIrd chromosome (T(2,3)Xa), which carries multiple inversions and the dominant marker allele Xa. These three chromosomes are individually identifiable, lethal when homozygous, and permit a severely restricted number of reciprocal crossovers. The IVth and sex chromosomes of these flies were made isogenic with the SAM  stock, by backcrossing each of the three balancers to the inbred SAM for over 15 (Cy), 12 (TM6(B)), and 9 (Xa) generations respectively. The (M)CyTM6(B)/Xa stock was
then generated from these isogenic strains by first crossing Cy/+ males to TM6(B)/+ females, then crossing the resultant CyTM6(B)/++ male progeny to Xa/++ females. The resultant strain was then multiplied for a further 2 or 3 generations prior to use in the experimental crosses.

(M)snw: This stock was identical to that outlined in 2.2. above.

P strains

HARWICH: A strong P strain, containing around 60 P elements per genome (Dr. A. Shrimpton, personal communication).

(P)CyTM6(B)/Xa: This strain was generated from the same balancer chromosomes as outlined above, by backcrossing the original stocks to a P strain derived from a strong P stock by approximately 30 generations of full sib mating (Mackay, 1987b), for 20 (Cy), 11 (TM6(B)) and 8 (Xa) generations respectively, prior to synthesising the stock. The intention was to create a stock with P contaminated balancer chromosomes in an inbred P strain background.

(P)snw: This stock is identical with the (P)snw stock outlined in 2.2. above.
Cytotype Assays

The cytotype of all stocks was checked using the snw tests of induced mutability (Engels, 1979a), as outlined in Section 2.2. above. Additionally, the ability of a particular line to prevent or induce gonadal dysgenesis in F1 hybrids from the appropriate crosses (Kidwell, Kidwell and Sved, 1977; Engels and Preston, 1979) raised at 29°C was determined. Two crosses were used, both taking place in controlled temperature incubators at 29°C ± 0.5°C. The first of these crosses, Cross A, consisted of mating virgin females from the unknown strain to males of a strong P tester strain (in this case, Harwich), and was used to quantify the ability of the strain to inhibit P element mobility (P cytotype). The second cross, Cross A*, tests for the ability of the lines to generate P dysgenesis by crossing males from the strain under test to females from a true M strain (Canton S). Regardless of the direction of the cross, the conditions were similar in that the males, aged for around 5 days, were crossed to virgin females aged for between 3 and 9 days, either in small batches (5x5) as bottle cultures, or as small groups of pair matings (5 to 10 pairs) in separate vials. Twelve days after being set up, the offspring were collected, aged at 25°C for a further 4 to 12 days, then scored. Scoring was performed by dissecting up to 50 females from a bottle culture or 20
from each vial in 30% ethanol solution, and the flies were classified according to the scale shown in Figure 3.1.

Figure 3.1 Morphology of the gonads used in assessing gonadal sterility in the female

Each female was scored on a scale of 0-2, by counting +1 for each ovary containing eggs. Redrawn after Engels (1988)
Culture Conditions

It was anticipated that the activity of the elements would create some weak lines, so all experimental crosses took place in 3"x1" vials, containing approximately 10ml of a rich, glucose/killed yeast (GY) medium seeded with 4-5 granules of baker’s yeast (J.S. Sainsbury’s Dried Yeast). To reduce dysgenic sterility while maintaining relatively short generation intervals, the flies were reared at 22-23°C in a constant temperature incubator.

This incubator was also used for the cytotype tests involving the snw assays, but those involving the assessment of gonadal dysgenesis were performed in a similar incubator set at 29°C. These latter tests were carried out in 1/3 pint milk bottles containing approximately 150ml of the GY medium, or in vials as described above.

Generation of Lines

The crossing scheme used to generate the lines is shown in Figure 3.2.

Over a three-week period, small mass matings (5(P) or 5(M) CyTM6(B)/Xa males crossed to 5 (M) XX females) were set up at a rate of one vial of each type every
Figure 3.2. The crossing scheme used to establish lines derived from inbred M strain IIInd and IIIrd chromosomes contaminated with a few P elements

\[
P_0 \quad XX_M \text{II}_M \text{III}_M \quad \text{---} \quad \text{----} \quad 1 \quad q \quad x \quad \text{---} \quad \text{----} \quad 1 \quad \delta \\
Y_M \text{II}_M \text{III}_M  \\

F_1 \quad X_M \text{CyO}_M \text{TM6}_M \quad \text{---} \quad \text{----} \quad 5 \quad q \quad x \quad \text{---} \quad \text{----} \quad 1 \quad \delta \\
X_M \quad Xa_M \\

F_2 \quad X_M \text{CyO}_M \text{TM6}_M \quad \text{---} \quad \text{----} \quad 5 \quad q \quad x \quad \text{---} \quad \text{----} \quad 1 \quad \delta \\
X_M \quad Xa_M \\

F_3 \quad X_M^o \text{II}_M^o \text{III}_M^o \quad \text{---} \quad \text{----} \quad 5 \quad q \quad x \quad \text{---} \quad \text{----} \quad 5 \quad \delta \\
X_M \quad Xa_M \\

G_0 \quad X_M^o \text{II}_M^o \text{III}_M^o \quad \text{---} \quad \text{----} \quad 10 \quad q \quad x \quad \text{---} \quad \text{----} \quad 10 \quad \delta \\
X_M \text{II}_M^o \text{III}_M^o \\

* these chromosomes are those potentially mutated in the gonads of the \( F_1 \) male and therefore made homozygous at generation \( G_0 \).
\( \circ \) these indicate mutations originating in the \( F_2 \) male's gonads that could be shared by some flies in a line.
This scheme only shows the fate of a single replicate from each \( F_1 \) derived line, although at least two vials were set up each generation. The other replicates not shown were discarded each generation until \( G_0 \), when they formed the basis of the two closed breeding pools maintained from then on.
two days. Flies eclosing before day 16 were discarded, and males were subsequently collected from day 16-18.

In the next generation (F₁ in Figure 3.2.), 10 individual dysgenic males and six control males were randomly selected from each set of vials on day 21, and individually crossed to 5 virgin (M) CyTM6(B)/Xa females aged for between 3 and 6 days. Two egg collections of two days' duration were obtained from each male group, and all offspring eclosing from both vials between day 14 and day 18 were collected.

In the F₂ generation, two replicates of each line were set up on day 21 by selecting 2 Xa/++ males at random from each male group, and mating each to a separate group of 5 (M) CyTb/Xa virgin females, aged for between 3 and 8 days. Again, two egg collections of two days were made.

In generation F₃, one of the two sets of two vials derived from each F₁ male was discarded. This was done at random unless one of the pairs produced no offspring, in which case the surviving pair was retained. All Xa/++ offspring eclosing from the retained pair of vials between day 14 and 19 were collected as virgins and pooled on day 21. From these pooled flies, two groups of 5 males and females were crossed inter-se and two egg collections of three days' duration obtained from each group.
The offspring eclosing from day 14 to 22 were collected, classified and their numbers recorded separately for both replicate vials. This chapter deals entirely with the flies obtained at this generation. However, from one vial of each G₀ line with live progeny, a group of ten males and ten females were collected at random and two egg collections of 7 days obtained (in some lines insufficient flies eclosed, but as long as there were at least five of each sex, these lines were set up with the number that did eclose). These replicate lines were then maintained as closed breeding pools. The details of the information extracted from these lines will be dealt with in the next chapter.

Characters scored
From each of the vials the following data were recorded:

1. The proportion of wild type and Xa flies eclosing during the first six days of eclosion.

2. The separate and combined bristle score on the right and left sternopleural plates from five males and five females. Unfortunately, quite a large proportion of the lines only produced flies in one of the two vials, so the number of lines with bristle scores for two replicates is rather smaller than had been anticipated. This occurred
as a result of various environmental factors, including contamination with fungi and bacteria, as well as the loss of some vials due to no egg hatch, or the absence of eclosed wild type flies.

Analysis

An estimate of the variation resulting from one generation of P induced mutations can be obtained from the variation among lines at this first homozygote generation (\(V_{B1}\)). This variation can be considered to have originated from 5 separate sources:

1. Mutations arising from the insertion of elements into the IIInd and IIIrd chromosomes within the germ line of the \(F_1\) flies. Only one set of such mutations is sampled in each line at the \(F_2\) generation so that all flies within a line share these in a homozygous state in the first analysis generation (\(G_0\)).

2. Mutation arising in the same autosomes of the \(F_1\) flies from sources other than P elements will also be shared in the homozygous state by all flies within a line.

3. Spontaneous and P induced mutation occurring in the \(F_2\) generation will also be shared to some extent by flies within a line due to common parentage.
4. Variation present in the original M strain chromosomes will contribute $2F_rV_g$ (Falconer, 1981) to the between line mean square, where $F_r$ is the probability of identity of alleles by common descent and $V_g$ is the variation segregating in the initial stock.

5. X chromosome variation, deriving both from the original stock and from mutations created de novo in the $F_1$ flies will also contribute to the variation between lines because all males in $G_0$ will share 50% and females 25% of their grandfather's X-derived sequences.

With the exception of points 1. and 5. the contribution from individual sources will be small, and shared equally by both the control and dysgenic lines. Subtraction of the variance between control lines from that between dysgenic lines will remove these sources from the variance estimates so they need be considered no further.

The between line mean square in the dysgenic population can then be considered to result from $P$ activity on the homozygous chromosomes and some, possibly confounding, effect from mutations on the X chromosomes.
P-induced mutation

P-induced mutations on the IIInd and IIIrd chromosomes will contribute $0.8 V_{M(P)}$, where $V_{M(P)}$ represents the total input of new variation contributed by one generation of P mutation on the whole genome.

Since the $F_2$ males derive M strain uncontaminated chromosomes from their mothers, those lines that contain no active P elements will have no mutational input beyond this. However, those lines in which the $F_2$ male harbours active P elements may be subject to further mutational input in this generation. It is difficult to assess the contribution from this source, since it is unknown exactly what proportion of lines will harbour active P factors and what effect the elements will have on the variance of gamete production. It has been noted that, even among apparently fertile $F_2$ offspring from a dysgenic cross, a reduction in the number of gametes produced (Kidwell, 1984) and alterations in their gross morphology (Mathews, 1981) can be found in the temperature range used here. On the one hand, this (possibly non random) death of cells may well reduce the size of the gamete pool within which mutations can occur; on the other, it might lead to an increase in the rate of inbreeding and thus increase the between line variation. It seems likely that most of the autosomal mutation in this generation will be segregating within
lines and therefore contributing very little to the between line mean square. However, if X linked mutations occurred in all lines then these would be shared by 50% of males and 25% of females within the flies in the analysed generation, leading to an expectation of a further $0.25V_M$ from these chromosomes. This is obviously an extreme overestimate: not only is it unlikely that all lines undergo mutation, but those that do are unlikely to contain as many P elements as the original $F_1$ and should thus be subject to lower levels of damage. It was decided to use an arbitrary figure of $0.1V_M$ to account for the effect of mutations arising from this source.

Variation within line

The separation of each line into two replicate vials splits the effect of differences in environmental effects into a between and within vial component. The subsequent analysis of variance, with between vials treated as a random effect nested within lines, means that these environmental effects should not contribute to the between line component.

Although theoretically possible, an estimate of $V_M^{(P)}$ from the variance within line was not attempted because it was felt that the confounding effects of environmental deviations could not be satisfactorily separated from the genetic causes of variation.
3.3. Results

Viabilities

The $Xa/++$ self cross at $G_3$ is expected to produce the zygotes $Xa/Xa$, $Xa/++$ and $++/++$ in the proportions 1:2:1. However, the $Xa$ chromosome is recessive lethal and therefore only two classes of adult are expected, $Xa/++$ and $++/++$, in the proportions 2:1. If these two genotypes differ in their egg to adult survival, the observed ratio of flies will differ from the expectation above, becoming $2r:(1-r)v$ where $r$ = the proportion of $Xa/++$ individuals and $v$ the viability of the homozygote relative to the heterozygote. From this a simple estimate of the viability of the homozygote

$$v = \frac{2(1-r)}{r}$$

can be obtained from each line.

If we assume that the heterozygote viabilities are similar between lines, then these estimates can be used to compare the viability of the extracted chromosomes.

The values obtained for the 101 dysgenic and 36 control lines are shown in Figure 3.3. The most notable feature of this distribution is the large proportion of apparently lethal (0.27) and semi lethal
Figure 3.3 Distribution of line means for viability. Data are pooled over both vials of a line where more than one vial produced offspring.

Viability of the lines estimated relative to the heterozygote as outlined in the text.

Key: ▼ Dysgenic flies    □□ Control flies
(defined as lines with viability lower than 0.1) (0.11) chromosomes extracted from the dysgenic lines. The comparison of the median viability of the two populations is significantly different (P<0.001 in a Mann-Whitney test). However, since this Figure includes those lines in which only one vial produced offspring (all lines producing more than 50 offspring were included), a certain bias may have crept into the analysis; for example, slightly more of the dysgenic lines are only represented by one vial. To counter this, a sample of the lines with two replicates were compared. Only viable lines were used and the sample was therefore biased against the detection of any induced viability modifiers with large effects in the dysgenic lines. These lines were in fact those used for the sternopleural bristle scores and, as Figure 3.4. shows, there was an apparent difference in the viabilities of the two groups. The viability of the dysgenics (0.372) proved to be significantly lower (P<0.01) than the controls (0.472) when compared using a two sample rank test (Mann-Whitney). This is an extremely conservative test and illustrates clearly the large amount of deleterious fitness mutations resulting from the elements' activity, over and above the obvious increase in lethal chromosomes.
Figure 34. Graph of mean sternopleural bristle score against average viability, both measured on two replicate vials per line.

Viability, as defined in text

- Dysgenic lines  + Control lines
Bristle score

Constraints on time and the low number of wild type flies hatching restricted the number of lines that could be scored for bristle traits. It was decided to record only the bristle count on the left and right sternopleural plates of 5 males and 5 females from each vial. These results were then analysed in a nested analysis of variance using the Least Squares and Maximum Likelihood algorithm of W.R. Harvey (Ohio State University, 1985). The analysis fitted sex as a fixed effect, then analysed vials as a random effect fitted within lines, also random.

The results of this analysis are presented in Table 3.1. These show clearly that there is a much higher level of variation between lines in the dysgenic population. An F test of the two between mean squares indicates that the observed difference is significant ($F = 4.304 \ P<0.01$). None of the other paired mean square comparisons indicate a difference larger than that expected by chance.

By equating these mean squares with those expected, a table showing the components of variance segregating in the populations can be obtained (Table 3.2).
Table 3.1. Results from an analysis of variance for sternopleural bristle score in the dysgenic flies.

<table>
<thead>
<tr>
<th>Source</th>
<th>Dysgenic</th>
<th>Non-dysgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df ms   prob sd*</td>
<td>df ms prob sd*</td>
</tr>
<tr>
<td>B line</td>
<td>19 8.588 *** 2.65</td>
<td>11 1.996 ns 0.78</td>
</tr>
<tr>
<td>B vial</td>
<td>20 1.959 ns 0.59</td>
<td>12 1.475 ns 0.56</td>
</tr>
<tr>
<td>Sex</td>
<td>1 7.810 ** 0.11</td>
<td>1 11.267 ***</td>
</tr>
<tr>
<td>Error</td>
<td>359 1.460 0.11</td>
<td>215 1.318 0.13</td>
</tr>
</tbody>
</table>

*These values are rough estimates of the variation of variance estimates calculated as;

\[
\sqrt{\frac{2(\text{ms}^2)}{\text{df}+2}}
\]

Significance estimates were obtained from an F test of variance with the appropriate numerator; *** P<0.01, ** P<0.02. Both analyses from counts of left and right sternopleural plates combined. Model fitted was;

\[Y_{ijk} = \mu + a_i + b_{ij} + S_k + E_{ijk}\]

where a and b represent the random effects of line and replicate, S the fixed effect of sex and E all sources of environmental deviations.
Table 3.2. Components of variation derived from the analyses presented in Table 3.1. above.

<table>
<thead>
<tr>
<th>Source</th>
<th>Component</th>
<th>SE</th>
<th>Component</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between lines</td>
<td>0.342</td>
<td>0.143</td>
<td>0.026</td>
<td>0.052</td>
</tr>
<tr>
<td>Between vials</td>
<td>0.052</td>
<td>0.063</td>
<td>0.016</td>
<td>0.062</td>
</tr>
<tr>
<td>Within vials</td>
<td>1.460</td>
<td>0.109</td>
<td>1.318</td>
<td>0.127</td>
</tr>
<tr>
<td>Total</td>
<td>1.854</td>
<td></td>
<td>1.360</td>
<td></td>
</tr>
</tbody>
</table>

* estimated standard errors of the components assuming Normally distributed variances;

\[
SE = \sqrt{\frac{2(\text{Var}_{MS1} + \text{Var}_{MS2})}{n^2}}
\]

where \(\text{Var}_{MS1}\) and \(\text{Var}_{MS2}\) represent the estimated variances of the two mean squares used in calculating the component and \(n\) is the number of individuals.

Table 3.3. Estimates of 'mutational heritabilities' \((V_h\text{ scaled by the environmental variation})\) from the data above.

<table>
<thead>
<tr>
<th>Source</th>
<th>(0.9V_h)</th>
<th>(V_h)</th>
<th>(V_h/V_e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysgenic - control</td>
<td>0.316</td>
<td>0.348</td>
<td>0.261</td>
</tr>
</tbody>
</table>

* The estimate of \(V_e = 1.33\) was obtained by pooling the between vial and within vial components from the analysis of the control lines.

The between and within vial components of variance include all environmental variation; thus, it is possible to consider the between line component as being entirely due to genetic variation. By
combining the components of variance within and between vials from the control population, an estimate of the environmental variance ($V_e$) can be obtained. Using this value it is possible to estimate a 'mutational heritability' (Table 3.3) that scales the observed mutational input by the environmental variation and permits comparison with other studies.

The estimate of $V_M$ obtained in this way may contain some amount of genetic variation, but since the chromosomes of these flies were inbred to start with, this error will be small and will tend to reduce the estimate if present. The value obtained (0.261), although not significantly different from zero, shows very clearly that the activity of the elements in the unrepressed dysgenic state generates high levels of mutational variance in excess of that generated by spontaneous mutation in an M strain background.

In the plot of average bristle score against viability (Figure 3.4) there is a suggestion that those dysgenic lines with lower viabilities are also those with the most extreme bristle scores. However, with such a small data set the observed distribution could easily have arisen by chance alone.

Visible major mutants

Several flies with extremely abnormal phenotypes were detected in the dysgenic lines in this generation.
Most occurred as single flies which proved to be infertile, suggesting that they were developmental aberrations. A small number proved fertile, and a few of these bred true in subsequent generations. One in particular proved to be of interest.

This mutant (27-Go) causes the removal or gross reduction in size of some or all of the major chaetae indicated in Figure 3.5. Initially found in 16 out of 29 wild type flies which hatched from this line, the frequency of the allele dropped during later generations until, at generation 7, 0 flies out of 180 had the phenotype. This suggested that the mutant was deleterious. At generation G₃, a sample of flies with this phenotype was extracted from this line, and used to found a homozygous population for study. Although initially mutable, this allele became stable within a few generations of being isolated.

To identify the position of the gene, males from the line were crossed to females from an XX stock carrying the autosomal recessive eye colour mutants brown (bw) and scarlet (st) (Lindsley and Grell, 1968). The results shown in Table 3.4. indicate that the gene is an autosomal recessive. The F₁ progeny were self-crossed and the results are also shown in Table 3.4.
Figure 15. A schematic illustration of the 27-G0 mutant phenotype.

The mutant creates a variable phenotype in which some or all of the bristles shown are either not present or are greatly reduced. In all cases it appears that the bristle pit is present, as the position of missing bristles are marked by 'holes'.
Table 3.4. A description of the major bristle mutant 27-G0

**Initial cross**

<table>
<thead>
<tr>
<th>Parents</th>
<th>♀♀ XX; bw;st x ♂♂ 27-G0</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 progeny</td>
<td>All wild type</td>
</tr>
</tbody>
</table>

**F1 Self cross**

**F2 progeny, observed numbers (expected numbers)**

<table>
<thead>
<tr>
<th>Eye colour</th>
<th>27-G0</th>
<th>Normal bristles</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>22 (109.8)</td>
<td>273 (219.8)</td>
</tr>
<tr>
<td>bw</td>
<td>19 (36.7)</td>
<td>144 (73.3)</td>
</tr>
<tr>
<td>st</td>
<td>0 (0.0)</td>
<td>101 (109.9)</td>
</tr>
<tr>
<td>white</td>
<td>0 (0.0)</td>
<td>27 (36.7)</td>
</tr>
</tbody>
</table>

1 Small batches of males and females were crossed at 23°C: if sex linked, then only male progeny should express the gene.

2 since no F1 progeny shows the bristle-less phenotype, this allele must be autosomal and recessive, a self cross will then permit the assessment of which autosome the gene is on.

3 calculated on the assumption of a recessive allele of equal fitness resident on the same chromosome arm as the st locus.
The total lack of any scarlet flies with the mutant suggests that the gene is located on the IIIrd chromosome, possibly quite close to the scarlet locus itself. However, a $\chi^2$ test of this hypothesis indicates that the data are not consistent with the predicted values ($\chi^2 = 163.25$, $P<0.001$). The table of O-E suggests that this deviation results from a dearth of flies carrying the mutant, again indicative of a detrimental fitness effect. Combining the data for wild type and brown flies, an estimate of 0.22 was obtained for the viability of the homozygote relative to the average viability of the heterozygotes. No further characterisation of this mutant has been pursued to date.

3.4. Discussion

As outlined in Chapter 1, previous studies of P induced mutations have shown large reductions in viability and/or fitness (see Mackay, 1989 for a detailed review). Other workers have looked at the rate of lethal induction on the X chromosome as a result of a single generation of dysgenesis and have obtained estimates of around 1-3% recessive lethals created per generation by direct insertional events.
The X chromosomes make up approximately 0.2 of the D. melanogaster genome and contain a similar proportion of euchromatin to the overall genome. It might therefore be predicted that this experiment should have produced around 4-12% lethals, values that are significantly different from the 27% recorded ($\chi^2_{1df}=18.75$, $P<0.01$, assuming an expected 12%).

The hemizygous exposure to selection in males and smaller effective breeding population of the X chromosomes might lead to the expectation that they will harbour a somewhat lower proportion of lethal mutable genes. From studies of the X it has been estimated that it carries about 600 lethal-mutable loci (Lefevre and Watkins, 1986). Using an estimate of 0.004-0.01 lethal events per element insertion, Eanes et al (1988) suggest that the observed levels of P-induced mutation to recessive lethals (1-3%) is only consistent with 76-186 such loci. There are reasons to expect that about 50% of all autosomal loci at which X-ray/EMS-inducible lethals can exist are also vulnerable to P-induced lethals (see Chapter 5 and Kidwell, 1986). This suggests that the elements are not causing as much damage on the X as might be expected on the assumption of random insertion. It may be that the proportion of genes on the autosomes that are lethal-mutable and have at least one element
insertion site is higher than on the X, or the higher rates observed here might merely reflect a higher density of lethal mutable genes on the autosomes. This may be especially true since two mutations that are independently sub lethal may interact synergistically when in the same fly resulting in a lethal phenotype, and this sort of lethal is much more likely to occur when the 0.8 of the genome represented by the autosomes is made homozygous than it is when the 0.2 of the X is.

The large number of apparently near lethal chromosomes may well be an artifact relating to the unstable nature of the insertion damage. Any lethal mutation caused by an insertion in the F₁ fly has a certain probability of undergoing precise excision in the gonads of the F₂ and F₃ flies; such an event might lead to the production of one or more viable offspring resulting in the line appearing to harbour a non-lethal, but seriously detrimental mutation. The comparison of the two samples of viable lines, although not conclusive, does suggest that the elements are creating some alleles that have only a small effect on fitness, as well as the major lethal mutations discussed above.

Turning now to the bristle data, the estimate of mutational variation obtained here is quite similar to the 0.3 of Mackay (1987, 1988), although it should be
remembered that the large errors attached to the estimate make it also consistent with no variation having been created. Lai and Mackay (1989) found no mutational variation in sternopleural bristle score created on M strain chromosomes exposed to one generation of dysgenesis. They did obtain estimates of 0.137 for abdominal bristles among the same group of M chromosomes. At the moment there is insufficient evidence to decide on whether this difference reflects a difference in the mutability of the two traits or is merely the result of chance.

From these and other studies the amount of variation resulting from one or two generations of element activity is also comparable with that observed in outbred laboratory strains of flies (Thoday and Boam, 1961; Sen and Robertson, 1964). One question which then arises concerns the similarity of the two types of variation. The design of this experiment does not permit an estimation of the dominance relationship of the induced variability, but previously it has been shown that P-induced mutations are similar in their degree of dominance to those obtained from other sources (Mackay, 1986, 1987). The relationship between viability and bristle score shown in Figure 3.4 is similar to that observed between these two traits in natural populations (e.g Mackay, 1985, figure 5, p62). However, although it is difficult to say anything
meaningful with such a small data set, it appears that most of the between line variation in bristle score occurs among those dysgenic lines with the lowest viabilities. In an integrated system that is as complex as a fruit fly, mutants of large effect on one phenotypic character can be expected to have pleiotropic effects that probably include a detrimental influence on fitness, as is the case for mutant 27-G₀ above. These mutants are unlikely to contribute greatly to the long-term evolution of a trait, since even if their main effect is favourable, selection against them should occur as a result of these pleiotropic effects (Lande, 1983). Some theoretical studies have shown that additive polygenic mutations with small effects on traits and fitness can be a powerful source of variation and long term evolutionary change (Lande, 1976a; Bulmer, 1972; Hill 1982a,b; Lynch and Hill, 1986). However, there is a lack of empirical evidence to support these studies including an almost total ignorance about the distribution of mutant effects introduced or even the amount of new variation created each generation (Turelli, 1984; Lynch, 1988). The P family of elements may provide a powerful source of new variation that can be used to test these theories, but this will require detailed study of the distribution of the individual sites of
new mutations and their phenotypic effects, which is outwith the scope of this thesis.

3.5. Conclusion.

Second and third chromosomes from an isogenic M strain stock were passed through dysgenesis and extracted using multiply inverted chromosome balancer stocks. Among the 101 pairs of chromosomes extracted from individual dysgenic F₁ males, 27% were found to be homozygous lethal and 11% near lethal. A sample of the chromosomes not in these two classes showed a significant decrease in fitness from 0.47–0.37 in comparison to a similar sample of uncontaminated chromosomes, suggestive of the induction of new mutations of relatively minor affect on viability.

The component of variation in sternopleural bristle score was found to be significantly higher among a sample of dysgenic lines than among a similar group of the control flies (0.34 as against 0.02). By subtraction of one from the other an estimate for \( V_M/V_e \) = 0.261 was obtained. However, although this is some hundred fold larger than that normally recorded for spontaneous rates, the small size of the sample means that it is not significantly different from 0.
CHAPTER 4.
Induced Bristle Variation

4.1. Introduction

The very existence of transposable elements in all eukaryotic animals so far studied raises interesting questions as to the origin of such sequences, their function, biology and effects on the rest of the genome.

As discussed in previous Chapters the P family of elements in Drosophila melanogaster provide an easily manipulated experimental model that can be used to gain an insight into the general effects of Transposable Elements.

Laboratory studies have already shown that hybrid populations composed of P and M sequences have a strong tendency to rapidly become P strains (Kidwell, Novey and Feeley, 1981), even at high temperatures (29°C), where a detrimental syndrome of effects, including sterility of hybrid females, would be expected to inhibit the spread of the elements (Kiyasu and Kidwell, 1984). Only a very small sample (Anxolabéhere et al, 1987) or a single functional element is required (Daniels et al, 1987; Preston and Engels, 1989), for evolution to high copy number and P cytotype (= repression of P activity found in P strains (Engels, 1989)) to occur.
The initial generations of element invasion seems to be capable of generating large amounts of variation in quantitative genetic traits such as bristle score and viability. However, as already discussed, there is some controversy over the scale of the effect.

This chapter reports an attempt to follow the changes created during the early stages of population expansion of elements introduced to long inbred (sib mated) and previously M strain flies. Individual lines were established by sampling pairs of IIInd and IIIrd chromosomes from dysgenic flies (hybrids in which element activity is high) using the Xa balancer scheme described in the last chapter. After making these chromosomes homozygous, each line was subdivided into two replicates which were maintained as closed breeding stocks with ten males and females selected as parents each generation. Periodically during the twelve generations following the initial cross, the activity of the elements and the variation present in abdominal and sternopleural bristle score were recorded and compared to a set of control lines whose chromosomes were originally isogenic with the experimentals and which differed only in that they contained no P DNA. A comparison of the two population could be made in several generations permitting the calculation of independant estimates of the variation induced on the chromosomes in the gonads of the F1 males.
Figure 4.1. The crossing scheme used to establish lines derived from inbred M strain IIInd and IIIrd chromosomes contaminated with a few P elements

\[
\begin{array}{c}
P_0 \\
XX_M \; \text{II}_M \; \text{III}_M \\
\text{---} \; \text{---} \; 1 \; p \; \times \; \text{---} \; \text{---} \; 1 \; \delta \\
Y_M \; \text{II}_M \; \text{III}_M \\
\end{array}
\]

\[
\begin{array}{c}
F_1 \\
X_M \; \text{CyO}_M \; \text{TM6}_M \\
\text{---} \; \text{---} \; 5 \; p \; \times \; \text{---} \; \text{---} \; 1 \; \delta \\
X_M \; Xa_M \\
\end{array}
\]

\[
\begin{array}{c}
F_2 \\
X_M \; \text{CyO}_M \; \text{TM6}_M \\
\text{---} \; \text{---} \; 5 \; p \; \times \; \text{---} \; \text{---} \; 1 \; \delta \\
X_M \; Xa_M \\
\end{array}
\]

\[
\begin{array}{c}
F_3 \\
X_M^* \; \text{II}_M^* \; \text{III}_M^* \\
\text{---} \; \text{---} \; 5 \; p \; \times \; \text{---} \; \text{---} \; 5 \; \delta \\
X_M \; Xa_M \\
\end{array}
\]

\[
\begin{array}{c}
G_0 \\
X_M^* \; \text{II}_M^* \; \text{III}_M^* \\
\text{---} \; \text{---} \; 10 \; p \; \times \; \text{---} \; \text{---} \; 10 \; \delta \\
X_M \; \text{II}_M^* \; \text{III}_M^* \\
\end{array}
\]

* these chromosomes are those potentially mutated in the gonads of the $F_1$ male and therefore made homozygous at generation $G_0$.

* these indicate mutations originating in the $F_2$ male's gonads that could be shared by some flies in a line.

This scheme only shows the fate of a single replicate from each $F_1$ derived line, although at least two vials were set up each generation. The other replicates not shown were discarded each generation until $G_0$, when they formed the basis of the two closed breeding pools maintained from then on.
4.2. Methods and Materials

Stocks and crossing scheme

For a description of the stocks used and the crossing scheme by which the lines were established, see Chapter 3 Section 2; the crossing scheme is redrawn in Figure 4.1 for reference purposes.

Maintenance of the Lines

After the initial generations described in Chapter 3 and shown in Figure 4.1, 10 males and 10 females (or as many individuals of each sex as actually eclosed up to a maximum ten of each) were selected from one replicate of each line that produced viable wild type progeny. After a seven day egg collection, each group was transferred to a fresh vial for a further seven days, thus establishing two replicate subpopulations per line, each derived from the same G₀ parents.

From each of these subpopulations, two samples of 10 males and 10 females were selected from the progeny eclosing between day 12 and 18, and these were placed in separate vials. Six days after initiation the parents were removed from the vials and rapidly frozen for storage at -70°C. From generation G₁ onwards one vial from each subpopulation was selected at random (unless fungal or bacterial contamination or other
experimental error ruled out one vial of the pair, in which case the other was used) and two samples of 10 males and 10 females collected from the progeny of this vial to set up the two vials of the following generation.

To facilitate handling of a large number of lines, their initial establishment had been staggered over a three week period; splitting the lines into overlapping subpopulations increased this spread to a four week period, as illustrated in Figure 4.2.

**Figure 4.2. Distribution of replicates over time**

```
WEEKS
   1   2   3   4
FLY GROUP
   \   \   \   \ 1
REPLICATE
   1   2   1   2   1
FOOD BATCH
   1   2   3   4
```

This figure illustrates how each line was represented by two replicates separated from each other by one week. Each replicate comprised two vials, only one of which contributed offspring to the next generation. After G3 all replicates from a single generation were set up on the same food batch, so that 'batch' effects really refer to those environmental effects that vary from week to week, and therefore affect the two replicates of a line differently.
Although every attempt was made to distribute a similar proportion of experimental and control lines in each of the four 'batches', unavoidable experimental factors meant that the dysgenic lines were more unevenly distributed than the controls. These factors included an uneven distribution of lethal lines and the total loss of one group of 6 dysgenic lines as a result of extreme fungal contamination.

To minimise variation resulting from this different distribution of lines over batch, from generation $G_3$ onwards a single batch of fly food was prepared and frozen at the start of each four week period and defrosted at $37^\circ C$, 1.5 hours prior to use.

Data Recorded

From each replicate population the following information was recorded:

- In generation $G_1$, the number of chaetae (bristles) on the last two abdominal sternites and on the left and right sternopleural plates were recorded for 10 $\delta\delta$ and 10 $\delta\delta$ from each vial.

- In generation $G_4$, $G_7$ and $G_{10}$, the number of chaetae on the last two abdominal sternites and on the left and right sternopleural plates were recorded for 10 $\delta\delta$ and 10 $\delta\delta$ from each vial, giving a total of 20 $\delta\delta$ and 20 $\delta\delta$ per replicate and 40 $\delta\delta$ and 40 $\delta\delta$ per line.
- In generation $G_2$, $G_5$ and $G_8$, the level of P activity and ability to control P activity (P cytotype) were assayed for all lines, including those for which only one replicate survived (these were excluded from the bristle score analysis). P activity was scored as the percentage of dysgenic gonads among the $F_1$ $\varnothing$ offspring of a cross of males from the line under test to $\varnothing$ from the standard M tester strain, Canton-S. Susceptibility was measured by scoring the dysgenic ovaries among $F_1$ $\varnothing$ progeny from a cross of Harwich males and $\varnothing$ from the strain under test (for details of both these tests see chapter 3.2).

Analysis

In $G_0$ the average bristle score among lines will differ as a result of mutations that arose in the $F_1$ flies. The dysgenic lines can be expected to differ from the control lines by approximately $0.9V_{M(P)}$, where $V_{M(P)}$ is the amount of variation produced as a result of mutations arising from element activity (for derivation see previous chapter). The dysgenic lines can also be expected to harbour extra variation within lines as a result of the P elements activity in the $F_2$ and $F_3$ flies, over and above that shared with the control lines from background effects.
By splitting each line into two replicates at $G_0$, any subsequent mutation and the effects of drift should influence the two replicates of a line independently and therefore add no extra deviation to the between line component of variation. It is then possible to say that the components of variation between line in generation $G_1$ onwards are independent estimates of the variation present between lines in $G_1$.

**Variation between lines in $G_1$**

Variation between line means in this generation will be similar to that present in generation $G_0$, since any new variation arising in the $G_0$ flies should be equally likely to affect the mean of replicate population one or two and therefore to contribute nothing to the between line component. The estimate of $0.9V_{M(P)}$ obtained for $G_0$ includes a component ($0.1V_{M(P)}$) to cover mutations on the sex chromosomes, it seems likely that the size of this component will actually become less in later generations as the sequences responsible become equilibrated between the sexes (in $G_0$ they are mainly shared by the males within a line where they have twice the effect that they have in the female). Subtraction of the between control lines component of variance from that between the experimental lines gives a conservative estimate of 0.9 times the variance resulting from P activity in the $F_1$ males.
Variation between replicates

Since the lines are maintained as two replicates of equal size from $G_1$ onwards it should be possible to estimate the expected divergence between the replicate sub populations in this and later generations. However, to do so would require the making of assumptions about the nature of $P$-induced variation, for example no dominance and neutral fitness, that are untenable with our current knowledge of their action.

Variation between lines in $G_4-G_{10}$

By splitting the lines into two replicates any dominance or fitness effects of the mutations should be entirely contained in the between replicate component of variance and should therefore not confound the estimates of between line components as would otherwise be the case. The expectation of the between line component in the later generations remains at the same value as that in generation $G_1$ and the estimates of variation obtained in these generation can be taken to be independent estimates of $0.9 \, V_{M(P)}$. Pooling these estimates should then give a better idea of the true value and the variation due to estimation errors.
Figure 4.3. showing the models fitted and the expected distribution of variance in the analyses

Models fitted

Generation $G_1$ Model fitted

$Y_{ijkl} = \mu + l_i + B_j + v_{ijk} + S_k + E_{ijkl}$

Generation $G_4$–$G_{10}$ Model fitted

$Y_{ijklmn} = \mu + B_i + l_j + r_{ij} + v_{ijk} + S_l + e_{ijklm}$

Key:-

$Y_{ijk} =$ the bristle score of the $n^{th}$ individual

$\mu =$ the true population mean

$B_i =$ the fixed effect of food batch $i$

$l_j =$ the random effect of line $j$

$r_{ij} =$ the interaction between line & batch (replicate effect)

$v_{ijk} =$ the random effect of vial $k$

$S_l =$ the fixed effect of sex $l$

$e_{ijklm} =$ all other random effects on ind. $m$

Expected distribution of variance components

Generation $G_1$

<table>
<thead>
<tr>
<th>Level of the analysis</th>
<th>Expected variance present</th>
</tr>
</thead>
<tbody>
<tr>
<td>between line</td>
<td>$V_{b1} = \sigma_e^2 + 20\sigma_v^2 + 40\sigma_1^2$</td>
</tr>
<tr>
<td>bet. vial : line</td>
<td>$V_{bv} = \sigma_e^2 + 20\sigma_v^2$</td>
</tr>
<tr>
<td>Within vial</td>
<td>$V_Z = \sigma_e^2$</td>
</tr>
</tbody>
</table>

120
**Generation G₄–G₁₀**

<table>
<thead>
<tr>
<th>Level of the analysis</th>
<th>Expected variance present</th>
</tr>
</thead>
<tbody>
<tr>
<td>between line</td>
<td>( V_{b1} = \sigma^2_e + 20\sigma^2_v + 40\sigma^2_r + K\sigma^2_i )</td>
</tr>
<tr>
<td>bet. rep: line</td>
<td>( V_{br} = \sigma^2_e + 20\sigma^2_v + 40\sigma^2_r )</td>
</tr>
<tr>
<td>bet. vial : rep</td>
<td>( V_{bv} = \sigma^2_e + 20\sigma^2_v )</td>
</tr>
<tr>
<td>Within vial</td>
<td>( V_E = \sigma^2_e )</td>
</tr>
</tbody>
</table>

**Details of analysis**

The mean squares were obtained by use of the Least Squares Maximum Likelihood algorithm of W.R. Harvey (Ohio State University, 1985). In generation one, a single mixed model analysis of variance (ANOVA) was performed with vials fitted within lines (both random) and sex and food batch treated as fixed effects.

In later generations two analyses were required. The first fitted food batch as fixed, lines as random and the replicate mean square as the interaction of these two cross classified effects. Sex was treated as a fixed effect. The mean squares for the lower levels were then obtained from a second analysis fitting lines as fixed effects with replicates nested within lines and vials within replicates (both random) then sex and batch as fixed.

By combining these two analysis the values shown above could be obtained. Since the lines were not evenly distributed across batches, the value for \( K \) was usually slightly less than 80 and was obtained from the analysis program.

**Computation of variance components**

The mean squares for the analysis and the estimation of variance components were obtained by use of the least square maximum likelihood algorithm of Harvey (Ohio State University, 1985). In generation \( G_1 \), a
single run fitting model4 was used. In later generations two separate runs were involved, the first fitting model6 and the second model5, with mean squares and components estimated as outlined in Figure 4.3.

Table 4.1. The average bristle score for generation G₁-G₁₀ split by sex

<table>
<thead>
<tr>
<th>ABDOMINAL</th>
<th></th>
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<td>Generation</td>
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<tr>
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<tr>
<td>Dysgenic</td>
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<tr>
<td>male</td>
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<td>male</td>
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<table>
<thead>
<tr>
<th>STERNOPLEURAL</th>
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<tbody>
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<tr>
<td>female</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The values in the brackets are approximate standard errors of the means shown, calculated from the average variation in males and females combined.
4.3. Results

Generation G₁

As Table 4.1 shows, the average bristle score in the two groups of lines are similar for both the sternopleural and abdominal traits in this generation.

Table 2a. ANOVA of generation G₁; bristle score on the last two abdominal segments combined

<table>
<thead>
<tr>
<th>level</th>
<th>CONTROL</th>
<th>DYSGENIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MS</td>
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<tr>
<td>line</td>
<td>16</td>
<td>66.17</td>
</tr>
<tr>
<td>batch</td>
<td>3</td>
<td>23.43</td>
</tr>
<tr>
<td>vial:line</td>
<td>14</td>
<td>19.25</td>
</tr>
<tr>
<td>sex</td>
<td>1</td>
<td>8884.95</td>
</tr>
<tr>
<td>error</td>
<td>645</td>
<td>7.69</td>
</tr>
</tbody>
</table>

Table 2b. ANOVA of generation G₁; bristle score on the combined sternopleural plates

<table>
<thead>
<tr>
<th>level</th>
<th>CONTROL</th>
<th>DYSGENIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MS</td>
</tr>
<tr>
<td>line</td>
<td>16</td>
<td>13.95</td>
</tr>
<tr>
<td>batch</td>
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<tr>
<td>sex</td>
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<td>3.53</td>
</tr>
<tr>
<td>error</td>
<td>645</td>
<td>1.65</td>
</tr>
</tbody>
</table>

The ANOVA illustrated in Table 4.2 suggests that, contrary to expectation, the control lines appear to
harbour more variation between lines than the dysgenic derived flies. However, this may well be an artifact of the experimental design resulting from the confounding of environmental factors such as family size and food batch with the line effect. An F test of $V_{b1}$ indicates that the values obtained for both the abdominal and sternopleural bristles are in fact consistent with both the dysgenic and control lines having been drawn from the same population. Since it is unclear to what extent the environmental and other effects are confounded with the genetic differences between lines in this generation it was decided to consider the between line estimates in $G_4-G_{10}$ independently.

Generations $G_4-G_{10}$ - Abdominal score

The results obtained for the number of bristles on the combined last two abdominal sternites are shown in Table 4.3. It is difficult to accurately calculate the error attached to these estimates since the distribution of mutational effects is unknown. The distribution of line means did not depart significantly from that expected for a Normally distributed trait ($\chi^2 = 8.2$ with 4df; $P>0.05$), but the small number of lines scored mean that it is impossible to rule out the possibility that the true distribution is non Normal. The standard errors shown were those expected assuming
<table>
<thead>
<tr>
<th>DYSGENIC FLIES</th>
<th></th>
<th>CONTROL FLIES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>level</td>
<td>G\textsuperscript{4}</td>
<td>df</td>
<td>ms</td>
</tr>
<tr>
<td>line</td>
<td>33</td>
<td>118.8</td>
<td>28.4</td>
</tr>
<tr>
<td>batch</td>
<td>3</td>
<td>16.6</td>
<td>4</td>
</tr>
<tr>
<td>rep:line</td>
<td>31</td>
<td>21.0</td>
<td>5.2</td>
</tr>
<tr>
<td>vial:rep</td>
<td>68</td>
<td>10.3</td>
<td>3.0</td>
</tr>
<tr>
<td>sex</td>
<td>2583</td>
<td>6.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Table 4.** ANOVA results for generations G\textsuperscript{4} to G\textsuperscript{10} for the combined score of chaetae on the ventral surface of the last and second last abdominal segments.
a Normal distribution, but it is possible that these may underestimate the true error variance since even slight departures from a Normal distribution would increase the variance of variance estimates.

The number of lines scored each generation varies and this might be expected to make comparison between generations difficult. However, most of this variation apparently resulted from random environmental effects (severe bacterial or fungal infection as well as occasional accidental release or over-etherisation of the flies prior to scoring) and therefore should have had no systematic effect on the results. Each generation should then give an unbiased estimate of the variation present in the lines in $G_1$. Examination of the between line values in this table shows that the estimates are very similar in each of these three generations. Indeed, a comparison of the between line mean squares using a test for heterogeneity of variance showed that in both the control and dysgenic lines the calculated values for the three generations are consistent with having been drawn from one population ($P>0.3$ for the dysgenic population and $P>0.05$ for the control population; Bartlett's (1937) test for heterogeneity of variances).

In contrast, a comparison of the variance present in the dysgenic lines relative to that in the controls illustrates that there is significantly more variation
present between the dysgenic lines in each of the
generations tested (Table 4.4). There is also
evidence for more variation between replicates within
the dysgenic lines particularly in the early
generations. The error variance, which is expected
to include some effect from the genetic variance
segregating within lines, is also consistently higher
in the dysgenic lines.

Table 4.4. Comparison of the mean squares (MS) using the F test
of the ratios of the mean square in the dysgenic lines with that
of the control lines in each generation. Values tabulated are
Dys.MS/Control MS with significance of the ratio

<table>
<thead>
<tr>
<th>Level</th>
<th>G₄</th>
<th>G₇</th>
<th>G₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>line</td>
<td>2.41 *</td>
<td>3.45 ***</td>
<td>3.44 ***</td>
</tr>
<tr>
<td>rep:line</td>
<td>2.39 *</td>
<td>2.59 *</td>
<td>1.50 ns</td>
</tr>
<tr>
<td>vial:rep</td>
<td>1.31 ns</td>
<td>1.05 ns</td>
<td>1.03 ns</td>
</tr>
<tr>
<td>error</td>
<td>1.26 ***</td>
<td>1.08 ns</td>
<td>1.20 ***</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01, *** P<0.001; P = probability of a ratio
of variance this great or greater arising by chance alone
under the assumption of a Normally distributed variable

The control population between replicate mean
squares appear to continue to rise in value throughout
the course of the experiment whereas the equivalent
dysgenic values do not show such a steady increase.
Given that the dysgenic flies are expected to have at least as much input from new mutation as the control flies over these generations, it is possible that the difference in behaviour results from a higher proportion of the variance in the dysgenic population being linked to detrimental fitness. The lower rates of increase in between replicate divergence in the later generations, despite the higher within replicate variance, might then be the result of more intense selection pressure leading to a rapid establishment of a mutation selection equilibrium in these lines. However, the very large errors attached to the estimates mean that it is equally possible that these trends do not actually exist and are merely observable by sampling.

Using the data in Table 4.5 it is possible to estimate the variance created in the F₁ flies and these estimates are shown in Table 4.6. In the last column of Table 4.6 the estimates of \( V_M \) have been scaled relative to proportion of the environmental variance estimated from the variation present in an inbred M strain population raised under similar conditions (\( V_e = 6 \), Mackay, personal communication). From the between line component of variance a combined mean value for the estimate of \( V_M/V_e = 0.124(±0.033)^* \) can be

* ±1 standard error calculated from the variance between the three estimates (\( G_4, G_7 \) and \( G_{10} \)).
Table 4.5. Components of Variance (and their standard errors) estimated from the ANOVA results shown in Table 4.4.

**DYSGENIC FLIES**

<table>
<thead>
<tr>
<th>Generation</th>
<th>G4</th>
<th>G7</th>
<th>G10</th>
</tr>
</thead>
<tbody>
<tr>
<td>line</td>
<td>1.26(0.361)</td>
<td>0.59(0.303)</td>
<td>0.91(0.324)</td>
</tr>
<tr>
<td>rep*</td>
<td>0.27(0.150)</td>
<td>0.81(0.273)</td>
<td>0.64(0.214)</td>
</tr>
<tr>
<td>vial</td>
<td>0.18(0.152)</td>
<td>0.22(0.097)</td>
<td>0.13(0.081)</td>
</tr>
<tr>
<td>error</td>
<td>6.75(0.188)</td>
<td>7.04(0.196)</td>
<td>7.21(0.195)</td>
</tr>
</tbody>
</table>

**NON DYSGENIC FLIES**

<table>
<thead>
<tr>
<th>Generation</th>
<th>G4</th>
<th>G7</th>
<th>G10</th>
</tr>
</thead>
<tbody>
<tr>
<td>line</td>
<td>0.54(0.215)</td>
<td>0.13(0.146)</td>
<td>0.10(0.170)</td>
</tr>
<tr>
<td>rep*</td>
<td>0.02(0.088)</td>
<td>0.15(0.180)</td>
<td>0.35(0.222)</td>
</tr>
<tr>
<td>vial</td>
<td>0.13(0.091)</td>
<td>0.22(0.144)</td>
<td>0.17(0.114)</td>
</tr>
<tr>
<td>error</td>
<td>5.34(0.204)</td>
<td>6.53(0.283)</td>
<td>6.02(0.244)</td>
</tr>
</tbody>
</table>

* The between replicate within line component of variance was estimated from the line by batch interaction and is thus possibly overestimated due to confounding effects.

Standard errors of the components were estimated assuming a Normal distribution of variance using the formula:

\[ se = \sqrt{\frac{V_{MS1} + V_{MS2}}{n^2}} \]

where \( V_{MS1} \) and \( V_{MS2} \) are the variances of Variance one and two respectively, and \( n \) is the number of individuals contributing to the observed Mean Square one.

Although the 95% confidence interval for this value (-0.018 to +0.266) just includes zero, this result provides evidence for the induction of large amounts of variance as a result of the activity of the P elements in the initial cross.
Estimates of induced variance based on the changes of variance within line are also theoretically possible (Lynch and Hill, 1986), but to do so would require assumptions about the nature of element induced mutation that are at present untenable.

Table 4.6. Estimates of the amount of variation in abdominal bristle score introduced in the initial dysgenic generation.

<table>
<thead>
<tr>
<th>Dysgenic - Control components</th>
<th>$V_M$</th>
<th>$V_M/V_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_4$</td>
<td>0.724</td>
<td>0.804</td>
</tr>
<tr>
<td>$G_7$</td>
<td>0.462</td>
<td>0.513</td>
</tr>
<tr>
<td>$G_{10}$</td>
<td>0.808</td>
<td>0.898</td>
</tr>
</tbody>
</table>

The values shown in brackets are the empirical standard errors calculated from the observed variation between the three estimates, for a full discription of the rational behind the calculations see the text.

A standard estimates of $V_e = 6$ bristles (from similar inbred lines under similar conditions) was used to obtain the 'mutational heritabilities' shown in the last column.

Distribution of line means

To examine the induced variation in more detail plots were prepared of the average score for the two segments separately and these are shown in Figures 4.4-4.6.
Figure 4.4. Graph of line means in generation G4. Last abdominal segment score is shown plotted against second last. Open squares represent control line values and closed circles the dysgenic derived lines. Values were obtained as the constant estimates from the ANOVA described in the text.
Figure 4.5. Graph of line means in generation G7. Last abdominal segment score is shown plotted against second last. Open squares represent control line values and closed circles the dysgenic derived lines. Values were obtained as the constant estimates from the ANOVA described in the text.
Figure 4.6. Graph of line means in generation G₁₀. Last abdominal segment score is shown plotted against second last. Open squares represent control line values and closed circles the dysgenic derived lines. Values were obtained as the constant estimates from the ANOVA described in the text.
Generation $G_4$--$G_{10}$. -- Sternopleural bristles.

Table 4.7 presents the results of the ANOVA of bristle scores on the left and right sternopleural plates combined.

Unlike the abdominal bristles it would appear that there is no large amount of extra variation between the dysgenic lines compared to the control lines ($V_M = 0.037 \pm 0.037$). However, the dysgenic $V_{bl}$ is larger than that between the control lines in all three generations, although not significantly so (Tables 4.8 and 4.9). This suggests that extra variation has arisen as a result of the P elements. Indeed, treating these paired comparisons as a binomial character with an expected mean of zero and a score of +1 for dysgenic variance greater than control and -1 for the opposite result, it can be shown that the number of cells in which the dysgenic lines exceed the controls (10.5) is greater than that expected by chance ($\chi^2_{1 \text{ df}}$).
Table 47. ANOVA results for generations G₄ to G₁₀ for the combined score of chaetae on the left and right sternopleural plates

**DYSGENIC FLIES**

<table>
<thead>
<tr>
<th>level</th>
<th>G₄</th>
<th>G₇</th>
<th>G₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MS</td>
<td>se</td>
</tr>
<tr>
<td>line</td>
<td>33</td>
<td>21.3</td>
<td>5.09</td>
</tr>
<tr>
<td>batch</td>
<td>3</td>
<td>58.8</td>
<td></td>
</tr>
<tr>
<td>rep:line</td>
<td>31</td>
<td>12.6</td>
<td>3.10</td>
</tr>
<tr>
<td>vial:rep</td>
<td>68</td>
<td>2.8</td>
<td>0.47</td>
</tr>
<tr>
<td>sex</td>
<td>1</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>error</td>
<td>2583</td>
<td>1.9</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**CONTROL FLIES**

<table>
<thead>
<tr>
<th>level</th>
<th>G₄</th>
<th>G₇</th>
<th>G₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MS</td>
<td>sd</td>
</tr>
<tr>
<td>line</td>
<td>17</td>
<td>15.2</td>
<td>4.93</td>
</tr>
<tr>
<td>batch</td>
<td>3</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>rep:line</td>
<td>15</td>
<td>6.7</td>
<td>2.30</td>
</tr>
<tr>
<td>vial:rep</td>
<td>36</td>
<td>3.7</td>
<td>0.85</td>
</tr>
<tr>
<td>sex</td>
<td>1</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>error</td>
<td>1367</td>
<td>1.8</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Therefore, although the estimated $V_M$ for this character is indistinguishable from zero, there is evidence that the elements have created variation in this trait as well as in the abdominal bristle trait discussed above.

Table 4.8. Components of Variance (and their standard errors) estimated from the ANOVA results shown in Table 4.7.

<table>
<thead>
<tr>
<th></th>
<th>Generation</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G4</td>
<td>G7</td>
<td>G10</td>
<td></td>
</tr>
<tr>
<td><strong>DYSGENIC FLIES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>line</td>
<td>0.113(0.076)</td>
<td>0.148(0.075)</td>
<td>0.124(0.071)</td>
<td></td>
</tr>
<tr>
<td>rep*</td>
<td>0.245(0.078)</td>
<td>0.151(0.040)</td>
<td>0.220(0.067)</td>
<td></td>
</tr>
<tr>
<td>vial</td>
<td>0.044(0.024)</td>
<td>0.035(0.022)</td>
<td>0.022(0.019)</td>
<td></td>
</tr>
<tr>
<td>error</td>
<td>1.905(0.053)</td>
<td>1.876(0.053)</td>
<td>1.878(0.051)</td>
<td></td>
</tr>
<tr>
<td><strong>CONTROL FLIES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>line</td>
<td>0.114(0.068)</td>
<td>0.058(0.042)</td>
<td>0.137(0.093)</td>
<td></td>
</tr>
<tr>
<td>rep*</td>
<td>0.075(0.061)</td>
<td>0.008(0.041)</td>
<td>0.178(0.085)</td>
<td></td>
</tr>
<tr>
<td>vial</td>
<td>0.098(0.043)</td>
<td>0.092(0.027)</td>
<td>0.020(0.025)</td>
<td></td>
</tr>
<tr>
<td>error</td>
<td>1.757(0.069)</td>
<td>1.726(0.074)</td>
<td>1.701(0.069)</td>
<td></td>
</tr>
</tbody>
</table>

* The between replicate within line component of variance was estimated from the line by batch interaction and is thus possibly overestimated due to confounding effects.

Standard errors of the components were estimated assuming a Normal distribution of variance using the formula:

$$se = \sqrt{\frac{V_{MS1} + V_{MS2}}{n^2}}$$

where $V_{MS1}$ and $V_{MS2}$ are the variances of Variance one and two respectively, and $n$ is the number of individuals contributing to the observed Mean Square one.
Activity of the P element in the lines.

Figures 4.7, 4.8 and 4.9 show the results of the gonadal dysgenesis work. Each point represents the mean score for around five vials from each replicate, giving an average number of ovaries scored per

Table 4.9. Comparison of Dysgenic and control populations using an F test for equivalence of variance.

<table>
<thead>
<tr>
<th>Level</th>
<th>G₄</th>
<th>G₇</th>
<th>G₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line</td>
<td>1.40 (ns)</td>
<td>2.86 (*)</td>
<td>1.07 (ns)</td>
</tr>
<tr>
<td>Rep:Line</td>
<td>1.88 (ns)</td>
<td>2.21 (ns)</td>
<td>1.21 (ns)</td>
</tr>
<tr>
<td>vial:rep</td>
<td>0.76 (ns)</td>
<td>1.24 (ns)</td>
<td>1.10 (ns)</td>
</tr>
<tr>
<td>error</td>
<td>1.00 (ns)</td>
<td>1.12 (*)</td>
<td>1.12 (*)</td>
</tr>
</tbody>
</table>

ns P>0.05, * P<0.05; P = probability of a ratio of variance this great or greater arising by chance alone under the assumption of a Normally distributed variable
Figures 4.7, 4.8 and 4.9.

The three figures on the following pages present the results of the gonadal dysgenesis tests. Each replicate of a line is represented separately, to allow for the independent evolution of the replicates.

Increasing P activity is shown on the Y axis as the percentage of dysgenic ovaries among the female F₁ offspring from cross A. Increasing sensitivity to P element activity is shown on the X axis as the percentage of dysgenic ovaries among the female F₁ progeny from the A* cross. M strains are expected to cluster in the bottom right hand corner (95-100% destroyed ovaries in cross A* and 0-5% dysgenic ovaries in cross A), and P strains in the top right (0-40% dysgenesis in cross A* and 95-100% in cross A). For details of conditions and crossing procedures refer to text.

Key: x = values for individual replicates
= distribution of control line values
= more than one replicate with the same values
Figure 4.9

P Activity

P sensitivity
replicate of 70 (G2), 155 (G5) and 129 (G8), with slightly more scored per rep in the controls than the dysgenics. It is quite clear from these Figures that most of the lines remain as M strain flies, although the number of lines with a small amount of ability to repress P activity appears to grow over the course of the experiment. Very few of the lines maintain P activity throughout the experiment and none of these show any tendency to become P strains over the observed time scale.

4.4. Discussion

Unlike earlier experiments (Torkamanzehi, Moran and Nicholas, 1989; Mackay, 1987) the use of a well defined control population permits the removal of all background sources of variation from the estimates of the effects of the elements. The mutational variance estimated for the abdominal trait (0.124 ± 0.033) is particularly large. In fact, this estimate conforms well to those obtained by Mackay (1987) for mutations generated on the IInd chromosome (V_H/V_e = 0.151) and Lai and Mackay (1989) (V_H/V_e = 0.137) for the variation created among 183 M strain X chromosomes. These three results are very similar and suggest that the elements are capable of generating approximately 100 times the
amount of variation that is thought to derive from spontaneous mutation processes (see chapter 1). This is consistent with the minimum expected 50 fold increase predicted from the observed increase in mutation rate to major alleles, as outlined in Chapter 1, section 4.

The lack of a significant difference in variation between control and dysgenic lines in sternopleural bristle score is surprising, given the results of previous chapters and the size of the abdominal effect. It is notable that Lai and Mackay, (1989), although recording a large increase in variation for this trait among 96 P strain X chromosomes passed through dysgenesis, also failed to detect a large difference among M strain chromosomes similarly exposed to element insertions. This could be merely chance, but might also reflect an inherent difference between the sensitivity of abdominal and sternopleural bristle trait loci to element insertional damage.

Previous studies have indicated that a rapid conversion to the P cytotype can be expected over very few generations (Kidwell, Novey and Feeley; 1981), even when only 5% of a population harbours P elements. However, in this experiment only elements that 'jumped' onto the M strain chromosomes were present in the lines. Thus the lines had more in common with those experiments involving the injection of one or a few intact elements, in which evolution to the P cytotype
takes an unpredictable, and often large number of generations (Daniels et al., 1987; Preston and Engels, 1989). Although it is as yet unclear why such a difference in time course exists, it seems likely that it is related to the importance of particular deletion products in cytotype regulation, which may already be present on P chromosomes, but have to 'evolve' in those flies with only a few, novel insertion sites containing a restricted spectrum of deleted versions of the element. As shown in chapter 5, many of the surviving lines did contain a small number of intact elements and these lines were probably subject to further mutational input during the course of the experiment. As outlined earlier, this variation will have been removed from the component of variation between lines by the design of the analysis, and will not have contributed to the estimates of mutational variation.

Previous studies have suggested that most of the new variation created by P elements results from a few lines of extreme phenotype. Although, the distribution of line means (Figures 4.6-4.8.) suggests that several of the dysgenic lines are more extreme than would be expected by chance, there is no evidence to conclude whether these result from single insertion events, or whether these particular lines harbour several inserts each of small, additive effect. This question will be addressed in Chapter 5. It should
also be pointed out that in this experimental design any mutants with both large effects on bristle score and fitness will be under-recorded, as they will tend to be removed either directly, if dominant or recessive lethal, or indirectly, as part of a compound lethal, prior to analysis.

4.5. Conclusion

Estimates of the amount of genetic variation between dysgenic derived lines potentially harbouring mutations and their M strain controls were obtained 1, 4, 7 and 10 generations after establishment of homozygous lines. Although the results from G1 were probably confounded with environmental effects, the three other generations provide independent estimates of the variation among the extracted chromosomes. An estimate of $V_M/V_e = 0.124 \pm 0.033$ was obtained for the abdominal bristle trait. No significant estimate of $V_M$ was obtained for sternopleural bristle score, but there was sufficient evidence to conclude that there was more variation in this trait also.
Chapter 5.

Elements: Experimental Evidence

5.1. Introduction

In the previous chapters it has been shown that a significant decrease in homozygous viability occurs in a group of autosomes passed through a single generation of dysgenesis. Similarly, an increase in the variance of abdominal and sternopleural bristle scores among lines derived from those chromosomes that were viable was also observed. This chapter set out to establish whether such effects could be attributed to the element 'load' associated with these lines.

5.2. Methods and Materials.

The lines studied here were a random sample of those described in Chapters 3 and 4. Genomic DNA was obtained from small batches of Heterozygous Xa/++ flies collected at G₀ (the first homozygous generation, see Chapter 3). By using only heterozygous flies it was possible to include all lines in the selection procedure, even those harbouring recessive lethal, near lethal and sterile genes. Flies from all lines had been rapidly frozen and stored at -70 °C for about 2 years prior to analysis. A structured sample was selected, choosing lines at random from each group of
lines. Where possible four lines were chosen from the six set up on each day. Some lines failed to give DNA and a replacement was chosen, if possible, from the remaining lines. In total DNA was obtained from 42 dysgenic derived and 11 control lines.

Approximately 20 flies (range 10-22) from each line were placed in liquid Nitrogen in a 1.5 ml tube, then homogenised with a yellow pipette tip in 200 μl of lysis mix (10 mM Tris PH 8, 10 mM EDTA, 50 mM NaCl + 2% SDS). After 6-24 hours digestion at room temperature, the DNA was phenol:chloroform extracted twice and precipitated in three volumes of ice cold ethanol. After resuspension in 50-100 μl TE buffer (10 mM Tris: 100 mM EDTA ) overnight, the solution was treated with RNAase for ten minutes at 37 °C, and stored at 4°C until required.

Aliquots containing 2-3 μg of DNA were then subject to digestion with appropriate restriction enzymes (see below) as outlined in the manufacturer's (Northumbrian Biologicals Ltd.) protocols. After size separation in 0.8% agarose gels, the restricted DNA was transferred to charged nylon membranes (Hybond-N+) by vacuum blotting in an alkaline (NaOH, NaCl) transfer buffer, as described in the manufacturer's protocol (Hybaid, vacuaid manual). Unless otherwise stated, filters were probed with whole plasmid DNA, oligolabelled (Feinberg and Vogelstein, 1983) with $^{32}$P dCTP (Amersham) to a
specific activity of $10^{-7}$-$10^{-8}$ Counts Per Minute per µg of probe DNA. A restriction map of the plasmid pm25.1 and the probes derived from it are shown in Figure 5.1.

Figure 5.1.
Structure of the plasmid pm25.1, containing a complete P-factor and some single copy Drosophila DNA from the hdp locus from which it was cloned. Relevant restriction enzyme recognition sites are indicated as are the probes used in this experiment.

**KEY**

* This approximately 4.8 kb probe contained both P element and Drosophila single copy DNA, and was used extensively.

** This 2.3 kb probe, obtained by digesting the plasmid with Dde 1 restriction enzyme, detects only P element signals and was used to reprobe a sample of the blots to confirm the identity of control (Drosophila derived bands) and P element derived bands, data not shown.

A = sites for Acc 1 digestion
B = sites for BamH 1 digestion, used to release probe from plasmid

Initially, blots were prepared using the enzyme BamH1, and probed with the complete pm25.1 probe. Since the P-factor contains no sites for this enzyme, as shown in Figure 5.1, these blots provide a crude estimate of the total number of elements in a line, with each band in a track representing a single element.
Figure 5.2. A typical Southern blot obtained by digestion of the Drosophila DNA with the enzyme BamH 1 and Dde 1 and probed with the complete pmt25.1 plasmid. Arrows indicate those bands due to the single copy DNA flanking the P-factor in this plasmid.

Letters beneath tracks refer to line numbers in table 5.1. Har = Harwich (P strain), P bal = the balancer stock used to contaminate the lines, M bal = the M strain balancer used to introduce the isogenic chromosomes. Sizes on left are approximate, in kilobase pairs.
in that line. This plasmid also picks up homology with Drosophila single copy DNA at the hdp locus, thus providing a track internal control for loading/digestion differences. Figure 5.2 shows the results from a sample of lines with the control bands indicated.

Other aliquots of DNA were digested with Ddel and the filters probed with the complete pm25.1 plasmid (Figure 5.2). These blots allow a crude classification of the elements within each line. Complete elements and those with deletions lying outside this fragment will give rise to a single band of 2.17 kb. Internally deleted elements that retain the \textit{inverted} repeats, and are thus capable of transposition, should give bands of less than 2.17 kb. Deletions that span one or other of these recognition sites could give rise to a larger or smaller fragment than that from the intact element, depending only on the amount of DNA deleted and the distance from the remaining \textit{P} element sequences to the nearest 'host' Ddel site(s).

A scanning densitometer (Chromoscan 3) was used to determine the density of silver grains forming the bands. Typical output for lines containing intact \textit{P}-elements and/or deleted elements is shown in Figure 5.3, alongside the output from a control M-strain
Figure 5.3
Printouts from a chromoscan 3 scanning densitometer, showing the density of silver grains in three southern tracks. A. represents the output from a control M strain (the M-balancer stock used in the line set up).
B. the results from a dysgenic derived line with several inserts (line M1). C. The results from line J2, hypothesised to contain only a single intact P factor.

line M5
P/C=0; no elements

Line M1
P/C=1.0; 2 intact elements

Line J2
P/C=0.6; 1 intact element

C = Drosophila single copy control bands
P = area used to estimate number of intact elements
D = peaks due to non intact P elements
track. This provides a quantitative estimate of the amount of DNA homologous to the probe of any particular size. The density of a band will depend both on the concentration of DNA and on the degree of homology with the probe. It was considered that only the intensity of the 2.17 band relative to the track internal control signal could be used as a reliable estimator of insert numbers. Three of the lines (G2, J2, and L3) that had been found to contain only one P-homologous band on the BamHI filters also gave only one homologous band, at approximately 2.2 kb, on the DdeI filters (data not shown). It was concluded that these lines contained a single P factor and that the ratio of the 2.17 kb band to the control bands in these tracks would thus be characteristic of a single inserted intact element. This gave a value of 0.55:1 (0.5, 0.5, 0.6 for the three lines), which was used to estimate the number of intact elements in the other lines. It was assumed that, as long as the autoradiograph was not over exposed, the relationship between this ratio and intact element numbers would be linear, as illustrated in Figure 5.3.

5.3. Results

It was found that most of the lines analysed did contain at least one band of P-element homology (Table 5.1), with at least one line containing more bands than could be counted accurately. It was found that an
Table 5.1: Summary of initial molecular studies showing the relationship between P-element number/type, A*/A cross activity and viability estimates. These lines represent a sample of those established at $G_0$

<table>
<thead>
<tr>
<th>Line</th>
<th>Number of elements</th>
<th>Element activity</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'intact'</td>
<td>Total</td>
<td>Initial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$N \times U$</td>
</tr>
<tr>
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<td>4</td>
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</tr>
<tr>
<td>A2</td>
<td>0</td>
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</tr>
<tr>
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</tr>
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<td>?</td>
<td>-</td>
</tr>
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<td>-</td>
<td>-</td>
</tr>
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Table 5.1 Continued.

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<th>Element activity</th>
<th>Viability</th>
</tr>
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<td>'intact'</td>
<td>Total</td>
<td>Initial</td>
</tr>
<tr>
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<td>U x P</td>
<td>H x U</td>
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<td>1</td>
<td>99</td>
</tr>
<tr>
<td>L4</td>
<td>0</td>
<td>2</td>
<td>94</td>
</tr>
<tr>
<td>M1</td>
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<td>0</td>
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</tr>
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<td>?</td>
<td>81</td>
</tr>
<tr>
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</tr>
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</tr>
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</tr>
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<td>ndB3</td>
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<td>0</td>
<td>100</td>
</tr>
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<td>ndC3</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
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<td>ndD1</td>
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<td>0</td>
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</tr>
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</tr>
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</tr>
<tr>
<td>ndH2</td>
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<td>0</td>
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</tr>
<tr>
<td>ndI3</td>
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<td>0</td>
<td>-</td>
</tr>
<tr>
<td>ndJ1</td>
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<td>0</td>
<td>100</td>
</tr>
<tr>
<td>ndN1</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

average of approximately 3.5 (n=45 sd=2.53) elements were present per line (exact counts from some of the lines were impossible due to poor digestion, or insufficient DNA, and a low estimate, based on the intensity of 2.17 Dde 1 band plus other bands larger than 0.5kb on this filter, was used.). Unfortunately, in a few of the lines there was insufficient DNA to prepare both sets of filters, or the DNA that exists has proved refractory to digestion with one or both enzymes; this has led to some gaps in the table.
In Chapter 3 it was suggested that the decrease in mean viability among chromosomes passed through a dysgenic fly might be the result of element insertions. If this were the case then it would be expected that there would be a negative relationship between the number of inserted elements and the viability of a line. As shown in Figure 5.4, this does seem to be the case. Indeed, a linear regression of these two parameters suggests that there is a significant negative correlation \( r = -0.388, \ P<0.01 \), with the line of best fit suggesting a drop of 3\% \( (b= -0.03 \pm 0.023) \) viability per inserted element.

In Chapter 4, it was argued, from genetic data, that it was unlikely that many of the lines surviving to generation \( G_{10} \) contained active elements. To test this a sample of these \( G_{10} \) lines were examined. The results are presented in Figure 5.5. It should be noted when examining these data that each line would be expected to contain two inserts for each found in the heterozygous \( G_0 \) flies. These data suggest that a large proportion of the examined lines do indeed contain active elements, with several lines having many faint bands indicative of 'new' element sites shared by a small proportion of flies in each line (for example lines E6 and A5, Figure 5.5.)
This raises the question of whether the elements inserted into the stem chromosomes represent a random sample of those present in the parent P-strain.

Figure 5.4. A graph of average viability (for derivation see Chapter 3) against element numbers.
Figure 5.5 Autoradiograph of DNA from generation $G_{10}$ flies, digested with BamHI and probed with the complete $P_{25.1}$ probe. The constant fragment identified by sequences from the $hdp$ locus is indicated.
Table 5.2  Summary results obtained from analysis of the generation 10 flies in relation to the generation 0 results shown in table 5.1. When making comparisons note that G0 flies were scored in the heterozygous state.

<table>
<thead>
<tr>
<th>Line</th>
<th>Generation 0</th>
<th>Generation 10</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>intact</td>
<td>total</td>
</tr>
<tr>
<td>A5</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>B4</td>
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<td>3</td>
</tr>
<tr>
<td>B5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>C5</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>E2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>F1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>G2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>H2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>H6</td>
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</tr>
<tr>
<td>J2</td>
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<td>1</td>
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<tr>
<td>K6</td>
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<td>2</td>
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<tr>
<td>M5</td>
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<td>?</td>
</tr>
<tr>
<td>N5</td>
<td>5</td>
<td>?</td>
</tr>
</tbody>
</table>

**KEY**

* indicates lines that had too many bands to score accurately.
?
indicates that these lines may have contained a few very faint bands that could not be unambiguously scored
> n indicates that at least n bands were detectible in this line

As Figure 5.2. illustrates, the parental P-strain contained too many elements to accurately count. However, on a longer run (data not shown), it was estimated that this strain contains about 15 intact elements and in excess of 35 bands on BamH1 digests. This gives a rough estimate of 40% of this strain's
elements being intact P-factors. This figure is similar to the 47% (70/127, using only those lines characterised on BamH1 and DdeI digests) of inserted elements recorded here, so there appears to be no evidence for differential insertion of the intact or deleted elements in these line.

It is notable that over the course of the experiment at least one line (G2) has apparently lost all P-element homology. It is likely that in this line the initial generation actually contained a mixture of chromosomes, some of which contained element(s) responsible for lethal phenotypes. Such a result is consistent with the low viability noted in this line, and suggests that not all of the lines were in fact homozygous for individual inserts.

It can be argued that, if the increase in variance in bristle scores noted in earlier chapters resulted from element insertions, there might be a positive relationship between the number of elements within a line and the deviation of that line's mean from the overall population mean. Plots were prepared for both bristle traits and these are shown in Figure 5.6 and 5.7. These values represent the deviation of each line from the overall population mean after accounting for the fixed effect of food batch, and were obtained as the constant estimates from the ANOVA analysis.
Figure 5.6 A Graph of average Abdominal bristle score plotted against the number of elements in the lines.
Figure 5.7 A graph of average sternopleural bristle score against element number.
Figure 5.8 A

Sternopleural versus element nos
Graph shows squared deviations of line means from overall population mean

Figure 5.8 B

Abdominal versus element nos
Graph of squared deviations of line means from overall population mean
described in Chapter 4 averaged over the three
generations scored. Regressions of the squared
deviations on the number of elements are significant
for both sternopleural ($b=0.023, r=0.238; F_{1,23}=5.87$
$P<2.5\%$) and combined abdominal ($b=0.131, r=0.268;$
$F_{1,23}=6.64, P<2.5\%$) bristle score.

The regression of squared deviations on insert number
can be used to estimate the contribution of an
individual element to the observed between line
variance. These values are summarised for both
bristle traits in Table 5.3.

<table>
<thead>
<tr>
<th>Trait</th>
<th>$V_{M}$/insert ($\pm t x s_b$)</th>
<th>$V_{M}/V_{e}$/insert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal Bristle score</td>
<td>0.131 ($\pm 0.128$)</td>
<td>0.022 ($\pm 0.021$)</td>
</tr>
<tr>
<td>Sternopleural Bristle score</td>
<td>0.023 ($\pm 0.023$)</td>
<td>0.017 ($\pm 0.017$)</td>
</tr>
</tbody>
</table>

5.4. Discussion

Viability

The results outlined above suggest that there is
indeed some relationship between the element numbers
inserting into a chromosome and the subsequent
homozygous viability of that chromosome. However, the
estimate of a 3% drop in viability associated with a single insertion is subject to some error. When estimating the number of inserts it was assumed that the chance of two independent insertions giving rise to bands of equal size was small. Although this is probably correct, it is certainly conceivable that on short gels, as prepared here, there may have been some undetected overlap. It is more likely that this will affect the estimate of numbers in those lines that contain several inserts, and indeed, it is this particular phenomenon that makes estimating the number and type of elements present in the P-progenitor strain so difficult. Similarly, estimates of total number of P-factors based on dosage could be inaccurate in those lines with most inserts. This will have the overall effect of underestimating the number of elements contributing to the most extreme lines, and hence of overestimating the effect of individual inserts.

Previous studies based on insertions into the X chromosome have suggested that around 0.2 insertion events per generation might be expected from each element in the donor host (reviewed in Engels 1988). Thus, if we assume a mean of 35 elements in the original P-strain, a mean of 5.6 inserts (0.8 x 0.2 x 35) might be expected per line. Since several of the lines in which large numbers of elements appear to have inserted were also those that could not be analysed on
BamH1 filters, this is not inconsistent with the 3.5 inserted elements observed. Thus, it would appear that there is little difference, if any, between the frequency of element insertion into the autosomes studied here and the X chromosomes studied by others (for example Eanes et al, 1988).

Figure 5.4 suggests that there is a significant linear relationship between element numbers and viability, accounting for 15% of the total variation. It is also notable that among those lines with less than 6 inserts any reduction in viability would appear to result from a few lethal lines. However, removing these lethal lines from the analysis has little effect on the regression (\( b = -0.026, r = -0.345, n = 44 \)), suggesting that a large part of the observed reduction in viability resulted from several mutations with non-lethal effects. This rules out the possibility that the observed relationship resulted only from lethal mutations that would be more likely to occur in those lines with most inserts. The estimate of 3% viability drop per insert is larger, although not significantly so, than estimates obtained from work on the X chromosome, in which an estimate of about 1% drop in viability per insert was obtained by Eanes et al (1988).
Recessive lethals

There are considerably more estimates of the proportion of inserts that induce recessive lethal phenotypes. In this experiment this number is difficult to estimate, since a line with several inserts may contain more than one lethal but would be scored only once. However, 9 lines scored here were lethal, giving a rough estimate of 9/156 or approx 6% of inserts giving rise to recessive lethals. This value is considerably higher than the consensus 1% derived for P-induced insertional mutagenesis involving the X chromosome (Eanes et al, 1988, Simmons et al, 1985). Very much higher rates of induced recessive lethals have apparently been reported in several experiments involving transformation of the autosomes by micro-injection, for example, 28% (Spradling and Rubin, 1985), 22% (Laurie-Ahlberg and Stam, 1987) and using chromosomally induced transformation systems. For instance, Cooley et al (1988) used a system called jumpstarter to generate about 10% lethal inserts from a sample of 1200 inserts examined. These experimenters did not make their stem chromosomes homozygous, so that it is impossible to calculate the part played by mutations already on the chromosomes prior to the transformations. However, Mackay, Lyman and Jackson (1991) found rates of approximately 3.8% recessive lethals per P element inserted into previously isogenic
third chromosomes, a value that is certainly consistent with that estimated above.

The results of this chapter, combined with those mentioned above, support the suggestion made in Chapter 4 that P-induced autosomal recessive lethals occur at a higher rate than X-linked recessive lethals, even when expressed on a per insert basis.

Bristle traits

In the previous chapters it has been concluded that the increased variation detected among the experimental lines when compared to the controls was the result of P-element mobilisation in the dysgenic progenitors of these lines. As shown above the number of inserts in a line is indeed positively correlated with the deviation of that line from the population mean. If this were entirely the result of element effects on fitness, then it would be expected that those lines with more elements would cluster above the population mean (due to reduced number of progeny giving rise to larger flies with more bristles). This is not apparent in either Figure 5.6 or 5.7, and it is only when the absolute deviation of a line is compared (by squaring to remove the sign) that a significant correlation is found between element numbers and a line's contribution to the variance. This suggests that the P-elements are responsible for the increased
variation by direct mutation, rather than via some indirect effect. It also suggests that the mutations created in this experiment are random in direction, with regard to increasing or decreasing the value of a trait.

The estimates of $V_H/V_e$ per element, shown in Table 5.3, are surprisingly close to one another, suggesting that around 2% of the environmental variance is created by each insert for both bristle traits. This compares well with the estimates of $V_H/V_e = 0.03$ per insert obtained by Mackay et al (1992) among stable insert lines created via the A 2-3 transformation system (Robertson et al, 1988). Using the average number of elements inserted per line calculated in this Chapter, it is possible to scale the component of variance detected between lines in Chapter 4. In this way an estimate of $V_H/V_e = 0.035 \pm 0.006$ is obtained for the abdominal bristle trait. Thus it would appear that a single element insert is capable, when homozygous, of producing 1-4% of the environmental variation in the bristle traits studied.

It is impossible to say much about the shape of the distribution of mutational effects based on the small number of lines available here. However, some of the more extreme bristle scores are observed in those lines with only one or a small number of inserts, suggesting that some proportion of the observed effect derives
from mutations of larger effect. Mackay et al (1992) have investigated a series of stable insert lines and have suggested that the distribution of line means in their experiments are not inconsistent with a leptokurtic distribution in which most variation is contributed by a relatively small proportion of inserts creating mutations of large effect.

5.5. Conclusion

In previous chapters several significant differences have been observed between lines containing chromosomes passed through dysgenesis, and those same chromosomes passed through a non-dysgenic cross. The data presented in this chapter show that the changes in fitness and in bristle score are directly related to the number of elements that transposed onto the chromosomes, presumably during the dysgenic cross. This is conclusive evidence that P-elements are a powerful natural mutagen of the drosophila genome.
Chapter 6.
Implications and Conclusions

6.1. Nature of Induced Variation

Transposable elements are potentially one of the most powerful tools for the future understanding of quantitative genetics. In the previous chapters, it has been shown repeatedly that the P element can create large amounts of new variation over very few generations. Can we say anything about the nature of this variation?

Viability effects

From the results of Chapter 2, it is difficult to draw many far-reaching conclusions about the nature of the variation in viability recorded, since, as discussed in that Chapter, mutations occurring late in the development of the $F_1$ flies' gonads will be shared by only a small fraction of the individuals within a line. The distribution of line mean viabilities, for example (see Chapter 2, Figure 2.3), is as consistent with several lines in which a few flies harbour recessive lethals as it is with several lines in which all flies harbour mutations of intermediate viability, and it is impossible to decide which is the true situation. Yukuihiro, Harada and Mukai (1985), and Mackay (1987) both found large numbers of lethal
chromosomes with few of intermediate viability. In contrast several experiments in which the chromosomes were examined soon after contamination have detected minor viability modifiers, as well as lethal chromosomes (Fitzpatrick and Sved, 1986; Eanes et al., 1988). In Chapter 3 (Figure 3.3) above, chromosomes of apparently intermediate viability were detected, as well as large numbers of lethals: the results of the work reported here are therefore consistent with those of Fitzpatrick and Sved and Eanes and co-workers, as might be expected given the methodological similarities; in particular, the chromosomes were studied shortly after mutation in these experiments. As discussed in Chapter 5, the estimates for the effects of an individual insertion on viability and the proportion generating recessive lethals are both higher than those obtained from studies of the X chromosome (Fitzpatrick and Sved, 1986; Eanes et al., 1988). This is consistent with other studies of insertions into the autosomes (Spradling and Rubin, 1983; Laurie-Ahlberg and Stam, 1987; Cooley et al. 1988 and Mackay et al., 1992) suggesting either that the autosomes contain a higher density of 'essential' genes, or that the genes present are somehow more vulnerable to P element damage.

Although inconclusive, the evidence presented here and in the other work discussed suggests that the
elements are indeed capable of generating a wide spectrum of viability effects.

Bristle data

The rapid responses and heterogeneity between replicates found in previous selection experiments used to estimate the mutational effects of P elements could easily be explained as the result of a few alleles of major effect. Similar results are not uncommon when selecting from X irradiated populations (Scosirolli and Scosirolli, 1959; Jones, 1967; Hollingdale and Barker, 1971) or indeed from outbred populations (Frankham, 1977; Spickett and Thoday, 1966). Thus, it seems possible that the mutations produced by P dysgenesis could have similar distributions of effects to those which are created by radiation or are indeed present in natural populations, that is a leptokurtic distribution with high variance.

Lai and Mackay (1989) detected a distribution of line means in which large amounts of variation were contributed by relatively few lines of similar but extreme phenotype. Indeed, several of the mutants detected by Lai and Mackay appear to map to similar sites on the X chromosome (C. Lai personal communication). Similarly Mackay and coworkers have shown for third chromosome mutations induced by P element inserts that the distribution of effects is
consistent with a highly leptokurtic distribution of effects. If it were the case that each chromosome contained only a limited number of genes for a given trait that were sensitive to element insertion, then the usefulness of the technique would obviously be limited. On the other hand individual quantitative trait loci may be capable of harbouring a wide range of effects, but that the frequency of occurrence of a particular mutant is inversely related to its effect on the trait. This predicts that de-novo induced variation will be dominated by a few mutations/loci with relatively large effect. However, it should be noted that mutations with large effects are also likely to be more highly selected against. Thus variation in natural population is more likely to be dominated by alleles of small effect that have not been removed by selection.

The experiments reported in Chapter 2 provide little information on the distribution of mutational effects, because, since all individuals within a line may not share the same mutation, the distribution of line means does not truly reflect the distribution of mutational effects. The results of Chapters 3, 4 and 5 appear to show no evidence for very extreme lines, although there is a tendency for a few dysgenic lines to lie outwith the range expected from the control populations (see Figures 4.4-4.6 and 5.6, 5.7). To gain an
insight into the distribution of the 'damaged' sites and the overall distribution of mutations would require the identification of all individual insertion sites and their correlation with the line mean. It would also be preferable to have revertants of the implicated insertions that lacked the detected phenotype. The experiments reported in this thesis were not designed in such a way as to facilitate this characterisation, and they can therefore only be said to be not inconsistent with a broad distribution of mutant effects.

6.2. The P Element as a Tool

The work reported in this thesis attempted to estimate the amount of variation created by a single generation of dysgenesis, and assumed that, by and large, variation resulted from insertion events. However, to assume that only a single round of transposition occurs in the gonads is somewhat suspect.

An insertion occurring during the first few hours of pole cell development may well be capable of movement at a later stage, either creating a new site of insertion or simply being lost from its previous site. Thus, the chromosomes carried by an $F_1$ individual's gametes may well contain a mixture of insertion and excision lesions resulting in the observation of variably sized clusters of lethal (Eeken
et al, 1985; Simmons et al, 1984b) and visible mutations (Green, 1977; Engels, 1981a) among the next generation. Evidence for this comes most directly from the work of Preston and Engels (1988), in which sib-mated lines initially containing only a single 2.9kb insert were maintained and studied using in situ hybridisation over several generations. In several of these lines, after an initial lag phase during which nothing happened, the number of located sites increased by up to 8 times the number in the previous generation, a result which could only have occurred within a single fly's germ line as a consequence of rapid replicative transposition.

The number of insertion/excision events on a particular chromosome may not be distributed at random, since it is possible that separate cell lines within the same fly are subject to high levels of element activity while others have relatively low levels. This is in part supported by the otherwise somewhat puzzling results of Exley and Eggleston (1989), who noted that, although the number of mutations is lower when a weak P strain is used, the clusters of allelic mutations from individual flies are rather larger. Put simply, the strong P strain may produce more cell lines in which conditions are favourable for transposition, but each active line may be more likely to produce dominant lethal secondary mutations.
To be most useful in the study of quantitative variation, all mutation sites must be detectable, i.e. it is desirable to have mutations created only by insertion events. To do this, the system must be capable of switching on transposition for a very short period of time. This is impossible with 'traditional' crossing schemes. A further disadvantage of dysgenesis-induced mutants is their susceptibility to loss by excision during their isolation and characterisation.

During the course of the work described in this thesis, two systems that could overcome these problems have been developed. Although the direct insertion of transposase-producing but insertion incompetent elements into early embryos carrying only defective elements has been used to produce single insert lines, this method is really too laborious to be easily used on a large enough scale for the study of quantitative characters.

Cooley et al (1988) developed a system based on a single P element-containing mutator line in which the P element is unable to produce transposase and harbours the bacterial resistance gene (neo⁸) to the antibiotic G418. When crossed to a second strain containing a single transposase-producing element (jumpstarter), the labelled element will transpose in a certain proportion of the germ cells, and progeny can be selected from
these by growing the flies on G418-containing media. Using this methodology, Cooley et al (1988) have created a 'library' of 1300 single-insert-carrying flies.

A similar system has been developed by Engels et al (1987; Robertson et al, 1988). In this system, the transforming strain contains a stable P element lacking the 2-3 intron (Δ2-3(99B)). This element produces as much transposase as is normally found in a strong P strain (Robertson et al, 1988). By introducing the IIInd chromosome containing this element into flies along with an 'ammunition' chromosome harbouring variable numbers of defective elements, a variable but predictable number of new insertion events can be created in a single generation. This system scores over the jumpstarter scheme in requiring less intensive stock management.

With both of these systems, it should be possible to create low numbers of stable inserts without the potentially confounding effects of multiple generations of activity by manipulating the temperature at which the flies are raised.

Thus far (1990), little work has been published utilising these systems in the study of quantitative variation. Interestingly, Cooley et al noted that around 11% of their IIIrd chromosome and 10% of their IIInd chromosome inserts produced lethal phenotypes, and

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they refer to two similar estimates, one from a scan of 1000 rosy-marked P insertions (Berg, McKearin and Spradling) and the second from 200 germ-line transformed lines (Thompson and Spradling). These results are at least a factor of 10 higher than the estimates from dysgenic crosses (Simmons et al., 1985; Eanes et al., 1988). It is possible that some of the lethals may have been present on the chromosomes prior to mutagenesis, since they were not made homozygous. However, the results reported in this thesis and by Mackay et al. (1991) indicate that even when this factor is controlled for, the elements appear to create more lethal mutations on the autosomes than would be expected from the previous X chromosome studies.

Given the results of Chapters 4 and 5, and those of other experiments discussed above, the generation of 'libraries' containing single P element inserts should permit a fuller understanding of the genetics underlying quantitative variation. Mackay et al. (1991) have already initiated this process by examining the effects of a series of insertion mutations induced on the third chromosome. Among the 94 lines examined, a few harboured mutants of relatively large effect on the traits examined (viability, abdominal and sternopleural bristle score). Estimates of induced recessive lethals (3.8% of inserts) and of induced bristle variance per homozygous insert \(V_H/V_e = 0.03\)
for both bristle traits) are very close to those obtained in this experiment. This suggests that inserts induced by artificial systems, such as Δ2-3 mutagenesis do not differ in effect from those induced by hybrid dysgenesis.

As to what proportion of polygenic loci could be investigated by P induced mutagenesis, it has been suggested that approximately 50% of all loci are vulnerable (Kidwell, 1986), and this figure compares well with the estimate of 563 lethal P-mutable genes on the IIIrd chromosome obtained by Cooley et al (1988).

6.3. The Importance of Elements in Evolution.

The results of Chapters 2, 4 and 5 suggest that a single, or at most two generations of dysgenesis can result in phenotypic variation comparable to that observed in outbred populations, and that a single inserted element has an average effect, when homozygous, equivalent to 10 times the spontaneous mutation rate. This raises the question of what effects such elements have in natural populations.

Early reports of rapid cytotype switching (Engels, 1979, 1981; Kidwell, 1981) suggested that the elements remain active for very few generations after invading a population. However, studies have shown that, starting from a single autonomous P factor (Daniels et al, 1987) or from a small number of elements,
establishment of P cytotype does not occur rapidly. Indeed, the results of Chapter 4 and 5 are in agreement with the supposition that many generations can be required for P cytotype establishment. This suggests that the initial invasion of the Drosophila population may well have been accompanied by several generations of dysgenesis induced insertion mutations.

At present, wild populations of D. melanogaster differ dramatically in their complement of P elements and in their dysgenic phenotypes (Todo et al, 1984; Sakoyama et al, 1985; Anxolabéhère et al, 1985; Boussy, 1987; Black et al, 1987). This is probably the result of the recent invasion of this element which appears to be absent from all sibling species (Brookfield et al, 1984) and even from strains of D. melanogaster trapped from the wild prior to the 1940s (Kidwell, 1979; 1983). Although all strains caught in the last two decades contain P elements, some populations have been detected in which both M and P cytotype flies have existed in the same locality for more than 5 years without change in the apparent frequency of each class (Izaabel, Ronsseray and Anxolabéhère, 1987). A geographical cline in cytotype also existed over a distance of a few tens of metres between 1983 and 1987 (Boussy et al, 1988).

These results, taken together with the estimates of induced variation reported here, suggest that the P
element family may have been contributing a substantial input of new variation to some populations over extended periods of time. Since two other elements families have also been implicated in hybrid-dysgenic-like syndromes (the I and Hobo elements; see Finnegan, 1988 and Blackman et al, 1987 respectively), the mutation rate in some wild populations may be much higher than that expected from estimates of per locus mutation rate calculated from single homogeneous laboratory strains. Indeed, although most element families studied appear to maintain relatively consistent copy numbers in different flies, the sites occupied are often highly variable from strain to strain (Leigh-Brown and Moss, 1987; Young, 1979; Montgomery, Charlesworth and Langley, 1987; Montgomery and Langley, 1983; Belyaeva et al, 1984). This suggests that in D. melanogaster at least there may be many different families of transposable elements that are actively undergoing mobilisation over relatively short time periods.

Conversely, the estimates obtained in these chapters refer to the homozygous influence of the mutations on the measured traits. Mackay et al (1992) have found that many of the P insertions are completely or at least semi-recessive. In natural populations most potential sites of insertion appear to be occupied at very low frequencies and most mutations will thus be in
a heterozygous state (Charlesworth and Charlesworth, 1983; Biémont, 1986). This is probably the result of natural selection acting on particular insertion events as a result of the recessive fitness effects detected in this and other studies (Chapters 2 and 3; Fitzpatrick and Sved, 1986; Eanes et al, 1988; Mackay et al 1994, Mackay 1989). Indeed, the decrease in the variation within dysgenic lines over the course of the experiment reported in Chapter 4 probably resulted from the influence of natural selection removing individuals harbouring mutations with an effect on fitness and bristle score. This implies that the contribution of TEs to overall population variation may be more closely related to the heterozygous effect of element induced mutations than to the (probably higher) homozygous effects measured here.

Selection pressure against elements may be less when they are inserted in heterochromatin sites, and, once movement of the elements becomes regulated, these may be the only sites in which elements are retained after the action of natural selection. Continued insertion of different families into these heterochromatic 'element graveyards' will result in the disruption of previously resident elements. The jumbling and reassortment of sequences in this region might be the driving force behind the creation of new element families which subsequently invade and spread through
the euchromatic sites. Eukaryotic species may therefore be subject to repeated waves of element invasion, and correspondingly periodic high levels of mutation.

6.4. In Conclusion

Quantitative geneticists have traditionally seen the response of a population to selection in terms of changes in the frequency of alleles present in the base stock, and have generally not attempted to grapple with the almost impossible problem of the nature of the underlying differences. Recent theoretical treatments have drawn attention to the importance of mutation, not only on an evolutionary timescale, but also over short-term, intensive selection experiments.

Mutation can be considered as any process resulting in alterations of the DNA or its expression. It can arise from base substitutions, additions and deletions, or from larger alterations in the number of copies of a gene, change in the position or control regions, reshuffling of domains, or insertion of other sequences. Transposable elements can be implicated in all of these processes.

The set of experiments reported in this thesis provide evidence that large amounts of variation can be created in at least some quantitative traits exposed to dysgenic conditions. Estimates of 25% lethal
chromosomes and $V_M/V_e = 0.124 \pm 0.033$ for abdominal bristles were obtained for the homozygous effect of mutations induced in dysgenic flies relative to inbred controls. A significant correlation was found between the number of elements within a line, its viability and its contribution to the overall variance. Estimates of $V_M/V_e = 0.02$ per element insert were calculated for abdominal and sternopleural bristle score, with the best estimate obtained being $V_M/V_e = 0.035 \pm 0.006$ for abdominal bristles. On average it was estimated that an individual inserted element reduced homozygous viability by 3% $\pm$ 2.3% with 6% of inserts being recessive lethal. Similarly dysgenesis was found to generate an extra $V_M/V_e = 0.06$ and 0.37 bristles, for the abdominal and sternopleural traits respectively, and to decrease fitness by 13% relative to control chromosomes passed through the reciprocal cross.

These results confirm that the P element is a powerful biological mutagen capable of generating high levels of variation in at least some quantitative traits. Much work remains to be done, but these elements potentially provide the mechanism by which the underlying biology of quantitative variation can be assessed and dissected, much as they have already contributed to the understanding of classical genetic mutants.
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