

The characterisation of P element-induced singed mutations
in Drosophila melanogaster, and an analysis of the
extent of mobilisation of transposable elements
in a P-M hybrid dysgenic cross.

Thesis submitted for the degree of Doctor of Philosophy to the
Faculty of Medicine, University of Leicester.

by

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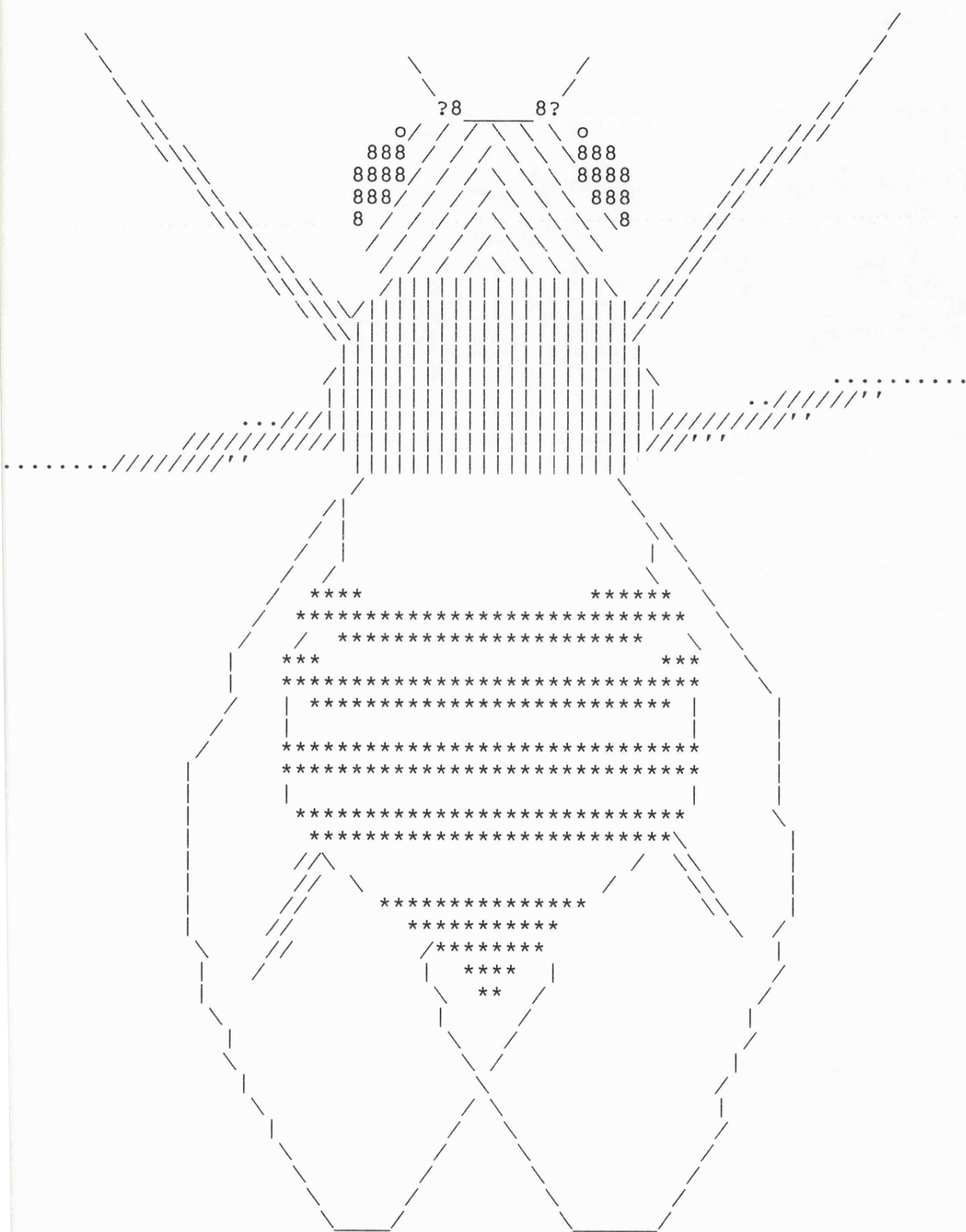
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To Elizabeth.

"Time flies like an arrow, but
fruit flies like a banana...."



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Index

Chapter 1 : Introduction	1
1.1 P elements	2
1.2 P-M hybrid dysgenic traits	4
1.3 Regulation of P element transposition	8
1.4 Tissue-specificity of hybrid dysgenesis	11
1.5 The mechanism of P element transposition	13
1.6 The origin of P elements	16
1.7 The use of P elements	19
1.8 Aims of the project	22
 Chapter 2 : Materials And Methods	 23
2.1 Materials	23
2.1.1 Fly stocks	23
2.1.2 DNA probes	23
2.1.3 Media	24
2.2 Methods	25
2.2.1 Maintenance of fly stocks	25
2.2.2 Phenol extraction of nucleic acids	25
2.2.3 Ethanol precipitation of nucleic acids	26
2.2.4 Large scale isolation of plasmid DNA	26
2.2.5 Preparation and transformation of competent bacteria	28
2.2.6 Agarose gel electrophoresis	29
2.2.7 Labelling of DNA probes	30
2.2.8 <u>In situ</u> hybridisation to <u>Drosophila</u> salivary gland polytene chromosomes	33

2.2.9 Isolation of <u>Drosophila</u> DNA	39
2.2.10 Southern blotting of DNA	41
2.2.11 Hybridisation of filters	41
2.2.12 RNA extraction from <u>Drosophila</u> pupae	43
2.2.13 Preparation of poly(A) ⁺ RNA from total <u>Drosophila</u> RNA	44
2.2.14 Northern blotting of RNA	45
2.2.15 Preparation of a bacteriophage λ L47.1 genomic DNA library	46
2.2.16 Scanning electron microscopy	52

Chapter 3 : An Analysis Of The Extent Of Mobilisation Of Transposable Elements In A P-M Hybrid Dysgenic Cross 53

3.1 Do transposable elements other than P elements transpose during P-M hybrid dysgenesis?	53
3.2 <u>Copia</u> -like and F transposable elements	54
3.3 <u>D. melanogaster</u> crosses	55
3.4 <u>In situ</u> hybridisation	56
3.5 Variable sites are present in the parental strains	60
3.6 Has P-M hybrid dysgenesis occurred in the dysgenic lines?	61
3.7 Have <u>copia</u> , <u>412</u> and F transposable elements been mobilised in the hybrid dysgenic cross?	62

Chapter 4 : The Characterisation Of P Element-Induced singed Mutations 65

4.1 P-M hybrid dysgenesis-induced <u>singed</u> mutations	65
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4.2	DNA molecular organisation of the <u>singed</u> mutations	70
4.3	Analysis of <u>singed</u> mRNA from the <u>singed</u> mutation strains	78

Chapter 5 : Attempts To Clone The P Element Insertions At The singed Locus In Strain singed-(very weak) 81

5.1	<u>singed-(very weak)</u> and <u>singed-weak</u>	81
5.2	The preparation and initial screening of <u>singed-(very weak)</u> genomic libraries	84
5.3	Analysis of genomic library plaques positive for CSsn9 genomic sequences	92

Chapter 6 : Discussion 95

6.1	Movement of <u>412</u> , <u>copia</u> and F transposable elements in P-M hybrid dysgenesis	95
6.2	Analysis of the P element-induced <u>singed</u> mutations	103
6.2.1	Bristle phenotypes	103
6.2.2	Germline reversion frequency	110
6.2.3	Somatic destabilisation	111
6.3	<u>singed-(very weak)</u>	113
6.4	Mutagenesis by transposable element insertion	115

Appendix 123

Chapter 7 : References 156

Chapter 1 : Introduction

Transposable elements have been defined as DNA segments which can insert into several sites in a genome (Campbell et al. 1977). They have been described in many organisms, ranging from IS and Tn elements in prokaryotes (Campbell et al. 1977), to the Ty elements of yeast (Cameron et al. 1979) and Ac elements in maize (McClintock 1950). The first elements recognised as being transposable were those of Zea mays, McClintock (1950, 1951, 1956) demonstrating that certain heritable nuclear components were responsible for changing the structure of maize chromosomes, consequently affecting gene expression. Since this discovery there has been extensive research into transposable elements in this and other systems.

Drosophila has been found to provide a first class experimental system for the study of these elements. We possess vast knowledge of its genetics making it very amenable to experimental manipulation. Drosophila also has an advantage over many other organisms in that it provides a technique for determining the genomic location of cloned DNA sequences. This method of analysis, termed in situ hybridisation, makes use of giant polytene chromosomes in the Drosophila third instar larvae which can be easily visualised under the light microscope. The Drosophila melanogaster haploid genome contains approximately 165000 kb of DNA (Rasch et al. 1971), of which about 9%, or 15000 kb, is made up of at least 50 different sequence families dispersed throughout the genome. These interspersed sequences were originally shown to be mobile transposable elements by using the in situ hybridisation technique, as their chromosomal positions were

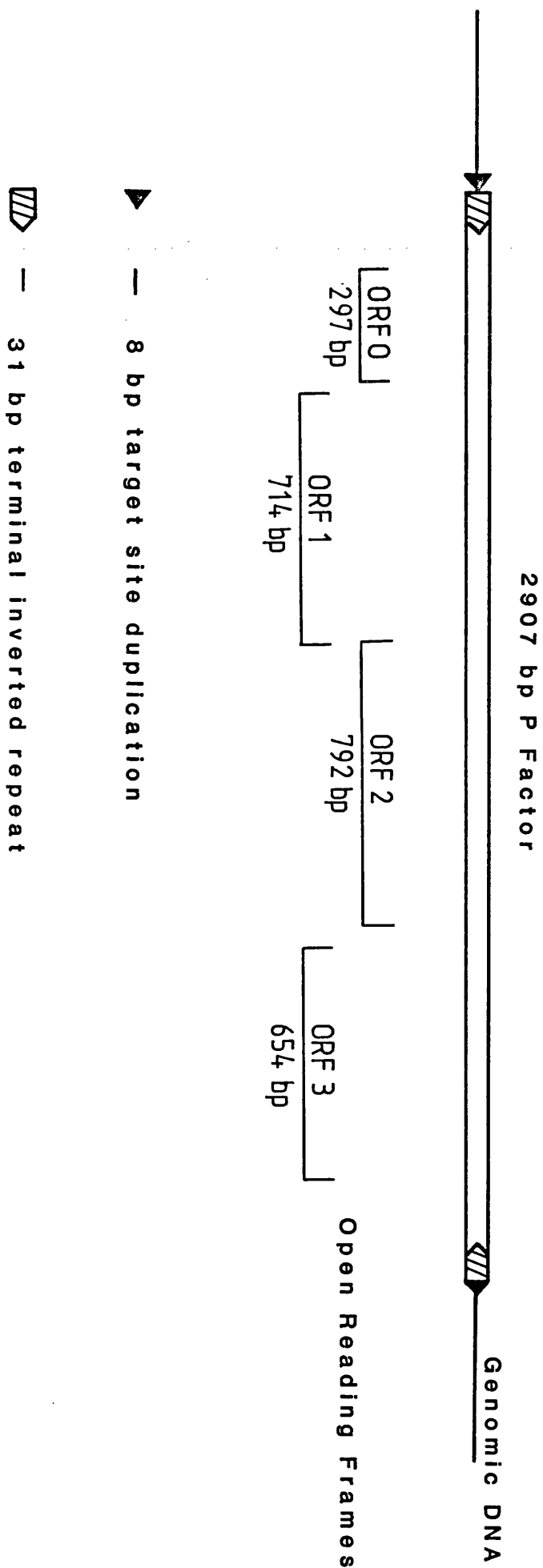
found to be highly variable between different stocks (Young 1979; Young and Schwartz 1981). Many spontaneous mutations in Drosophila have been found to be due to transposable element insertions. However, one can also actually use transposable elements to experimentally mutate certain D. melanogaster loci by means of a phenomenon known as hybrid dysgenesis.

Hybrid dysgenesis has been defined as a syndrome of correlated genetic traits that is spontaneously induced in hybrids between certain mutually interacting strains, usually in one direction only (Kidwell et al, 1977). These traits can include sterility, male recombination, elevated mutation rates, chromosomal rearrangements and transmission ratio distortion.

1.1 P Elements

One system of hybrid dysgenesis, the P-M system, involves certain transposable elements called P elements. The molecular structure of the P element has been studied extensively, and the entire element has been sequenced (O'Hare and Rubin 1983). An intact P factor has been isolated which consists of 2907 bp with precise 31 bp inverted terminal repeats (fig. 1). Smaller P elements also exist within the genome. They possess internal deletions of between a few hundred base pairs and over 2 kb, but still retain their terminal repeats. The 2.9 kb P factor possesses four major open reading frames (ORF's) on one of the DNA strands (O'Hare and Rubin 1983), each of which is thought to contribute to a single polypeptide of 87000 dalton molecular weight termed transposase (Karess and Rubin 1984; Rio et al. 1986). This protein is presumed to act on the 31 bp

Figure 1 Summary of the structure of the 2.9 kb P factor



terminal sequences to catalyse the transposition of P elements, although, in addition to these repeats, a cis-acting sequence required for transposition is also present in the first 140 bp of the P element (Rubin and Spradling 1983). The elements are flanked by an 8 bp direct duplication of genomic DNA for which a weak consensus sequence of GGCCAGAC has been proposed (O'Hare and Rubin 1983). The production of a small repeat at the site of insertion appears to be a general property of transposable elements and is thought to be the result of staggered nicks made at the target site by the transposase (Calos and Miller 1980).

D. melanogaster strains can be classed on the basis of whether or not they possess copies of the intact 2.9 kb P factor. P strains usually contain about 30-50 P elements (Bingham et al. 1982) which are probably made up of 10 copies of the P factor in addition to approximately 30 deleted elements (O'Hare 1985). M strains, on the other hand, are those which lack P factors (Rubin et al. 1982), but may in some cases contain the deleted, defective elements (Bingham et al. 1982), these flies being termed M' or pseudo-M strains. P elements can exist in the germline in either a quiescent or active state (Engels 1979). When quiescent, the elements are said to be in a cellular environment known as the P cytotype, this state being exhibited in P strain flies. Transposition in this condition occurs at a low level (Preston and Engels 1984). The active state, however, is observed in M strains possessing an M cytotype, and is characterised by high rates of P element transposition (Engels 1979). P factor activity is seen as the excision of P elements from chromosome sites and as the insertion of elements into new sites. P factors in the

sperm of a P strain male are transformed from a nondysgenic to a dysgenic state when they fertilize an M strain egg, and thus they become active. Hybrid dysgenesis is thus observed when males of a P strain are mated with females of an M strain, but is not observed in the reciprocal cross between an M male and P female, nor in a P male with P female or M male with M female cross (Engels 1983).

1.2 P-M Hybrid Dysgenic Traits

Gonadal dysgenic (GD) sterility is observed as a dysgenic trait in the P-M system. Both sexes may be affected, rudimentary gonads of reduced size and amorphous structure being produced (Yannopoulos 1978; Engels and Preston 1979; Schaefer et al. 1979). The courtship and mating behaviour of such flies appears normal but no gametes are actually produced. In some cases individuals are observed with one normal and one rudimentary gonad. These flies may be sterile in some cases and fertile in others. GD sterility exhibits temperature specificity, the highest level in dysgenic females occurring at 29°C, whilst being almost absent at 24°C (Engels and Preston 1979; Kidwell and Novy 1979). There is a sharp, approximately linear increase in sterility through the intermediate temperatures. The frequency of GD sterility in males is only one quarter of that seen in females, and also requires higher temperatures for its expression. The temperature-sensitive period for the sterility occurs during late embryonic development and continues into the first and second larval instar stages (Engels and Preston 1979; Schaefer et al. 1979).

GD sterility is thought to result from massive cell death in the germ line when P elements are undergoing transposition at high frequency (Kidwell 1983a; Engels 1983). The precise role of the elements, however, is unclear. The elements might perhaps simply insert into a specific sterility locus at high frequency. Alternatively, the actual site of insertion may not be of such importance, but instead the internal structure of certain P elements may be the key factor in causing sterility. Such elements could produce their sterility effects when destabilised at a number of chromosomal locations, causing chromosome fragmentation, arrest of germline cells, and thus GD sterility (Kidwell 1983a). However, the frequency of such chromosome breakage would have to be extremely high to cause the loss of all germ cells (Engels and Preston 1979). Thus, perhaps the sterility effect is due to a temperature-sensitive specific interaction between some area of the P factor transposition machinery and an aspect of the metabolism of the rapidly dividing germ cells (Engels 1983).

A second type of sterility termed embryo lethality (EL) sterility has also been observed in P-M hybrid dysgenesis (Kidwell 1984). This sterility is due to the death of embryos produced from both male and female F1 generation flies of dysgenic crosses. The embryo development has been found to be arrested in the latter stages of its cleavage divisions. The temperature-sensitive period for EL sterility occurs in the latter half of the developmental period, after the time at which that of GD sterility has been completed.

The mutations observed in P-M hybrid dysgenesis are usually caused by one of three mechanisms - P element insertion, P element excision or chromosome rearrangement. The

frequencies of P element insertions are found to vary between different loci examined (Simmons and Lim 1980; Simmons et al. 1984) and also between different target sites within the same locus (O'Hare and Rubin 1983; Chia et al. 1986; O'Hare 1986; Roiha et al. 1987). Mutations caused by P element insertion can revert in some circumstances to partially or completely restore the wild-type phenotype. These events are due to imprecise or precise excisions of the elements, thus resulting in the loss of most or all of the P element from the site of insertion (Voelker et al. 1984; Daniels et al. 1985; Searles et al. 1986).

Imprecise excisions can also occasionally cause mutations (Engels 1983). This can occur if the element is originally located at a position near a gene such that the locus is not affected. If imprecise excision of the element subsequently occurs removing some flanking sequences which extend into essential regions of the gene, then a mutation can be produced.

The frequency of P element excision can in fact be used as an assay to test the ability of a P strain to cause hybrid dysgenesis. Singed-weak (sn^W) is a P element insertion mutation affecting bristle morphology which, in hybrid dysgenic crosses, mutates by excision either to a more extreme or to a wild-type phenotype at frequencies as great as 50% (Engels 1979). Thus, the potential of a strain for hybrid dysgenesis can be deduced by crossing its males to M strain sn^W females and observing the frequency of mutability (Engels 1984).

Many of the mutations produced in P-M hybrid dysgenesis are caused by chromosomal rearrangements, the breakpoints of

which usually occur at or very near the sites of preexisting P elements (Simmons and Lim 1980; Engels and Preston 1981b; Engels and Preston 1984). The most common events are inversions and reciprocal translocations, although more complex rearrangements can also occur possessing multi-point breaks. The P elements present at the breakpoints are often lost during the rearrangement event by P element excision, although occasionally there can also be an apparent gain of P elements. Sometimes, both breakpoints of an inversion can retain their P elements, and in these cases the inversion is capable of reverting at high frequency to the original sequence or something very similar (Engels and Preston 1984).

Male recombination is not normally observed in D. melanogaster, but has been found often to be associated with GD sterility in P-M hybrid dysgenesis (Kidwell and Kidwell 1975; Yannopoulos 1978; Engels and Preston 1980). This trait may occur in both second and third chromosomes (Kidwell and Kidwell 1976; Woodruff and Thompson 1977), and is usually premeiotic in nature (Hiraizumi 1979). Most of the breakpoints that occur in male recombination are found in or adjacent to the centric heterochromatin, thus differing from the position of meiotic female recombination breakpoints (Slatko and Hiraizumi 1975; Kidwell and Kidwell 1976). Transmission ratio distortion was originally observed as a negative correlation between the male recombination frequency and the transmission ratio of chromosomes derived from M and P strains (Hiraizumi 1971; Kidwell and Kidwell 1976; Kidwell et al. 1977). It was found that in P-M dysgenic hybrids M strain-derived chromosomes appeared to be transmitted at a higher frequency than the chromosomes derived from the P strain, thus resulting

in loss of the P chromosomes from the progeny (Hiraizumi 1977).

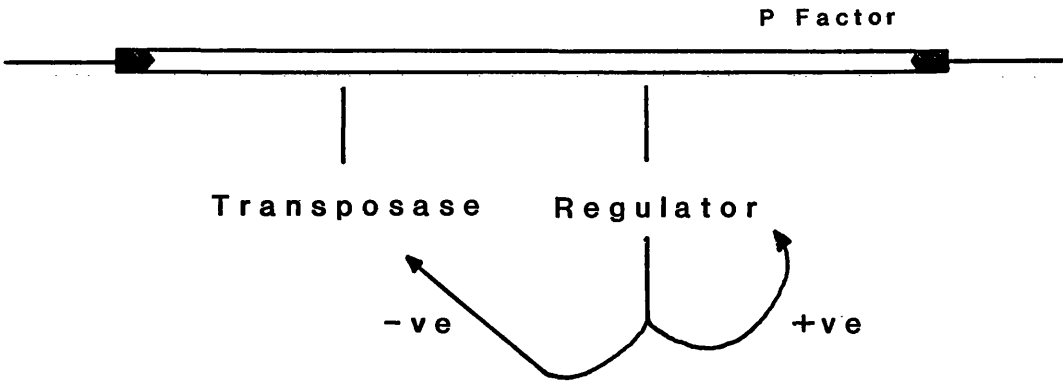
There is variability between P strains in the extent to which they cause the different dysgenic traits (Engels and Preston 1980; Kocur et al. 1986). In some strains all traits are observed at a similar level, whilst in others certain of the traits are more pronounced. One such example occurs in certain P strains known as Q strains. These strains are unable to cause GD sterility but possess normal levels of activity for the other dysgenic traits (Simmons et al. 1980; Engels and Preston 1981a; Kidwell 1981).

1.3 Regulation Of P Element Transposition

P element transposition is regulated, suppression occurring in the P cytotype, but derepression when present in M strains. It is thought that regulatory factors are present in the eggs produced by P strain females, but are not in the sperm of P males, nor in either the eggs or sperm of M strain flies. Thus the cross between a P strain male and M strain female results in hybrid dysgenesis, P elements from the sperm of the male entering an environment lacking the repressor, whereas the P eggs of the reciprocal cross contain the regulatory molecules repressing the transposition process. It has been suggested that the repressor is in fact a protein regulator encoded by the 2.9 kb P factor, and O'Hare and Rubin (1983) have proposed a model which incorporates this hypothesis (fig. 2). The regulator is postulated to feedback positively on itself stimulating its own activity, but negatively on the production or activity of the transposase.

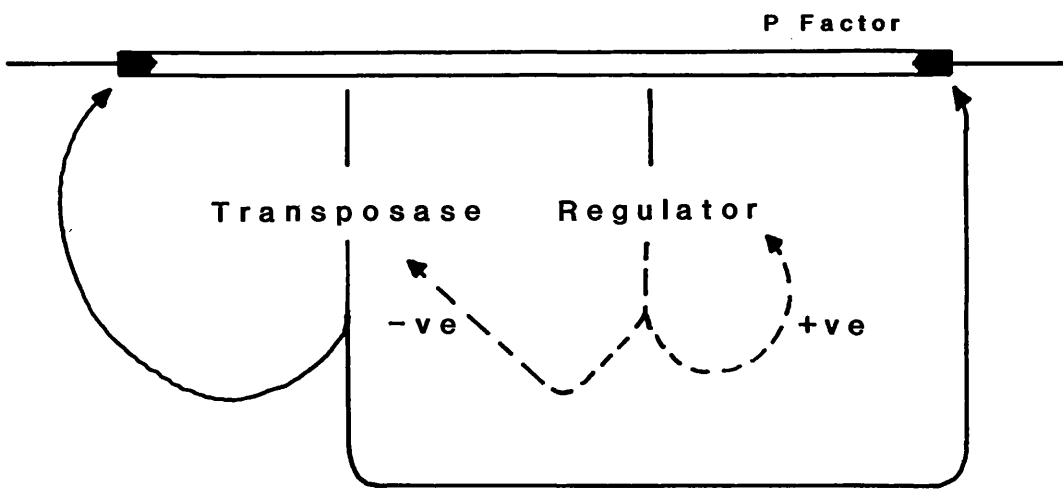
Figure 2 Model for the regulation of P element transposition (O'Hare and Rubin,1983)

P CYTOTYPE



The regulator feeds back positively on itself and negatively on the transposase thus repressing transposition.

M CYTOTYPE



The amount of regulator is insufficient to repress the transposase and thus transposition of P elements occurs. With time, the regulator accumulates to a level at which the cytotype is switched to P.

Thus, in the P cytotype, where regulator is present in high abundance, P element transposition is switched off. On the other hand, when P elements are first introduced into the M cytotype from a P sperm, the amount of regulator is inadequate to repress the transposase produced by the P factors, and thus initially transposition occurs. Eventually, however, due to positive feedback of the regulator on its own activity, a level is reached such that transposition is switched off and the cytotype is converted to P. In certain crosses this switch of cytotype may take only a single generation, but in other cases several generations may be required (Kidwell 1981).

This model raises the question of what form the repressor of transposition takes. One could conceivably imagine that a defective transposase produced from a deleted P element could function in some way as a repressor, perhaps binding to the 31 bp terminal repeats thus interfering with the action of the active transposase. Such a molecule, or its parent P element, however, would have to be in some way transmitted from generation to generation in tandem with the P factors, and in the case of the P element, without undergoing any further deletions.

Simmons and Bucholz (1985) have proposed a model hypothesizing that the regulator is in fact the P element itself. This "transposase titration" model suggests that extrachromosomal defective P elements are present in the P cytotype which bind the transposase thus preventing it from causing hybrid dysgenesis. These defective elements are not present in the M cytotype, and thus when P elements are introduced from a P sperm transposition can occur. Extrachromosomal elements are produced by the action of

transposase such that with time the P cytotype is achieved. This model fits in with observations that the offspring of M' strain with P strain hybrid dysgenic crosses show a lower frequency of sterility (Kidwell 1983b) and a reduced sn^w mutability (Simmons and Bucholz 1985) than progeny of normal M strain, P strain crosses. These reductions could be due to the defective P elements of the M' strains titrating out the transposase to some extent. Certain deletion derivatives termed KP elements, however, have been observed in M' strains at an abundance of up to 30 copies per haploid genome (Black et al. 1987). The elements are 1154 bp long and yield a 0.8 kb poly(A)⁺ RNA with the coding capacity for a 207 amino acid polypeptide. It is thought that these elements may be responsible for the weak repression of P element transposition exhibited in M' strains, as strains constructed, possessing different numbers of KP elements, reduce both transposition rates and levels of gonadal sterility, even when present in low copy-number (Black et al. 1987; Jackson et al. 1987). Whether this reduction is due to "transposase titration" by the KP elements or to some other mechanism is unclear. These elements are not present in American P and Q strains, however, and thus can not be involved in regulation of P transposition in these strains, and may not be the major factor in the repression in M' strains.

A second model of transposition regulation based again on extrachromosomal P elements has been proposed by Sved (1987). However, in this case the extrachromosomal species are in fact 2.9 kb P factors circularised by joining of the 8 bp direct repeat target site. In this model two forms of P factor transposition are hypothesized depending on the cytotype. In

the P cytotype it is suggested that the extrachromosomal P factors are present, and that transposition occurs preferentially to the target sites of these extrachromosomal P factors rather than to chromosomal sites. The structures formed by insertion are each resolved into two extrachromosomal factors, and thus hybrid dysgenesis is not produced, P factor transposition in the P cytotype leading only to the production of further extrachromosomal P factors. In the M cytotype, however, the extrachromosomal P factors are absent and thus transposition occurs to the chromosomal target sites, resulting in hybrid dysgenesis. Extrachromosomal P factors are hypothesized to be produced at a low probability in M cytotype transposition and thus switching to P cytotype is eventually achieved. One problem with this model, however, is how such extrachromosomal factors would repress the excision of elements.

As yet, no extrachromosomal P elements have been visualized, but such copies of the Drosophila transposable element copia have been observed (Flavell and Ish-Horowicz 1981; Flavell and Ish-Horowicz 1983; Shiba and Saigo 1983). It should be emphasised, however, that these are thought to be intermediates in the mechanism used for copia transposition.

1.4 Tissue-Specificity Of Hybrid Dysgenesis

P element transposition is also regulated in that it is tissue specific, transposition in hybrid dysgenesis being restricted to the germline (Engels 1983; McElwain 1986). This tissue specificity has been found to be controlled at the level of mRNA splicing (Laski et al. 1986). Karess and Rubin

(1984) have analysed the poly(A)⁺ transcripts produced from a D. melanogaster line carrying a single copy of the 2.9 kb P factor but no deleted P elements. Two mRNA species were observed, a major transcript of 2.5 kb and a minor type of 3.0 kb. It was thought that one of these transcripts might encode the transposase polypeptide. However, on analysis (Laski et al. 1986), it was found that the two transcripts were identical except at their 3' ends where in the 3.0 kb mRNA species the message had extended through the normal P element polyadenylation signal and was terminated at a downstream site in the DNA flanking the P factor. It was also discovered that the intron between ORF2 and ORF3 was not spliced out and consequently the protein products of both species would be identical, terminating at the 3' end of ORF2 and having an estimated molecular weight of 66000 daltons. A polypeptide of the correct molecular weight was identified as the product of the transcripts, but this was found to be lacking in transposase activity (Rio et al. 1986). A construct was thus made containing a P factor without the ORF2-ORF3 intron, and this was used to transform Drosophila flies. A strong promoter was also added, and Drosophila cell cultures transformed for further analysis. It was found that a polypeptide of the correct 87000 dalton estimated molecular weight was produced in cells, and that in transformed flies this possessed transposase activity in both germline and somatic tissue (Laski et al. 1986; Rio et al. 1986). Thus it was proposed that the tissue specificity is due to differential ORF2-ORF3 intron splicing. In somatic cells, splicing of this intron does not occur, and thus the 576 amino acid residue, 66000 dalton polypeptide, inactive as a transposase, is produced. In

the germline, however, a splicing event joins ORF2 to ORF3, such that the 751 amino acid residue, 87000 dalton transposase is synthesized and transposition of P elements occurs. The spliced transcript was not detected by Karess and Rubin (1984) because the mRNA pool would have been dominated by somatic mRNA species. The actual mechanism for the splicing is not known at present, although it is thought to be due to either the presence of a germline specific factor necessary for the splicing event, or to a germline specific alteration of the P transcript tertiary structure that is needed for splicing to occur.

The alternative mRNA splicing may also be the key to answering the question of how P element transposition is regulated. Both the 66000 and 87000 dalton polypeptides possess a putative DNA binding domain at amino acid residues 308-327 (Rio et al. 1986). A model can therefore be proposed in which both proteins retain site-specific DNA binding activity but only the 87000 dalton polypeptide possesses transposase activity. The 66000 dalton polypeptide may perhaps compete with the transposase for binding to the P element terminal repeats, or may directly interfere with the transposase by protein-protein interactions. The 66000 dalton protein thus may in fact be the repressor of the O'Hare and Rubin (1983) model.

1.5 The Mechanism Of P Element Transposition

One area of P-M hybrid dysgenesis that still comparatively remains a mystery is the actual mechanism of transposition. A fundamental question is whether the process

is replicative or conservative. Most of the evidence seems to support a replicative mechanism involving the transfer of sequence information, rather than the physical transfer of a P element from one site to another. Benz and Engels (1984) have analysed the loss and gain of P elements during hybrid dysgenesis (Simmons and Kares 1985). A P element-bearing X chromosome was maintained for a number of generations by crossing to attached-X M strain females. It was found that new insertions on the X chromosome outnumbered excisions by a factor of 1.5-3.0, thus providing some evidence for replicative transposition. W. R. Engels and C. R. Preston have followed the fate of single P factors in otherwise pure M strain genomes (Simmons and Kares 1985; Simmons and Bucholz 1985). In one line the P elements grew slowly at first, and then explosively until the line died out. In a similar experiment Daniels et al. (1987) observed the accumulation of numerous elements in 3 such lines over a period of about 7 generations. Such rapid gains in P element numbers again argues for a replicative model.

P elements have been found to function largely independently of the host D. melanogaster DNA repair mechanisms (Slatko et al. 1984), suggesting that the elements code for the major product(s) (the transposase) necessary for their movement. What, however, is the method of movement? Some insight has been obtained from studying the chromosome rearrangements produced by hybrid dysgenesis. Some classes of multi-point rearrangements were found to be too complex to have been generated by sequential two-break events, suggesting that there must have been a number of breakpoints at several P element sites, followed by a random reassortment of the

fragments, and joining of the broken ends (Engels and Preston 1984). This result tends to rule out models for rearrangement production that involve homologous recombination or co-integrate structures (Shapiro 1979; Calos and Miller 1980; Galas and Chandler 1981).

Certain Drosophila transposable elements, termed copia-like elements, are thought to transpose via a full length RNA transcript of the element followed by reverse transcription to produce the new DNA copy (Flavell and Ish-Horowicz 1981; Flavell and Ish-Horowicz 1983; Shiba and Saigo 1983; Flavell 1984; Mount and Rubin 1985) thus following the mechanism used by retroviruses (Bishop 1978; Weinberg 1980; Varmus 1983). There are a number of arguments, however, against this mechanism being used in P element transposition. The structure of P elements differs from those transposable elements which transpose using an RNA intermediate, an example being the lack of a reverse transcriptase-like gene DNA sequence. The major P transcripts lack the first 87 nucleotides, and also intervening sequences, and thus are very unlikely to be transposition intermediates (Karess and Rubin 1984; Laski et al. 1986).

A "cut and paste" mechanism of transposition (Kleckner 1981) is thought to best fit the data at present (O'Hare 1985). Double-stranded cuts are first produced at the target site of the genomic DNA and at one or both ends of a P element, and then the element sequence is either copied (if replicative) or physically transposed (if conservative) into the new site. If a replicative model is used and the copying mechanism is prone to slippage of the replication fork, then deletion of the element could occur, thus accounting for the

production of the deleted P elements from the P factor (O'Hare and Rubin 1983).

1.6 The Origin Of P Elements

There has been speculation concerning the origin of P elements within D. melanogaster. It is thought that P elements only appeared in the D. melanogaster genome in the last 30-60 years as true M strains are mostly found to be stocks kept in laboratories since before that time, whereas flies caught in the wild during the last 15 years are usually P (Bingham et al. 1982; Kidwell 1983b). This "recent-invasion" hypothesis suggests that P elements may have initially been introduced into D. melanogaster by some type of rare interspecific gene transfer, and have subsequently spread through natural populations by vertical transmission. Geographical investigations reveal a lack of M strains in natural populations of America becoming more abundant in Europe and Asia (Kidwell 1983b; Anxolabéhère et al. 1984) thus perhaps suggesting that the original invasion may have occurred on the American continent.

A number of studies have looked for the presence of P elements in other Drosophila species and these have suggested a possible source for the horizontal transfer of the P elements. P elements have been found to be widely distributed in the subgenus Sophophora (Lansman et al. 1985; Daniels and Strausbaugh 1986) which includes the species groups melanogaster, obscura, willistoni and saltans (Throckmorton 1975). They are completely absent from the D. melanogaster sibling species, and from other members of the melanogaster

subgroup (Brookfield et al. 1984), and yet are present in a number of species of the more distantly related montium and ficuspila subgroups of the melanogaster species group (Lansman et al. 1985), and also in the very distantly related species groups obscura, willistoni and saltans (Daniels et al. 1984; Lansman et al. 1985; Daniels and Strausbaugh 1986). P-homologous fragments were found to be present in lower numbers in the willistoni and saltans group species when compared to D. melanogaster P strains, and also showed characteristic hybridisation patterns within a number of these species (Daniels and Strausbaugh 1986). These results, together with the result that most of the willistoni and saltans species possess P elements, suggest that the P elements may have had a long evolutionary history in the willistoni and saltans species groups, being resident perhaps before they diverged from the melanogaster lineage. Natural populations of D. nebulosa, D. willistoni and D. sucinea, of the willistoni species group, extend geographically into the southern portion of North America where the P elements are suspected to have arisen in D. melanogaster. In conjunction with the observation that P element sequences in the willistoni group species have similar restriction enzyme sites to D. melanogaster P elements, this has led Daniels et al. (1984) to suggest that a species from the willistoni group may have been the source for the P element invasion of D. melanogaster.

Functional P elements have been introduced into the D. melanogaster sibling species D. simulans (Scavarda and Hartl 1984; Daniels et al. 1985) and into a very distant relative of D. melanogaster, D. hawaiiensis (Brennan et al. 1984), by

interspecific DNA transformation. The behaviour of the elements appears to be strikingly like their behaviour in D. melanogaster, thus suggesting that the action of P elements may not be significantly influenced by Drosophila species differences. Thus, one would in fact expect that P elements would be invasive when introduced into a new species.

How could the original interspecific transfer into D. melanogaster have occurred? Miller and Miller (1982) have observed a copia-like transposable element, from the lepidopteran species Autographa californica, inserted into the genome of a large DNA virus that infects this species. Thus, it is possible that the P element was transferred into D. melanogaster by integrating into the genome of a virus which had both species of the gene transfer in its host range. Such a transfer would have to be a sufficiently rare event such that no transfer of P elements has yet occurred into D. simulans (Engels 1986).

Engels (1983, 1986) has proposed an alternative model to the "recent-invasion" hypothesis, in which he states that P elements were in fact present in D. melanogaster well before any laboratory stocks were established. It is suggested that P strain populations were originally only situated in relatively isolated parts of the world and that early laboratory samples may have excluded these isolates by chance. Due to enhanced Drosophila migration rates, however, brought about by human activity this century, the elements have recently spread into previously uninfected populations.

1.7 The Use Of P Elements

P elements can be used as tools for the cloning of genes in which the elements have inserted. This method of molecular cloning by "transposon tagging" was first utilised by Bingham et al. (1981) to clone the white (w) locus with the transposable element copia. A mutant allele, white-apricot (w^a) was observed to have arisen by the insertion of copia into the w gene. A genomic library was produced and a number of copia-containing clones identified. These were then analysed for w flanking sequences by in situ hybridisation to fly stocks, wild-type for w. P elements can also be used in a similar manner to clone genes (Searles et al. 1983; Engels 1985; Chia et al. 1986). These transposable elements, however, have a distinct advantage over copia in that hybrid dysgenesis can be used to induce mutations at gene loci by P insertion or chromosomal rearrangements. The only limitation to this method is the site specificity of P elements, some loci being mutated at too low a frequency for the method to be practical (Engels 1983).

Once a gene has been cloned, P elements can also be used to return it to the genome. Spradling and Rubin (1982) found that P factors could transpose from plasmid vectors into the D. melanogaster genome. A bacterial plasmid containing a 2.9 kb P factor, together with flanking Drosophila sequences, was microinjected into the posterior pole of embryos from an M strain. P factor DNA introduced in this manner became integrated into the genomes of a number of posterior pole cells. These cells are the germ-line precursor cells, and thus the progeny of injected embryos would be expected to have

incorporated P factors. It was found that P factors could be integrated into a number of the germ cells of at least half of the injected fertile adults. The inserted elements occurred at widely scattered positions and were not accompanied by any flanking Drosophila or plasmid DNA, thus suggesting that integration had occurred by transposition.

Rubin and Spradling (1983) have exploited the above finding and produced a number of plasmid vectors which allow the transfer of genes into Drosophila. Their initial experiment (Rubin and Spradling 1982) involved a synthetic transposon, pry1, in which a copy of the wild-type rosy (ry) gene, coding for xanthine dehydrogenase, was incorporated into a 1.2 kb deleted P element cloned in a bacterial plasmid. This construct was co-injected into ry mutant flies with a plasmid carrying the 2.9 kb P factor for transposase production. The transposable element was found to efficiently transfer the gene into the germ-line of the fruit flies thereby correcting the genetic defect in their progeny, even though the ry gene integrated at a variety of chromosomal locations in different flies. The experiment has been successfully repeated with a variety of other genes (Scholnick et al. 1983; Spradling and Rubin 1983; Goldberg et al. 1983).

The development of such a transformation system in Drosophila has led to the production of an alternative method to "transposon tagging" for cloning genes (Steller and Pirrotta 1985). A transposon called Icarus-neo has been constructed in which the P transposase gene is under the control of the Drosophila heat shock hsp70 promoter. The construct also contains a resistance gene for neomycin as a selectable marker for transformants, and a bacterial plasmid

vector pUC9 (Vieira and Messing 1982) between the P terminal inverted repeats. On heat shocking a transformed fly containing this construct transposition is increased 10-fold, thereby generating insertional mutations. Once the desired mutation is detected, the construction of the transposon allows the direct recovery of the sequences flanking the insertion site by means of plasmid rescue (Perucho et al. 1980).

The P element gene transfer vectors provide numerous applications for the study of gene expression in Drosophila. They allow the analysis of the DNA control signals required for the developmental and tissue-specific regulation of gene expression. In vitro mutagenesis of sequences around these cloned genes will enable one to see which DNA is required for their correct expression. One can also analyse dosage compensation, using the transformation system to find out whether X-chromosome DNA regulates the expression of adjacent genes. The study of the expression of genes from other Drosophila species in D. melanogaster is also feasible, and due to the fact that interspecific transformation can be achieved (Brennan et al. 1984; Scavarda and Hartl 1984; Daniels et al. 1985), also the study of genes transferred between any two Drosophila species. Recent results have suggested that transposase can be expressed in cultured mammalian cells (Rio et al. 1986), and thus it is possible that P element vectors could be used for transforming organisms other than Drosophila.

1.8 Aims Of The Project

A number of experimental results (Gerasimova 1981; Rubin et al. 1982; Gerasimova et al. 1984a, 1985; Clark et al. 1986; Côté et al. 1986) have suggested that hybrid dysgenesis, in addition to the increase it produces in the transposition rate of P elements, may also increase the rate of transposition of other transposable element families to a certain extent. I have used in situ hybridisation techniques to investigate the changes in the location of the transposable elements copia, 412 and F during a hybrid dysgenic cross, in order to provide further data on this aspect of P-M hybrid dysgenesis.

One area of interest in P-M hybrid dysgenesis involves the question of how P elements actually mutate the genes into which they insert. A number of singed (sn) mutations have been induced in D. melanogaster by P-M hybrid dysgenesis (Brookfield and Mitchell 1985) due to insertion of P elements. The mutations possess different properties in terms of their phenotypes and frequencies of P element excision, and have been analysed at the molecular level to try and correlate any variations with their observed characteristics. This may, perhaps, be reflected in the P element insertion sizes, positions and orientations.

Chapter 2 : Materials And Methods

2.1 Materials

2.1.1 Fly Stocks

D. melanogaster strains used were Canton-Special (Canton-S) obtained from B. Burnet, and Harwich from M. Ashburner.

2.1.2 DNA Probes

Sequences used as probes were p π 25.1 (containing a complete P factor cloned into pBR322), pBB2 (with a 2LTR copia circle cloned into pAT153), and cDm2042 (with 412 cloned into pBR322), kindly supplied by G. M. Rubin (Flavell and Ish-Horowicz 1983; Montgomery and Langley 1983; O'Hare and Rubin 1983), 19-27 (with an F element cloned into pBR322) obtained from P. P. DiNocera (DiNocera et al. 1983), and CSsn9 (containing a region of the sn locus cloned into pBR322) and pBSP6 (with a sn cDNA sequence in a Bluescribe vector) supplied by K. O'Hare (Roiha et al. 1987).

Both of the cloning vectors pBR322 (Bolivar et al. 1977) and pAT153 (Twigg and Sherratt 1980) possess the selectable markers Ap^r (ampicillin resistance) and Tc^r (tetracycline resistance), whilst the Bluescribe vectors (Vector Cloning Systems) possess Ap^r and the lacZ gene.

2.1.3 Media

Oatmeal-treacle-agar Drosophila medium

10 l	distilled water
1.3 kg	medium ground oatmeal
110 ml	Nipagin (25 g Nipagin M (Tegosept M, p-hydroxybenzoic acid methyl ester) in 250 ml ethanol)
400 ml	black treacle (Silver Spoon)
65 g	agar (Steetley)

Luria broth (LUB)

1% (w/v)	tryptone (Oxoid)
0.5% (w/v)	yeast extract (Oxoid)
0.5% (w/v)	NaCl
Adjusted to pH 7.0 with 1 M NaOH	

Luria agar (LUA)

LUB, solidified with 1.5% (w/v) agar (Sterilin)

Nutrient broth (NB)

2.5% (w/v)	nutrient broth no.2 (Oxoid)
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Lambda buffer (LB)

6 mM	Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.2
10 mM	MgSO ₄
0.005% (w/v)	gelatin

BBL bottom layer agar (BLA)

1% (w/v)	trypticase peptone (BBL)
0.5% (w/v)	NaCl
1.7% (w/v)	agar (Sterilin)

BBL top layer agar (BTL)

1% (w/v)	trypticase peptone (BBL)
0.5% (w/v)	NaCl
0.7% (w/v)	agar (Sterilin)

2.2 Methods

2.2.1 Maintenance Of Fly Stocks

D. melanogaster stocks were maintained in 1/3 pint milk bottles on oatmeal-treacle-agar medium overlayed with a drop of active live yeast suspension. Unless otherwise stated, all stages were kept at 26°C.

2.2.2 Phenol Extraction Of Nucleic Acids

This technique is used to extract proteins from nucleic acid solutions. Phenol/chloroform stock solution is made up of

100 ml chloroform, 0.1 g 8-hydroxyquiniline, 100 g phenol, and 4 ml isoamyl alcohol, and overlayed with 100 ml of 10 mM Tris-HCl, pH 7.5.

An equal volume of phenol/chloroform was added to the DNA solution, and mixed until emulsified. For small volumes, this was spun for 5 minutes at 13 krpm in an MSE Micro Centaur, and for large volumes, in a Sorvall HB4 rotor at 10 krpm, 4°C, for 10 minutes (Sorvall RC-5B centrifuge). The upper, aqueous layer was removed and retained, and the lower, phenol layer, reextracted with a small volume of 10 mM Tris-HCl, pH 7.5. The aqueous layers were pooled.

2.2.3 Ethanol Precipitation Of Nucleic Acids

0.1 volume of 2 M sodium acetate (pH 5.6 for DNA; pH 7.0 for RNA) and 2.5 volumes of ethanol were added to the nucleic acid solution and mixed gently. In the case of DNA, if a clot was produced, this was transferred to a tube by means of a pipette tip. For RNA, however, and in the case of DNA where a precipitate was not visible to the naked eye, the solution was incubated at -70°C for one hour, and then spun for 15 minutes at either 13 krpm in an MSE Micro Centaur, or at 10 krpm in a Sorvall HB4 rotor, 4°C (Sorvall RC-5B centrifuge). Unless otherwise stated the nucleic acid precipitate was then drained, washed with 70% (v/v) ethanol, vacuum dried, and redissolved in the appropriate solution.

2.2.4 Large Scale Isolation Of Plasmid DNA

This method was based on the triton X-100 lysis procedure of Pratt (1984). Bacterial cells were grown overnight at 37°C in 400 ml of LUB containing ampicillin (Sigma) at 50 µg/ml.

The bacterial cells were harvested from the overnight cultures by centrifugation (Sorvall GS3 rotor at 5 krpm for 10 min. at 4°C in a Sorvall RC-5B centrifuge) and resuspended in 10 ml 0.7% (w/v) Na₂HPO₄, 0.4% (w/v) NaCl, 0.1% (w/v) KH₂PO₄, and 0.01% (w/v) MgSO₄.7H₂O. Cells were again harvested, but by centrifugation in a Sorvall SS34 rotor at 8 krpm for 10 minutes at 4°C (Sorvall RC-5B centrifuge). The pellet produced was resuspended in 3 ml of TS (25% (w/v) sucrose, 50 mM Tris-HCl, pH 8.0) and incubated for 10 minutes at room temperature with 0.5 ml of 10 mg/ml lysozyme (Sigma; dissolved in TS) and 30 µl of 5 mg/ml ribonuclease A (Sigma). 1 ml of 0.25 M ethylenediaminetetraacetic acid (EDTA) was added, and the mixture incubated for a further 10 minutes at room temperature. After the addition of 4 ml of 2% (v/v) triton X-100 in 50 mM Tris-HCl (pH 8.0) the resulting solution was spun in a Sorvall SS34 rotor at 18 krpm for 20 minutes at 4°C (Sorvall RC-5B centrifuge). The supernatant was decanted and incubated on ice for 1.5 hours with 2/3 the volume of 25% (w/v) polyethylene glycol (PEG) 6000, 1.25 M NaCl. The solution was centrifuged in a Sorvall HB4 rotor (10 minutes at 5 krpm, 4°C in a Sorvall RC-5B centrifuge) and the resulting pellet resuspended in 1.1 ml of TES (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 5mM EDTA). 4 ml of CsCl mix (80 g CsCl, 52 ml TES, 8 ml 5 mg/ml ethidium bromide; refractive index 1.399) was pipetted into a 5 ml Beckman Quick-Seal centrifuge tube, and overlaid with the plasmid suspension. This was spun in a Beckman VTi65 rotor at 50 krpm, 15°C overnight (Beckman L5-65 ultracentrifuge) to band the DNA.

The overnight centrifuge tube was observed in a darkroom under UV light, and the plasmid DNA (lower band) collected

using a 1 ml syringe with a 0.8 mm x 25 mm needle. An equal volume of isopropyl alcohol (maintained over 5 M NaCl) was added to the DNA solution, mixed and then spun in an MSE Micro Centaur at 13 krpm for 5 minutes to remove the ethidium bromide. The lower layer was collected and reextracted with isopropyl alcohol until the originally pink solution had cleared. This was then dialysed against 750 ml sample volumes of TE (1 mM EDTA, 10 mM Tris-HCl, pH 8.0) for 30 minutes, 1 hour and 2 hours respectively. The resulting plasmid DNA solution was phenol extracted twice, ethanol precipitated, and the dried DNA redissolved in 1 ml TE and stored at -20°C.

2.2.5 Preparation And Transformation Of Competent Bacteria (adapted from Mandel and Higa 1970)

Plasmids were maintained in Escherichia coli strain 5K (F⁻, thi-1, thr-1, leuB6, lacY1, tonA21, supE44, λ^- , hsdR_K; Hubacek and Glover 1970).

10 ml LUB was inoculated with E. coli 5K, and grown at 37°C to mid-logarithmic growth phase (OD₆₀₀ = 0.35). The suspension was incubated on ice for 10 minutes, and the cells harvested in universal tubes at 4 krpm for 10 minutes at 4°C in an MSE Chilspin 2. The bacterial pellet was resuspended in 5 ml of precooled 0.1 M MgCl₂ and spun as before at 4 krpm for 10 minutes, 4°C. The pellet was resuspended in 5 ml of precooled 0.1 M CaCl₂ and incubated on ice for 30 minutes. The suspension was again centrifuged at 4 krpm for 10 minutes, 4°C, and the resulting pellet resuspended in 600 μ l of precooled 0.1 M CaCl₂. 200 μ l of the competent cells was taken and 10 μ l of 0.1 μ g/ μ l plasmid DNA added. This was incubated for 1 hour on ice, and heat shocked in a 42°C water bath for 5

minutes. 2 ml of NB was added, and the cells incubated at 37°C for 90 minutes to allow expression of the ampicillin resistance of the plasmid. Cells were spread onto 50 µg/ml ampicillin (Sigma) LUA plates, 100 µl of suspension per plate, and grown inverted at 37°C overnight. Transformants were resuspended in 5 ml of 50 µg/ml ampicillin (Sigma) LUB and shaken overnight at 37°C. 800 µl of culture was added to 200 µl of glycerol and stored at -70°C.

2.2.6 Agarose Gel Electrophoresis

Electrophoresis of DNA was carried out using TAE buffers (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0) and SeaKem HGT agarose as described by Maniatis et al. (1982). DNA was visualised on the gels by immersing in 5 µg/ml ethidium bromide for 10 minutes and washing in water (Sharp et al. 1973). The ethidium bromide contains a planar group that intercalates between the stacked bases of DNA such that dye bound to DNA displays an increased fluorescent yield compared to that in free solution. UV-irradiation absorbed by the DNA at 260 nm and transmitted to the dye, or irradiation absorbed at 300 and 360 nm by the bound dye itself, is emitted at 590 nm in the red-orange region of the visible spectrum, and thus can be visualised by eye. Endonuclease restriction digests were performed under the conditions specified by the manufacturers (BRL). Gel-loading buffer used was 0.04% bromophenol blue, 0.04% xylene cyanol, 25% ficoll 400.

Electrophoresis of RNA was as described (Maniatis et al. 1982; Amersham 1985) using 1% agarose (SeaKem HGT), 1 x MOPS buffer (10 x MOPS buffer is 0.2 M 3-(N-Morpholino) propane sulphonic acid, 0.05 M sodium acetate, pH 7.0, 0.01 M EDTA),

38% (w/w) formaldehyde gels run in 1 x MOPS buffer.

2.2.7 Labelling Of DNA Probes

(i) Labelling with α -[^{32}P]-dCTP (Amersham; 3000 Ci/mmol, 10 $\mu\text{Ci}/\mu\text{l}$)

Nick translation (based on Rigby et al. 1977): The following were mixed on ice; 50-100 ng DNA , 2.5 μl nick mix (0.5 M Tris-HCl, pH 7.8, 0.1 M 2-mercaptoethanol, 50 mM MgCl_2), 2 μl each of dATP, dGTP and dTTP (Sigma; dissolved in 3 mM Tris-HCl, 0.2 mM EDTA, pH 7.0, at a concentration of 50 μM), 8 pg deoxyribonuclease I (Sigma), 4 units E. coli DNA polymerase I (Pharmacia), 2 μl α -[^{32}P]-dCTP, 2 μM spermidine (Sigma) and distilled water to make up a final reaction volume of 25 μl . This was incubated for 1 hour at 15°C, and the reaction stopped with 25 μl quench mix (2% (w/v) sodium dodecyl sulphate (SDS), 50 mM EDTA, 10 mM Tris-HCl, pH 7.5). The solution was phenol extracted once, and ethanol precipitated twice (to remove unincorporated nucleotides) after the addition of 0.1 mg high molecular weight salmon sperm DNA (Sigma). The labelled DNA was finally redissolved in 500 μl 10 mM Tris-HCl.

Oligo-labelling (adapted from Feinberg and Vogelstein 1984): The reaction was performed for 5 hours, or overnight, at room temperature by adding the following reagents in the order given; distilled water (to a final reaction volume of 15 μl), 3 μl OLB, 0.6 μl 10 mg/ml bovine serum albumin (BSA;

Sigma), 25 ng DNA (denatured by boiling for 5 minutes and cooling on ice), 1.5 μ l α -[32 P]-dCTP, and 1.2 units of Klenow (Pharmacia). Oligo-labelling buffer (OLB) was made from solutions A, B and C in the ratio of 2:5:3 respectively (solution A = 625 μ l 2 M Tris-HCl, pH 8.0, 25 μ l 5 M MgCl₂, 350 μ l distilled water, 18 μ l 2-mercaptoethanol, and 5 μ l each of dATP, dTTP and dGTP (Sigma) at a concentration of 0.1 M in 3 mM Tris-HCl, 0.2 mM EDTA, pH 7.0; solution B = 2 M hepes (Sigma), titrated to pH 6.6 with NaOH; solution C = 90 OD units/ml hexadeoxyribonucleotides (Pharmacia) in 3 mM Tris-HCl, 0.2 mM EDTA, pH 7.0). The reaction was stopped with 35 μ l of stop solution (20 mM NaCl, 20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1 μ M dCTP (Sigma), 0.25% (w/v) SDS). It was not necessary to remove unincorporated nucleotides due to the high incorporation achieved by this method.

(ii) Labelling with biotin-11-dUTP (BRL; 0.4 mM)

Nick translation: Protocol I - The nick translation method for labelling DNA with α -[32 P]-dCTP as above was followed except that 8 μ M biotin-11-dUTP was used in place of the dTTP, the α -[32 P]-dCTP was diluted ten-fold, and 4 μ M dCTP (Sigma) was added. The spermidine concentration was also increased to 4 μ M and the reaction incubated for 2 hours. The phenol extraction step was omitted as this interferes detrimentally with the biotin. Protocol II - The α -[32 P]-dCTP nick translation was modified by using final concentrations of 25 μ M of the nucleotides dATP, dGTP and dCTP, 2.5 μ M of α -[32 P]-dCTP, 50 μ M of biotin-11-dUTP, 40 ng/ml of deoxyribonuclease I, and 40 units/ml of E. coli DNA polymerase

I in the reaction, which was incubated for 2 hours. The phenol extraction step was omitted.

Oligo-labelling: The α -[^{32}P]-dCTP oligo-labelling method of above was modified by doubling the concentration of BSA, labelling 300 ng of DNA and replacing the α -[^{32}P]-dCTP with 20 μM biotin-11-dUTP. The dTTP in the OLB was also replaced by an equivalent concentration of dCTP. Incubation of the reaction was carried out overnight, and after addition of stop solution the DNA was ethanol precipitated once and redissolved in TE to a concentration of 25 ng/ μl .

(iii) Labelling with tritiated-TTP ([^3H]-TTP; Amersham; 2.04 TBq/mmol, 37 MBq/ml)

The nick translation method used for labelling with α -[^{32}P]-dCTP was modified by using final concentrations of 8 μM of the nucleotides dATP and dGTP, 8 μM of dCTP in place of the α -[^{32}P]-dCTP, 8 μM of [^3H]-TTP in place of dTTP, and 10 pg/ μl of deoxyribonuclease I in the reaction, which was incubated for 2 hours.

(iv) Testing percentage incorporation of label

Percentage incorporation was determined before removal of unincorporated nucleotides by use of a liquid scintillation spectrometer (Packard Tri-Carb). 1 μl of the stopped nick translation or oligo-labelling reaction was spotted onto 25mm diameter nitrocellulose membrane filter (Whatman) and baked under a UV-lamp (0.5 J/m²/s) for 5 minutes. The radioactivity

on the filter was measured in a liquid scintillation spectrometer (in the case of [^3H]-TTP the filter was immersed in scintillation fluid when counted, and then completely dried out before proceeding to the next step). The filter was washed briefly in distilled water, and then for 2 minutes in 5% (w/v) trichloroacetic acid, to remove unincorporated nucleotides. The filter was again rinsed in distilled water and then allowed to completely dry. The radioactivity count was again measured and the percentage incorporation, when compared to the first count, calculated.

2.2.8 In Situ Hybridisation To Drosophila Salivary Gland Polytene Chromosomes

(i) Preparing slides (modified from Brahic and Haase 1978)

One third instar larva was used per slide. Coverslips were siliconized by treating with 2% dimethylchlorosilane in 1,1,1-trichloroethane for 10 minutes, drying and then rinsing in distilled water. Slides were coated with SSC-Denhardt's solution (0.45 M NaCl, 0.045 M sodium citrate, 0.02% (w/v) polyvinylpyrrolidone, 0.02% (w/v) nuclease-free BSA (Sigma), 0.02% (w/v) ficoll 400) by incubating in the solution for 2.5 hours at 65°C, rinsing briefly in distilled water, and fixing in ethanol-acetic acid (3:1) for 20 minutes at room temperature.

Larvae were dissected in 45% acetic acid and the salivary glands transferred to a drop of lacto-acetic acid (1:2:3 - lactic acid: distilled water: acetic acid) on a siliconized coverslip for 5 minutes, and cleaned by rubbing with tissue

and blowing with air from a bench oxygen line. The coverslips were each picked up with a slide, and the coverslips gently nudged so that one corner overlapped the edge of the slide, this movement helping to break up the salivary glands and distribute the cells. The chromosomes were spread by squashing the slide with thumb pressure, coverslip down, onto paper towels. After refrigeration overnight at 4°C, to further flatten the chromosomes, slides were immersed in a dry ice-ethanol bath for 1 hour. The coverslips were flipped off with a scalpel, by making use of the overhanging corners, and the slides immediately placed back in the cold ethanol. Once warmed up to room temperature (about 2 hours) the slides were removed from the ethanol and air dried. Slides were heat treated for 30 minutes at 65°C in 2 x SSC (20 x SSC is 3 M NaCl, 0.3 M sodium citrate), and then transferred to 65°C 70% (v/v) ethanol and left for 10 minutes at room temperature. They were then systematically immersed in fresh 70% (v/v) ethanol and 95% (v/v) ethanol, for 10 minutes each at room temperature, and air dried. Slides were stored at 4°C until hybridisation.

(ii) In situ hybridisation and detection of labelled probe

"ABC" protocol with biotinylated probes (Hsu et al. 1981; Montgomery et al. 1987): Polytene chromosomes were denatured by immersing in 0.07 M NaOH for 2 minutes, and rinsed in 2 changes of 2 x SSC for 6 minutes. They were dehydrated by passing twice through 70% (v/v) ethanol, and once through 95% (v/v) ethanol, for 5 minutes each and air dried. 0.4 µg of hybridisation probe was prepared in 1 x SSC, 10% dextran

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sulphate, 50% formamide, and 0.4 mg/ml sonicated salmon sperm DNA (Sigma), denatured by boiling for 5 minutes and cooled on ice. 20 μ l of probe was applied to each slide and hybridised overnight at 37°C in a petri dish over a water bath. Damp tissue was placed in the petri dishes to ensure the slides did not dry out. The slides were washed to remove unhybridised probe by two 10 minute washes in 2 x SSC at 37°C and two similar washes at room temperature. They were kept in phosphate buffered saline (PBS; 0.13 M NaCl, 7 mM Na₂HPO₄, 3 mM NaH₂PO₄) at room temperature for 15 minutes.

The probe was detected on the chromosome squashes with the Vectastain ABC kit (Vector). The "ABC" complex was mixed by adding equal volumes of each component (avidin DH and biotinylated horseradish peroxidase H) to 125 times the volume of 50 mM Tris-HCl pH 7.6, 4% (w/v) BSA. After 5 minutes 50 μ l of complex was added to each slide which was covered with a siliconized coverslip and incubated at 37°C for 30 minutes in a moist chamber. After incubation, slides were washed 3 times in PBS for 30 minutes at room temperature. 0.5 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB) in 0.05 M Tris-HCl, pH 7.6, was freshly prepared and, immediately before use, 50 μ l of 30% hydrogen peroxide added per 5 ml of reagent. 250 μ l of this solution was added to each slide, covered with a siliconized coverslip, and incubated for 30 minutes at 37°C. Slides were washed for 10 minutes in PBS and stained with Giemsa (Sigma; Macgregor and Varley 1983). Giemsa stock solution was made up of 5 g of Giemsa powder in 33 ml of glycerol, heated for 2 hours at 60°C and mixed with 33 ml of methanol. Slides were immersed in 60 ml 4.4 mM KH₂PO₄, 6.6 mM Na₂HPO₄, pH 7.0, into which 5 ml of Giemsa stock was mixed.

After 10 minutes staining the Giemsa was flushed out with distilled water, and the slides air dried. Preparations were made permanent by treating for 5 minutes in xylene and mounting in a drop of hystomount (Hughes and Hughes Ltd.) under a coverslip. They were examined under bright field objectives.

Alkaline phosphatase protocol with biotinylated probes (Langer et al. 1981; Leary et al. 1983; Shrimpton et al. 1986): Polytene chromosomes were denatured by heating slides for 3 minutes at 100°C in 10 mM Tris, 5 mM MgCl₂, and washed 3 times for 5 minutes each in 2 x SSC. Slides were dehydrated by 2 rinses in 70% (v/v) ethanol for 5 minutes each followed by once for 5 minutes in 95% (v/v) ethanol, and then air dried. The probe was prepared for hybridisation by adding 0.72 µl of labelled 0.025 µg/µl DNA to 2.7 µl of deionised formamide and 2.58 µl of hybridisation carrier mix (15% dextran sulphate, 0.65 mg yeast tRNA (Sigma), 1.5 mg sonicated salmon sperm DNA (Sigma), 9.8 x SSC) per slide. It was denatured by heating at 75°C for 10 minutes and cooling on ice. 6 µl of probe mix per slide was pipetted onto an air-dusted siliconized coverslip and picked up with the denatured chromosome preparation. The slides were incubated overnight at 37°C in a moist chamber (as above in the "ABC" protocol). After hybridisation the unhybridised probe was removed by washing the slides twice for 10 minutes each in 2 x SSC at 37°C, followed by two 10 minute washes in 2 x SSC at room temperature and two similar washes in PBS.

The hybridised probe was detected using an alkaline phosphatase DNA detection kit (BRL). Slides were immersed in

pre-warmed 2% (w/v) BSA in BRL Buffer 1 (0.05% (v/v) triton X-100, 0.1 M NaCl, 4 mM MgCl₂, 0.1 M Tris-HCl, pH 7.5) for 20 minutes at 42°C, followed by an additional 10 minutes at room temperature. 2 µl of streptavidin (BRL kit) was mixed per 1 ml of BRL Buffer 1 and 50 µl of this solution added to each drained slide. After 5 minutes at room temperature with occasional gentle agitation a second 50 µl of streptavidin solution was added per slide and left for 5 more minutes. The streptavidin solution was removed by first pouring BRL Buffer 1 over the slides, and then washing them 3 times for 3 minutes each in more BRL Buffer 1. 1 µl of poly AP (biotinylated calf intestinal alkaline phosphatase; BRL kit) was mixed per 1 ml of BRL Buffer 1, and 50 µl of the solution added per drained slide for 5 minutes at room temperature. A second 50 µl was added to each slide for an additional 5 minutes after which the slides were washed by flushing with BRL Buffer 1 and immersing for 3 minutes each, twice in BRL Buffer 1 and twice in BRL Buffer 3 (0.1 M NaCl, 0.1 M MgCl₂, 0.1 M Tris-HCl, pH 9.5) at room temperature. Stain solution was prepared by adding 4.4 µl of NBT (nitro-blue tetrazolium; BRL kit) to 1 ml of BRL Buffer 3, mixing, and then adding 3.3 µl of BCIP (5-bromo-4-chloro-3-indolyl phosphate; BRL kit). 30 µl of solution per slide was added to a clean siliconized coverslip and picked up with the slide. Slides were incubated for 2 hours at room temperature in the dark and then the reaction stopped by rinsing in distilled water. Slides were air dried and stored at 4°C. Examination was carried out under phase contrast by adding a drop of water and a coverslip when required. If needed the chromosomes were stained with orcein. This was prepared by dissolving 1.8 g orcein (Gurr) in 30 ml

hot glacial acetic acid, and when cool adding 30 ml lactic acid and 30 ml distilled water, and filtering. Staining was achieved by diluting the orcein solution ten-fold in 45% acetic acid, and pipetting a few drops onto the chromosomes. Slides were rinsed with distilled water after a couple of seconds.

Protocol for labelling with [^3H]-TTP (Macgregor and Varley 1983): Prepared slides were incubated for 2 hours at 37°C in a solution containing 50 $\mu\text{g/ml}$ of ribonuclease A (Sigma) and 100 units/ml of ribonuclease T1 (Sigma) in 2 x SSC, and then washed 3 times in 2 x SSC for a total of 30 minutes. The polytene chromosomes were denatured in 0.07 M NaOH at 20°C for 3 minutes, dehydrated by immersing 3 times in 70% (v/v) ethanol and twice in 95% (v/v) ethanol for 5 minutes each, and air dried. The labelled probe DNA was ethanol precipitated and redissolved in 0.1 M NaOH (10 μl for every 10^6 counts/minute of probe). Formamide and 20 x SSC were added to give final concentrations (after addition of HCl) of 50% and 4 x SSC respectively, together with any necessary volume of water (to give a final volume of 100 μl for every 10^6 counts/minute of probe). After cooling on ice for 5 minutes 0.1 M HCl was added at the correct volume (previously determined) to neutralise the 0.1 M NaOH. The reaction mix was applied to the slides, 30 μl per preparation, which, after addition of coverslips, were incubated overnight at 37°C in a moist chamber. After hybridisation the slides were washed for 15 minutes in 2 x SSC at 65°C and twice in 2 x SSC, 10 minutes each, at room temperature, to remove unhybridised probe. They were then immersed in 5% (w/v) trichloroacetic acid at 4°C for 5

minutes, and again washed twice in 2 x SSC at room temperature for a total of 20 minutes. The slides were dehydrated by washing twice in 70% (v/v) ethanol, 10 minutes each, and twice in 95% (v/v) ethanol for the same period of time, and then air dried.

The labelled probe was detected on the chromosomes by autoradiography. Kodak NTB2 nuclear track emulsion was diluted 1:1 with distilled water and melted for 30 minutes at 45°C. Slides were dipped in the solution for 10 seconds each and stood in a rack for 1 hour to dry. The dried slides were placed in a light tight storage box with a dessicant, and autoradiographed for 2 to 4 weeks at room temperature. Test preparations were used to determine the best exposure time. The slides were developed in Kodak D19 developer for 2.5 minutes, washed briefly in distilled water, and fixed for 10 minutes in Kodak Photostat fixer number 4, all stages being carried out at 20°C. After washing in distilled water the slides were stained with Giemsa and made permanent for examination (see above with "ABC" protocol for biotinylated probes).

2.2.9 Isolation Of Drosophila DNA (Bingham et al. 1981)

Adult flies were flash frozen in liquid nitrogen and homogenised in nuclear isolation buffer (NIB; 60 mM NaCl, 10 mM EDTA, 0.15 mM spermidine (Sigma), 0.15 mM spermine (Sigma), 0.5% triton X-100, 10 mM Tris-HCl, pH 7.4), 25 ml per gramme of organisms, using 10-20 passes at moderately fast drive speed of a motor driven teflon homogeniser (Camlab Tri-R Stir-R). The homogenate was spun in a universal tube at 1500 rpm for 15 seconds in an MSE Centaur 1 to pellet the

macroscopic material. The supernatant was spun at 7 krpm in a Sorvall SS34 rotor for 7.5 minutes at 4°C (Sorvall RC-5B centrifuge) to pellet the nuclei which were resuspended in fresh NIB (25 ml per gramme of flies). The nuclei were pelleted again, resuspended in NIB (6.4 ml per gramme of organisms), and 10% Sarkosyl (the sodium salt of N-lauroyl sarcosine) added to give a final concentration of 2%. The suspension was gently agitated and mixed with 6.2 g of CsCl. The refractive index of the solution was adjusted to 1.3995 with 2% Sarkosyl in NIB, and then centrifuged in a Beckman Quick-Seal tube at 55 krpm overnight at 15°C in a Beckman VTi65 rotor (Beckman L5-65 ultracentrifuge). A Beckman Fraction Recovery System was used to collect fractions from the tube which were tested for DNA by spotting 1 µl each onto 0.5 µg/ml ethidium bromide in a 0.8% SeaKem HGT agarose gel and checking for fluorescence under UV light. D. melanogaster DNA fractions were pooled and dialysed against three 1 litre sample volumes of TE for 24 hours total. The DNA solution was concentrated by adding equal volumes of butan-2-ol, spinning 5 minutes in an MSE Centaur 1 at 4 krpm or an MSE Micro Centaur at 13 krpm, and collecting the lower aqueous layer. The concentration procedure was repeated until the volume was approximately 300 µl. Any remaining butan-2-ol was extracted twice by adding 100 µl ether, mixing, spinning for 1 minute at 13 krpm in an MSE Micro Centaur, and blowing off the ether with a pasteur pipette. The DNA solution was phenol extracted twice, ethanol precipitated, and redissolved in 1 ml TE. It was stored at -20°C.

2.2.10 Southern Blotting Of DNA (based on Southern 1975)

DNA was electrophoresed overnight at 25 volts on a 0.8% agarose gel, approximately 1 μ g per lane. The DNA was denatured by immersing the gel twice in 0.25 M HCl, shaking occasionally, for 15 minutes each, followed by similar treatment in 0.5 M NaOH, 1 M NaCl. The gel was rinsed briefly in distilled water and then neutralised in 2 washes of 0.5 M Tris-HCl, pH 7.5, 3 M NaCl, 15 minutes each. The DNA was transferred onto a nitrocellulose (Sartorius) or Hybond-N nylon filter (Amersham), which were manipulated using Millipore forceps. The transfer was completed over 2 hours using 20 x SSC and a constructed Southern transfer apparatus (Maniatis et al. 1982), changing the wad of paper towels every 15 minutes. The filter was washed in distilled water, air dried, and then either baked at 80°C for 1 hour, if nitrocellulose, or wrapped in Dow "Saran Wrap" and co-linked for 5 minutes on a standard UV transilluminator, if nylon. Filters were stored at 4°C until hybridisation.

2.2.11 Hybridisation Of Filters

In each protocol the solutions were preheated to 65°C in a water bath, and all steps were carried out in a 65°C shaking water bath. Hybridisation of filters was carried out in perspex air-tight hybridisation boxes (18 x 5.5 x 1.5 cm), and washing after hybridisation in sealed plastic sandwich boxes (19 x 19 x 6.5 cm). 0.1 μ g of nick translated or 25 ng of oligo-labelled α -[³²P]-dCTP probe was used in each case. It was denatured before use by boiling for 5 minutes and cooling on ice.

(i) Protocol I (Jeffreys and Flavell 1977)

Filters were washed systematically in 30 ml 3 x SSC for 15 minutes, and 30 ml 1 x Denhardt's mix (0.2% (w/v) BSA, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) ficoll 400), 30 ml filter hybridisation mix (1 x Denhardt's, 100 mg alkali denatured salmon sperm DNA (Sigma), 0.1% (w/v) SDS, 0.7 x SSC), and 30 ml filter hybridisation mix plus 9% (w/v) dextran sulphate for 1 hour each. Hybridisation of filters was carried out overnight in the latter solution, after addition of denatured probe.

After hybridisation, filters were washed 4 times for 15 minutes each in filter hybridisation mix, and 2 times in 0.5 x SSC, 50 µg/ml salmon sperm DNA (Sigma), 0.1% (w/v) SDS for 15 minutes and 30 minutes respectively. Filters were rinsed in 3 x SSC at room temperature and air dried on Whatman 3 MM filter paper at 37°C.

(ii) Protocol II (adapted from Reed and Mann 1985)

Filters were incubated for 1 hour in 30 ml hybridisation mix (1.5 x SSPE (0.15 M NaCl, 12 mM NaH₂PO₄.2H₂O, 1.5 mM EDTA, pH 7.4), 5 mg/ml Marvel (Cadbury), 6% (w/v) PEG 8000 (Renz and Kurz 1984), 1% (w/v) SDS) and then hybridised overnight after addition of the denatured probe.

Filters were rinsed briefly 4 times in 3 x SSC, 0.1% (w/v) SDS, followed by two 10 minute washes in 3 x SSC, 0.1% (w/v) SDS and four 10 minute washes in 0.5 x SSC, 0.1% (w/v) SDS. They were dried by blotting on Whatman 3 MM filter paper before autoradiography.

(iii) Protocol III (Church and Gilbert 1982)

Filters were pre-hybridised for 2 hours in 40 ml of 7% (w/v) SDS, 1 mM EDTA, 0.5 M Na_2HPO_4 , pH 7.2, degassed with a vacuum pump before use. Denatured probe was added and hybridisation carried out overnight.

Filters were washed twice in 0.5 M Na_2HPO_4 , pH 7.2, 1% (w/v) SDS for a total of 1 hour, and 3 times in 0.5 x SSC, 0.1% (w/v) SDS for 25 minutes each. They were drained briefly before autoradiography.

(iv) Autoradiography of filters

Filters were aligned on clingfilm-backed cardboard and wrapped totally in clingfilm. They were autoradiographed at -80°C for the desired length of time (depending on the activity) using Kodak X-Omat S, or Amersham Hyperfilm MP, with Philips Ultra intensifying screens. Film was developed for 3 minutes in Kodak LX24 developer, rinsed briefly in dilute acetic acid, fixed for 3 minutes in Kodak FX40 fixer and washed for 15 minutes in water.

2.2.12 RNA Extraction From Drosophila Pupae (based on Barnett et al. 1980)

D. melanogaster pupae were frozen in liquid nitrogen in a mortar, and ground to a fine dust with a pestle. 7.5 ml of extraction buffer (0.15 M sodium acetate, 5 mM EDTA, 1% (w/v) SDS, 50 mM Tris-HCl, pH 9.0) per gramme of organisms, plus diethylpyrocarbonate (DEPC) added to 1% (v/v) just before use, was pipetted into the mortar followed by the same volume of

cold phenol/chloroform, and homogenised for a further 30 seconds. Subsequent steps were carried out on ice unless otherwise specified. The homogenate was spun in a Sorvall HB4 rotor for 10 minutes at 10 krpm, 4°C (Sorvall RC-5B centrifuge) and the aqueous phase reextracted twice with equal volumes of phenol/chloroform. The RNA was ethanol precipitated overnight at -20°C (note that the sodium acetate concentration is already 0.15 M) and the precipitate resuspended in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1% (w/v) SDS, 2.5 ml per gramme of pupae. Proteinase K (Sigma) was added to 100 µg/ml and the solution spun in 0.1% (v/v) DEPC pre-rinsed centrifuge tubes at 45 krpm in a Beckman SW50.1 rotor for 45 minutes, 4°C (Beckman L5-65 ultracentrifuge) to pellet any insoluble material. The clear supernatant was decanted, incubated 20 minutes at 37°C, phenol extracted twice, and ethanol precipitated. The RNA pellet was redissolved in 1 ml 0.1% (v/v) DEPC and stored at -20°C.

2.2.13 Preparation Of Poly(A)⁺ RNA From Total Drosophila RNA (based on Aviv and Leder 1972)

50 mg of oligo (dT)-cellulose type 3 (Collaborative Research Inc.) per mg of total RNA was suspended in elution buffer (0.01 M Tris-HCl, pH 7.5, 0.05% (w/v) SDS, 1 mM EDTA) and pipetted into a DEPC-treated sterile siliconized pasteur pipette (which was mounted on a retort stand and plugged with sterile polyallomer wool). This was washed with 10 oligo (dT)-cellulose column volumes of binding buffer (0.5 M LiCl, 0.5% (w/v) SDS, 1 mM EDTA, 0.01 M Tris-HCl, pH 7.5). Total RNA was heat shocked for 3 minutes at 65°C and cooled on ice. An equal volume of 2 x binding buffer was added to the RNA

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solution, and this was loaded onto the column. The eluate was collected, heat shocked and reapplied to the column twice. 15 ml of binding buffer was run through the column, followed by 5 ml of elution buffer which was collected in a DEPC-treated siliconized 30 ml Corex tube. This poly (A)⁺ RNA fraction was ethanol precipitated and pelleted for 45 minutes at 4°C in a Sorvall HB4 rotor at 10 krpm (Sorvall RC-5B centrifuge). The RNA was redissolved in 400 µl of 0.1% (v/v) DEPC.

To reuse the oligo (dT)-cellulose the column was washed with 10 volumes of elution buffer followed by 10 of 0.1 M NaOH. If reused immediately the column was first equilibrated with elution buffer and then binding buffer, but if not, it was washed with 0.1% (v/v) DEPC followed by ethanol and then vacuum dried overnight. The oligo (dT)-cellulose was stored in a closed container at -20°C.

2.2.14 Northern Blotting Of RNA (Amersham 1985)

0.2-2.0 µg of D. melanogaster poly (A)⁺ RNA, per lane of gel, was ethanol precipitated and redissolved in 4.8 µl of 0.1% (v/v) DEPC. This was added to 10 µl deionised formamide, 3.2 µl formaldehyde and 2 µl 10 x MOPS buffer, heated at 65°C for 5 minutes and cooled on ice. 5 µl of 50% (w/w) glycerol, 0.1 mg/ml bromophenol blue was added, followed by 1 µl of 1 mg/ml ethidium bromide, and the RNA solution was loaded onto the gel for running overnight at 25 volts. The excess ethidium bromide was chopped out before it had left the gel, and the buffer was circulated whilst running. After electrophoresis the RNA was blotted for 4 hours with 20 x SSC onto a Hybond-N nylon filter (Amersham) using a constructed Southern transfer apparatus (Maniatis et al. 1982). The filter was dried at room

temperature, wrapped in Dow "Saran Wrap" and co-linked on a standard UV transilluminator for 4 minutes. It was stored at 4°C until hybridisation.

2.2.15 Preparation Of A Bacteriophage λ L47.1 Genomic DNA library

(i) Plating out bacteriophage λ

5 ml of overnight E. coli culture in LUB and 10 mM MgSO_4 was diluted with 15 ml of similar medium and shaken for 3 hours at 37°C. BLA plates were poured, set, and dried inverted at 42°C. BTL plus 10 mM MgSO_4 , was microwaved, cooled to 60°C, and pipetted in 3 ml volumes into capped tubes in a 60°C heating block. An appropriate volume of phage suspension was mixed with 150 μl of the E. coli culture (ED8910 (supE44, supF58, recB21, recC22, hsdS, metB, lacY1, galK2, galT22; Loenen and Brammar 1980) or WL87 (803, supE, supF, hsdR_K, hsdM_K, tonA, trpR, metB; obtained from W. J. Brammar) for screening libraries or growing bacteriophage λ L47.1; WL95 (P2 lysogen of WL87; obtained from W. J. Brammar) for library plates) and incubated for 15 minutes at room temperature. 3 ml of 60°C 10 mM MgSO_4 BTL was added, and this then poured onto a BLA plate. The plates were incubated inverted overnight at 37°C.

(ii) Isolation of DNA from bacteriophage λ L47.1 and recombinant λ clones (based on Blattner et al. 1977; Yamamoto et al. 1970)

An unbaffled 2 litre conical flask was acid washed and autoclaved with 200 ml LUB plus 10 mM MgSO_4 , 20 $\mu\text{g/ml}$ thymine (Sigma). 6 bacteriophage $\lambda\text{L47.1}$ (Loenen and Brammar 1980) or recombinant λ plaques were picked from an overnight plate of phage grown on E. coli ED8910 or WL87, blown into the flask and gently shaken overnight at 37°C on an orbital shaker. 40 μl of 5 mg/ml pancreatic deoxyribonuclease I (Sigma) and 10 μl of 10 mg/ml pancreatic ribonuclease A (Sigma) were added to the lysate which was incubated for 15 minutes at room temperature. NaCl was added to 1 M, dissolved, and left on ice for 30 minutes. This was spun in a Sorvall GS3 rotor at 8 krpm for 10 minutes, 4°C (Sorvall RC-5B centrifuge), and polyethylene glycol 6000 dissolved into the supernatant to 10% (w/v)--by slow stirring on a magnetic stirrer at room temperature. Once dissolved, the solution was incubated on ice for 1 hour to precipitate the phage, which were spun down in a Sorvall GS3 rotor at 8 krpm for 10 minutes at 4°C (Sorvall RC-5B centrifuge). The phage pellet was drained completely and resuspended in 3 ml LB at 4°C. The suspension was spun in a Sorvall HB4 rotor at 8 krpm for 2 minutes at 4°C (Sorvall RC-5B centrifuge) and the supernatant collected. The pellet was resuspended in 1.5 ml LB, spun in a similar manner, and the supernatants pooled. A CsCl step gradient was made up in a 13.5 ml Beckman Ultra-Clear tube with 2 ml of 1.7 g/ml, 3 ml of 1.5 g/ml, and 2 ml of 1.3 g/ml CsCl respectively. This was overlaid with the phage suspension and spun in a Beckman SW40.1 rotor for 1 hour at 35 krpm, 20°C (Beckman L5-65 ultracentrifuge) without brake. The bluish phage band (at the 1.5 g/ml and 1.3 g/ml interface) was collected by side puncture into a 1 ml syringe using a 0.8 mm x 25 mm needle,

and diluted with 10 ml LB in a 13.5 ml Beckman Ultra-Clear tube. The phage were pelleted in a Beckman SW40.1 rotor at 35 krpm for 1 hour, 20°C (Beckman L5-65 ultracentrifuge), and resuspended in 1 ml LB. The phage DNA was released by lysis of the phage with 100 µl of 10% (w/v) SDS. It was phenol extracted twice, ethanol precipitated, and the DNA clot redissolved in 300 µl 10 mM Tris-HCl, pH 7.5. 6 µl of 10 mg/ml pancreatic ribonuclease A (made deoxyribonuclease-free by boiling for 5 minutes in 10 mM Tris-HCl, pH 7.5, 15 mM NaCl and cooling slowly to room temperature) was added and incubated for 10 minutes at 37°C. The solution was phenol extracted twice, ethanol precipitated, and the DNA redissolved in 200 µl 10 mM Tris-HCl, pH 7.5, and stored at -20°C.

(iii) Recovery of DNA from agarose gels

Electroelution onto dialysis membrane (adapted from Maniatis et al. 1982): The DNA on the gel was observed in the electrophoresis tank, by means of a hand-held UV-lamp, and a slit cut in front of the DNA of interest. A strip of dialysis membrane was inserted into the gap and the DNA electroeluted onto the membrane by electrophoresis at 150 volts. Any DNA not required, which may run onto the membrane, was cut from the gel before running. Once electroeluted completely onto the dialysis membrane the DNA and membrane were quickly transferred to a universal tube by means of Millipore forceps. One corner of the membrane was trapped with the lid of the tube, and the DNA solution was spun down at 4 krpm in an MSE Centaur 1. The DNA was butan-2-ol concentrated, ether extracted, phenol extracted and ethanol precipitated as in the

Drosophila genomic DNA isolation procedure (section 2.2.9), and then redissolved in the required volume of 10 mM Tris-HCl, pH 7.5, and stored at -20°C.

Electroelution in a Schleicher and Schnell BT 1000 biotrap: The DNA of interest was cut out of the gel, and the gel slice inserted into the elution chamber of the biotrap. Membranes BT2 and BT1 (Schleicher and Schnell) were positioned to form the smallest trap volume, and the DNA electroeluted at 200 volts through membrane BT2 onto BT1. After elution the voltage was briefly reversed, and the DNA solution in the trap removed with a pipette. The DNA was phenol extracted and ethanol precipitated as above, and redissolved in the required volume of 10 mM Tris-HCl, pH 7.5, and stored at -20°C.

(iv) Preparation of packaging extracts (adapted from Scalenghe et al. 1981)

Freeze Thaw Lysate (FTL): Three 500 ml cultures of E. coli BHB 2688 (N205 recA⁻ [λimm⁴³⁴, cIts, b2, red⁻, Eam, Sam/λ]; Hohn and Murray 1977) in LUB were grown at 30°C until OD₆₀₀ equalled 0.3. The cells were induced at 45°C, without shaking, for 15 minutes, and grown at 37°C for 1 hour with shaking at high speed. Cultures were cooled on ice and the cells harvested by centrifugation in a Sorvall GS3 rotor at 9 krpm for 10 minutes, 4°C (Sorvall RC-5B centrifuge). The supernatant was drained off thoroughly and the pellets resuspended in 0.5 ml of 10% (w/v) sucrose, 50 mM Tris-HCl, pH 7.5, per 250 ml of original culture. The suspensions were pooled into 2 tubes, each mixed with 75 μl of fresh lysozyme

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solution (Sigma; 2 mg/ml in 0.25 M Tris-HCl, pH 7.5), frozen in liquid nitrogen, and stored overnight at -70°C. The cells were completely thawed at room temperature, cooled on ice, and 75 µl of buffer M1 (18 mM MgCl₂, 15 mM rATP (Sigma; neutralised with NH₄OH), 0.03 M spermidine (Sigma), 0.06 M putrescine (Sigma), 0.2% (v/v) β-mercaptoethanol, 6 mM Tris-HCl, pH 7.5) mixed in gently. The suspensions were spun down in a Sorvall SS34 rotor at 17 krpm for 50 minutes at 4°C (Sorvall RC-5B centrifuge) and the supernatants retained. The solution was distributed into precooled tubes in 25 µl aliquots, quick frozen in liquid nitrogen and stored at -70°C.

Sonicated Extract (SE): Three 500 ml cultures of E. coli BHB2690 (N205 recA⁻ [λimm⁴³⁴, cIts, b2, red⁻, Dam, Sam/λ]; Hohn and Murray 1977) in LUB were grown, induced and harvested as for FTL. The pellets produced were resuspended in 0.6 ml of buffer A (20 mM Tris-HCl, pH 8, 3 mM MgCl₂, 1 mM EDTA, 0.05% (v/v) β-mercaptoethanol) per 250 ml of original culture, pooled, and diluted with 2.6 ml of buffer A. The suspension was sonicated, without foaming, using 3 second blasts of an MSE 150 Watt Ultrasonic Disintegrator (high power, amplitude setting 4) until no longer viscous, and the debris spun in a Sorvall SS34 rotor at 6 krpm for 6 minutes, 4°C (Sorvall RC-5B centrifuge). The supernatant was distributed into precooled tubes in 50 µl aliquots, quick frozen in liquid nitrogen and stored at -70°C.

(v) Ligation of bacteriophage λL47.1 annealed BamHI arms to Sau3A partially digested genomic DNA (Maniatis et al. 1982)

1 μ g λ L47.1 arms, 0.5 μ g genomic DNA, 1 unit T4 DNA ligase (BRL), 1 x ligation buffer (5 mM MgCl_2 , 1 mM ATP (Sigma), 5mM dithiothreitol, 66 mM Tris-HCl, pH 7.5), and 4 mM spermidine (Sigma) were mixed on ice in a total volume of 20 μ l. This was incubated overnight at 15°C and stored at -20°C.

(vi) In vitro packaging of ligated DNA (Scalenghe et al. 1981)

10 μ l ligated DNA, 7 μ l buffer A (section 2.2.15.(iv)), 2.5 μ l buffer M1 (section 2.2.15.(iv)), 15 μ l packaging extract SE and 25 μ l packaging extract FTL were mixed on ice and incubated for 1 hour at 25°C. The packaged DNA was either plated out immediately or stored at -20°C.

(vii) Lifting of bacteriophage λ plaques onto membrane (Amersham 1985)

A Hybond-N membrane filter (Amersham) was carefully placed on the surface of the plate, and the membrane and agar marked using a sterile needle to ensure correct orientation of the plaques. The membrane was removed after 1.5 minutes and transferred, plaque side up, to sterile filter paper. It was placed, plaque side up, on a pad of absorbant filter paper soaked in 1.5 M NaCl, 0.5 M NaOH. After 7 minutes the membrane was transferred to a similar pad soaked in 1.5 M NaCl, 0.001 M Na_2EDTA , 0.5 M Tris-HCl, pH 7.2. This was left for 3 minutes, and then an additional 3 minutes on a fresh pad soaked in the same solution. The membrane was washed in 2 x SSC, and dried plaque side up on filter paper for 20 minutes at 65°C. The

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membrane was wrapped in Dow "Saran Wrap" and co-linked, plaque side down, on a standard UV transilluminator for 5 minutes. The whole process was repeated with a fresh membrane to produce a replica. The membranes were stored at 4°C until hybridisation.

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2.2.16 Scanning Electron Microscopy

D. melanogaster adult flies were dehydrated progressively for 10 minutes each in 20%, 50%, 70% and 90% (v/v) acetone, and then twice in 100% analar acetone for a total of 20 minutes. The samples were dried using a Samdri-780 critical point drier (Tousimis) and coated with gold-palladium in a Polaron sputter coater. Specimens were observed and photographed using a Cambridge S.100 scanning electron microscope.

Chapter 3 : An Analysis Of The Extent Of Mobilisation Of Transposable Elements In A P-M Hybrid Dysgenic Cross

3.1 Do Transposable Elements Other Than P Elements Transpose During P-M Hybrid Dysgenesis?

There has been speculation as to whether transposable elements other than P elements are also mobilised in a hybrid dysgenic cross. Transposition bursts, in which many mobile elements may simultaneously excise or insert, have been described in a genetically unstable D. melanogaster strain isolated during the course of P-M hybrid dysgenesis (Gerasimova et al. 1984a; Gerasimova et al. 1985). In such bursts or explosions, dramatic changes in the location of the copia-like elements mdg-1, mdg-2, mdg-3 and copia, and in the location of fold-back (FB) elements and P elements were detected, all of which occurred in the same germ cell. Are such transposition bursts related to hybrid dysgenesis, or does some other trigger mechanism exist? The genetically unstable D. melanogaster strain was found to contain an unstable mutation in the cut locus (Gerasimova 1981). The mutation was determined to be due to the insertion of a copia-like transposable element mdg-4 (Gerasimova et al. 1984b; Mizrokhi et al. 1985). A strain homozygous for the insertion mutation was found to be unstable for approximately 50 generations, after which the instability rapidly decreased. However, on crossing with the P strain originally used in the P-M hybrid dysgenic cross, the instability at cut increased to a level exceeding the initial one. In two separate P-M hybrid dysgenic crosses the transposable genetic element copia has

also been observed to transpose into the white (Rubin et al. 1982) and rosy (Clark et al. 1986; Coté et al. 1986) loci of D. melanogaster. In these cases, however, the resulting mutations were found to be stable. Such observations raise the possibility that P-M hybrid dysgenesis may, in addition to the large increase it produces in the transposition rate of P elements, also increase, to a certain extent, the rate of transposition of copia-like elements.

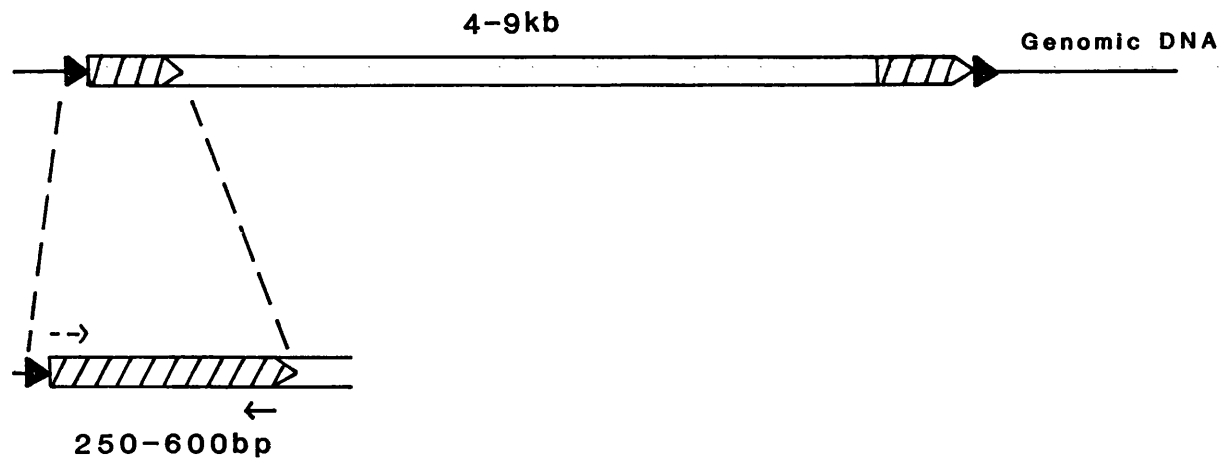
In situ hybridisation to polytene salivary gland chromosomes was used in an experiment designed to investigate changes in the location of the transposable elements copia, 412 and F during a hybrid dysgenic cross. Isofemale lines derived from the offspring of such a cross were scored for the chromosomal positions of these elements, together with lines derived from the reciprocal nondysgenic cross.

3.2 Copia-Like And F Transposable Elements

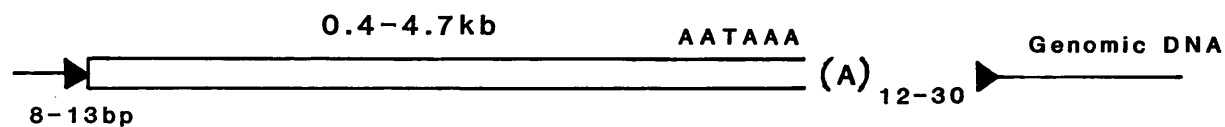
Copia and 412 both belong to a class of transposable elements termed copia-like elements. There are thought to be over 30 different families of this type of element (Rubin 1983) which, although nonhomologous in nucleotide sequence, share certain structural properties (figure 3). They range in size from 4 to 9 kb, copia being 5.15 kb long (Mount and Rubin 1985) and 412 being 7 kb (Finnegan et al. 1978). They possess long direct terminal repeats (LTR's) of between 250 and 600 bp in length, the LTR's of copia being 276 bp (Levis et al. 1980) whereas those of two different 412 elements analysed have been determined to be 481 and 571 bp (Will et al. 1981). The LTR's also themselves possess imperfect terminal inverted repeats

Figure 3 Summary of the structure of copia-like and F transposable elements

COPIA-LIKE ELEMENTS



F ELEMENTS



► Target site duplication

▨ Long direct terminal repeat

--> ← Imperfect inverted repeats

which differ between families. Such inverted repeats investigated have revealed lengths of 13 and 17 bp for different copia transposable elements, and 8 and 10 bp for different 412 elements. The lengths of the target site direct repeats flanking the inserted copia-like elements are also found to vary between the different families, the repeat of copia being 5 bp whilst that of 412 is 4 bp.

F elements differ from both P and copia-like elements in that they possess neither inverted or direct repeats at their termini (figure 3). This class of transposable elements was initially discovered interspersed within the chromosomal copies of type I ribosomal insertions (Dawid et al. 1981). DiNocera et al. (1983) have analysed 5 different F elements, and found that 3 of the elements had a length of 4.7 kb, whilst the other 2 were smaller with lengths of 0.4 and 1.3 kb. The elements were found to terminate at their one end with a stretch of between 12 and 30 A residues, and this was preceded by a polyadenylation signal AATAAA. The target site duplications were found to vary from between 8 and 13 bp.

3.3 D. melanogaster Crosses

D. melanogaster strains used were a P strain, Harwich, and an M strain, Canton-S. 10 hybrid dysgenic crosses of 2 Harwich males to 2 Canton-S females per vial were performed, and 8 reciprocal crosses (2 Canton-S males and 2 Harwich females per vial) as a nondysgenic control. 10 isofemale lines were produced from each F1 generation, and maintained for 15 generations of sib-mating to allow a switch to P cytotype and to render sites of new insertions homozygous.

All stages were maintained at 22°C to minimise gonadal dysgenic sterility effects. However, even kept at this temperature, high levels of sterility were observed in the F1 flies of the dysgenic cross. Sublines were thus taken at various stages to increase the number of lines examined. Previously published data concerning dysgenic crosses between Harwich and Canton-S stocks have indicated no sterility effects at temperatures below 24°C (Kidwell and Novy 1979; Bregliano and Kidwell 1983). A similar sterility effect at 22°C, however, has been observed by J. F. Y. Brookfield (unpublished). F1 generation males from such a dysgenic cross were mated with compound (1) double X yellow forked (C(1)DXyf) virgin females. Very few F2 lines resulted, suggesting a high male sterility in the F1 generation.

3.4 In situ Hybridisation

It was decided to use biotin-11-dUTP, in preference to [³H]-TTP, for the labelling of the transposable element probes. Biotin-11-dUTP is a dTTP analogue with the small molecular weight vitamin, biotin, attached at the 5-position of the pyrimidine base by an 11-atom linker (Brigati et al. 1983). A glycoprotein, avidin, has a high affinity for biotin producing an essentially irreversible interaction. There are 4 binding sites for biotin on the avidin molecule, and thus enzymes conjugated with biotin molecules can be bound to avidin at the same time as biotin-11-dUTP. These features provide a technique for detecting biotin-11-dUTP labelled DNA sequences hybridised to target DNA. Biotinylated enzymes can be bound to the label via avidin molecules, and substrate dyes

added for visualisation of the target DNA.

Biotin-11-dUTP has a number of advantages over [^3H]-TTP for use in in situ hybridisation protocols. In addition to more precise bands when observed under the light microscope, the former method also possesses a vast advantage concerning the experimental time required for detection of the hybridised probe. The techniques available for detecting biotin-11-dUTP labelled probes can, in many cases, take less than 4 hours to complete, whereas the exposure of the autoradiographs, required for use with [^3H]-TTP, can take from 2 to 4 weeks.

Various techniques were tried in an attempt to label the probes p π 25.1, cDm2042, 19-27 and pBB2 with biotin-11-dUTP. Initially, a method based on the nick translation protocol for labelling with α -[^{32}P]-dCTP was used (section 2.2.7(ii); protocol I). The highest percentage incorporation of biotin-11-dUTP achieved (determined by measuring that of the small amount of α -[^{32}P]-dCTP in the reaction mix), however, was found to be only 0.8%. The technique was thus modified by varying different parameters such as enzyme and nucleotide concentrations, and the length of the reaction incubation period. By using protocol II (section 2.2.7(ii)) an increased incorporation of 7.5% was attained. This, however, was still considerably lower than the 20-30% routinely achieved when nick translating with α -[^{32}P]-dCTP only. Nick translation of the probes with [^3H]-TTP was thus attempted, again using a modified α -[^{32}P]-dCTP nick translation technique. An incorporation of 65% was accomplished, and an in situ hybridisation attempted with this labelled probe to some test chromosome squashes. Unfortunately, however, no signal was detected after development of the autoradiographed slides.

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This negative result presented a problem as to which area to continue with the greatest effort of work. However, as I was still primarily in favour of using biotin-11-dUTP in the experiments, it was decided to concentrate on trying to overcome difficulties with this technique, rather than devoting time to the [³H]-TTP in situ hybridisation procedure, even though labelling of the probes had been attained with this latter method.

I thus tried a technique termed oligo-labelling, which routinely produced incorporations of α -[³²P]-dCTP as high as 80%. The procedure was thus modified for use with biotin-11-dUTP and an experimental test attempted. An incorporation of 60% was achieved which was found to be reproducible in further labellings.

Two techniques were available for the in situ hybridisation and detection of the biotin-11-dUTP labelled probes. The Vectastain ABC Kit (Vector) was developed by Hsu et al. (1981) for immunohistochemical staining of sections against histologically significant antigens. The sections were incubated with a primary antiserum raised against the antigen of interest, and a biotin-labelled secondary antibody added which bound to the primary antibody. An avidin: biotinylated horseradish peroxidase complex could then be added which bound to the biotin of the secondary antibody. The antigen could thus be localised by incubating in a peroxidase substrate solution, such as DAB, to give a stain (brown in the case of DAB). The alkaline phosphatase DNA detection kit (BRL) was developed by Leary et al. (1983) for detection of biotin-labelled DNA probes hybridised to nucleic acids bound on nitrocellulose filters. The method makes use of

streptavidin and biotinylated polyalkaline phosphatase to bind to the biotinylated probes, and NBT and BCIP dyes as a phosphatase substrate to produce a blue stain for colourimetric visualisation.

The Vectastain ABC kit was initially tried by adapting it for use in in situ hybridisations. The avidin: biotinylated horseradish peroxidase complex was used to detect and stain for the chromosome squashes, as opposed to the biotinylated secondary antibody of the original ABC protocol. No staining, however, could be detected, and thus the alkaline phosphatase kit was attempted. This technique has an advantage in that it was actually designed for detection of biotinylated DNA probes, even if for use with hybridised DNA on nitrocellulose filters and not on chromosome squashes. The polyalkaline phosphatase present in the kit provides greater sensitivity than the horseradish peroxidase of the ABC kit, and the streptavidin, which demonstrates the same high affinity for biotin as avidin, exhibits less nonspecific binding. In experimental tests with oligo-labelled biotin-11-dUTP probes, the alkaline phosphatase protocol was found to work well, giving blue bands on the chromosome squashes which could be visualised under the microscope to the greatest effect using phase contrast objectives. Thus, the alkaline phosphatase DNA detection kit, in conjunction with the oligo-labelling technique, were used for analysis of the D. melanogaster lines.

The number and cytogenetic locations of the transposable elements copia, 412, F and P were determined for the parent strains and the dysgenic and nondysgenic sublines. 2nd, 3rd and X chromosomes were scored, one larva being examined per

subline and two for each of the parental strains.

3.5 Variable Sites Are Present In The Parental Strains

Figures 4 and 5 show the sublimes produced from the dysgenic and nondysgenic crosses, respectively, that were analysed after generation F14 by in situ hybridisation. The experiment was designed to screen for new transposable element sites not originally present in the parental strains. The locations of the transposable elements copia, 412, P and F, in the parental strains and the sublimes, can be seen in figures 6-45. Of the new sites observed which were not found in the parental lines, many were found to be shared by a number of the experimental and control lines. It was concluded that these sites represented variable sites originally present in the parental stocks. This theory was confirmed by analysing two larvae for each of the transposable elements of the parents, whereby it was found that element sites did indeed vary. Sites found in only one of the two larvae examined for Harwich and Canton-S are shown underlined in figures 6 and 7. The variable sites were discounted from being new transposable element insertion sites in the analysis. Transposition initially occurs in the F1 generation of a cross and is thus represented in the genomes of F2 generation flies. If two different lines derived separately from the F2 generation contain a site, not present in the parental larvae scored, but shared by each of the two lines, then this probably represents a variable site originally present in the parental stocks, as the two lines will possess different new transposable element sites. Thus, any such sites were deleted from the analysis.

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Figure 4: Isofemale lines produced from Harwich male with Canton-S female crosses. Two males and two females were mated in each of the original vials. Single fertilized females were taken to form each subsequent generation e.g. two fertilized females were isolated from the F3 generation of line Aa and separated to form two F4 sublines Aa1 and Aa2. All 27 lines were scored for the elements F, copia and 412, and all apart from Ab2(b)III \bar{Z} and Ac3(a) for the P element.

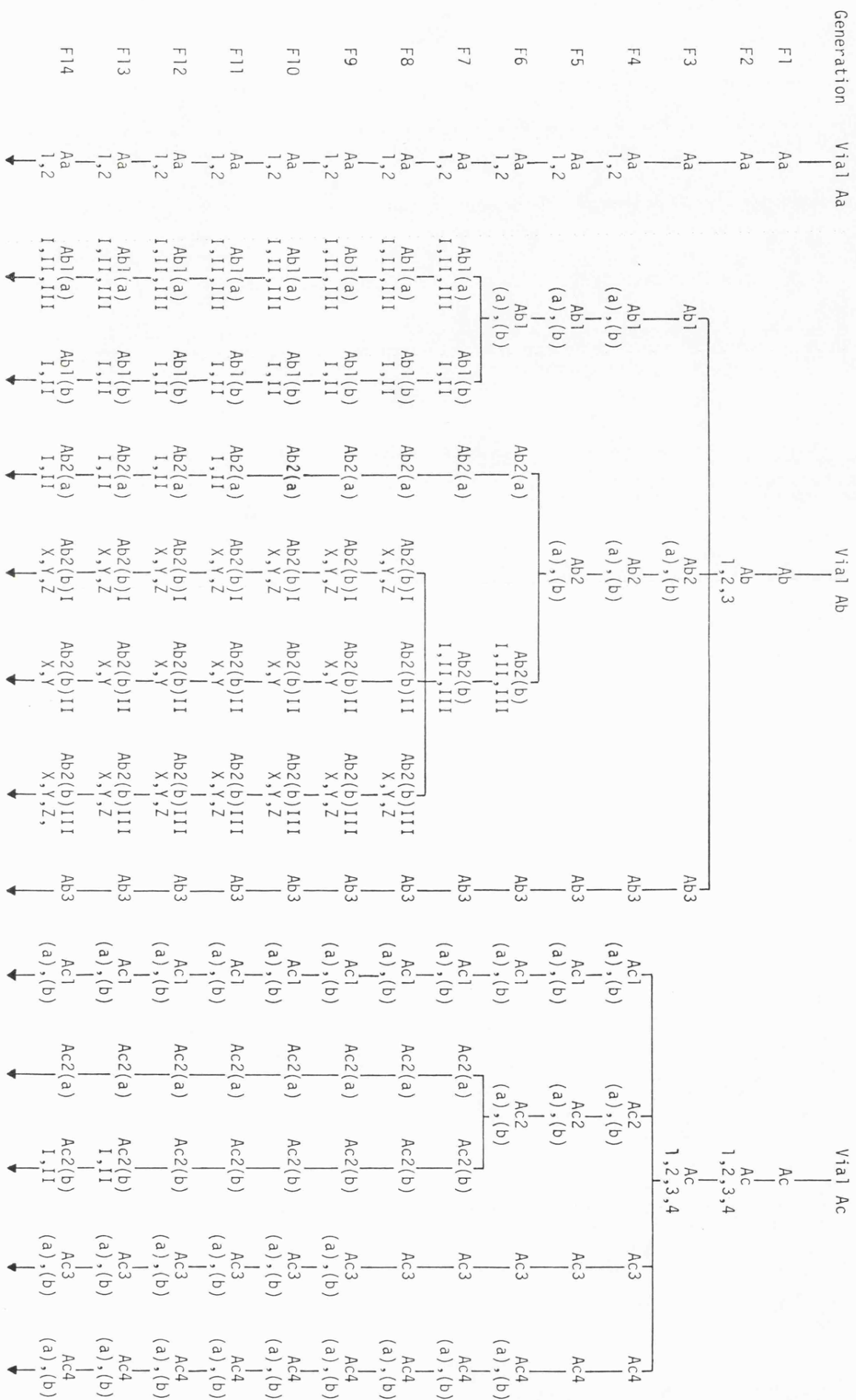


Figure 5: Isofemale lines produced from Canton-S male with Harwich female crosses. Two males and two females were mated in each of the original vials. Single fertilized females were taken to form each subsequent generation. All 11 lines were scored for the elements F, copia and 412, and all apart from Bd2 for the P element.

Generation	Vial Ba	Vial Bb	Vial Bc	Vial Bd	Vial Be	Vial Bf
F1	Ba	Bb	Bc	Bd	Be	Bf
F2	Ba 1,2	Bb 1,2	Bc 1,2	Bd 1,2,3	Be	Bf
F3	Ba 1,2	Bb 1,2	Bc 1,2	Bd 1,2,3	Be	Bf
F14	Ba 1,2	Bb 1,2	Bc 1,2	Bd 1,2,3	Be	Bf
	↓	↓	↓	↓	↓	↓

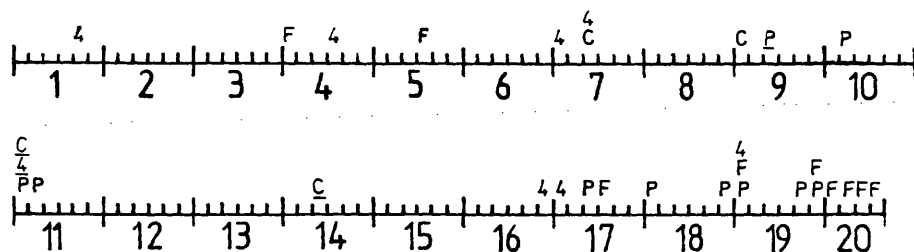
Figures 6-45: Locations of the transposable elements copia, 412, P and F in the genomes of Harwich and Canton-S, and in the dysgenic and non-dysgenic sublines. Chromosomes 2, 3 and X were scored, 2L and 3L representing the left arms of chromosomes 2 and 3 respectively, and 2R and 3R the right arms. The X chromosome and the arms of chromosomes 2 and 3 are each divided up into 20 sections according to the banding patterns of Bridges (1935), and each section again into 7 further divisions, A-F. One larva was examined for each of the sublines and two for each parental strain. Sites found in only one of the two larvae examined for Harwich and Canton-S are underlined (figures 6 and 7).

Figure 6

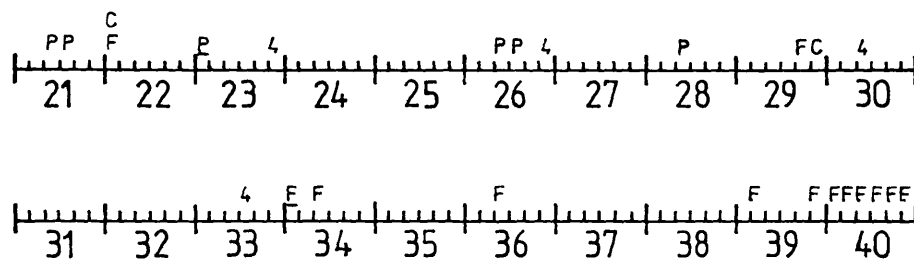
Positions of transposable element insertions in the chromosomes
of D. melanogaster strain Harwich

C - copia / F - F element / 4 - 412 / P - P element

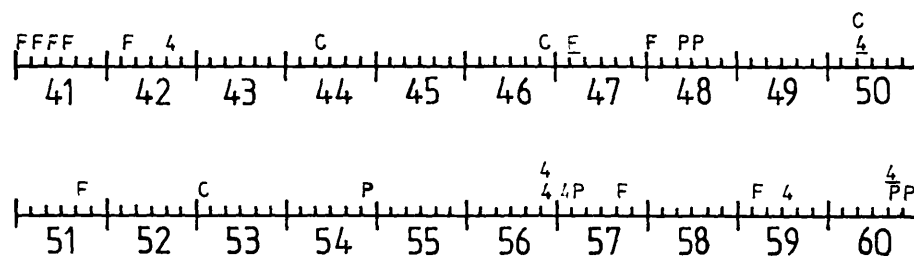
1=X



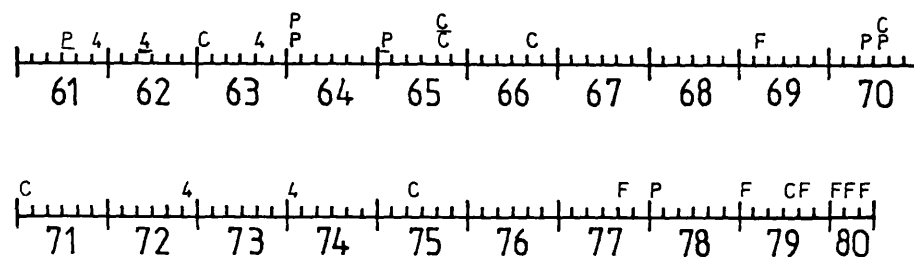
2L



2R



3L



3R

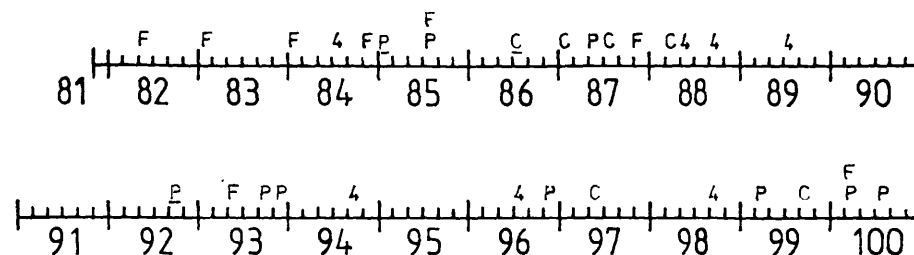


Figure 7

Positions of transposable element insertions in the chromosomes
of D. melanogaster strain Canton-S

C - copia / F - F element / 4 - 412 / P - P element

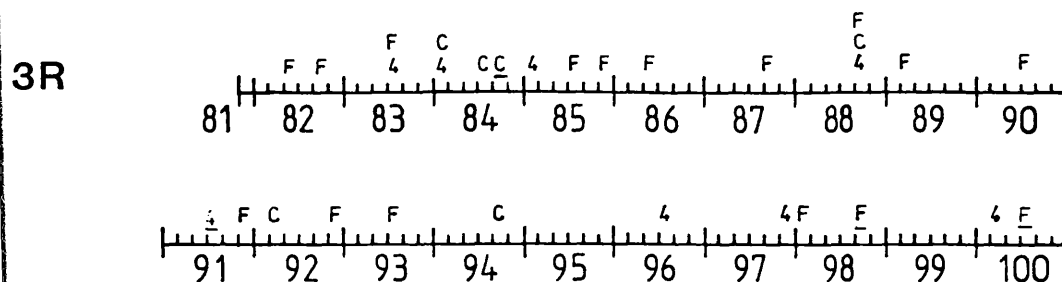
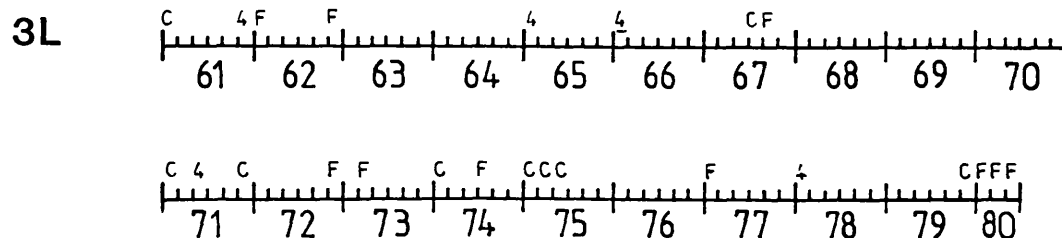
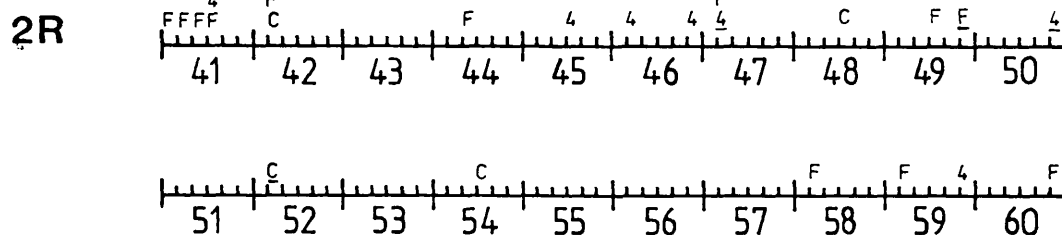
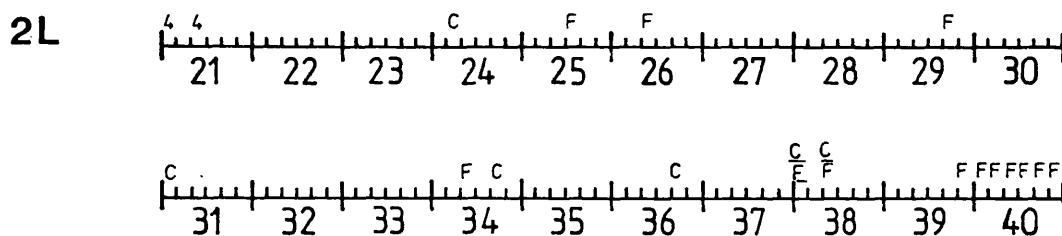
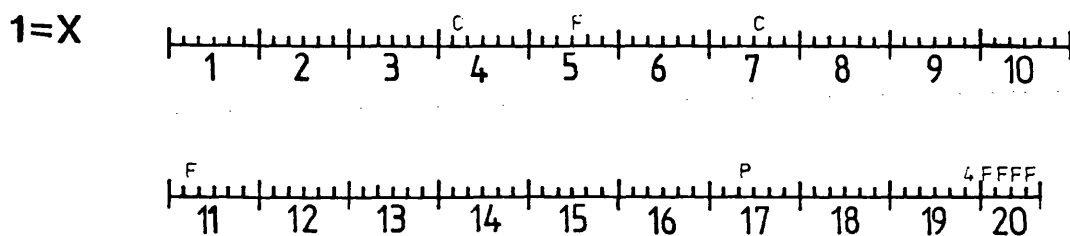
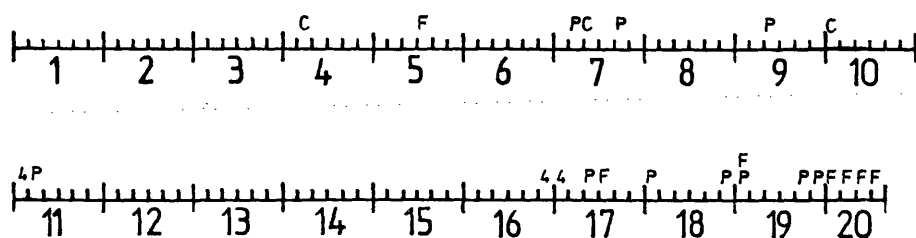


Figure 8

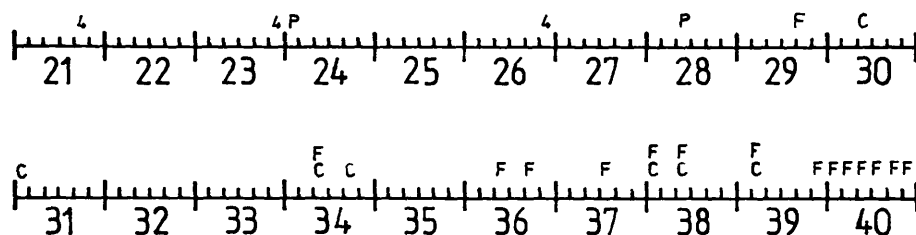
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Aa1

C - copia / F - F element / 4 - 412 / P - P element

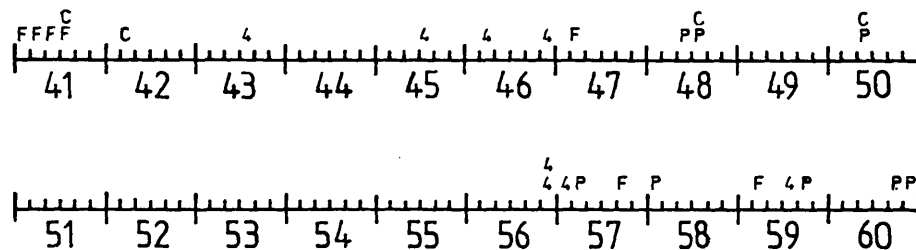
1=X



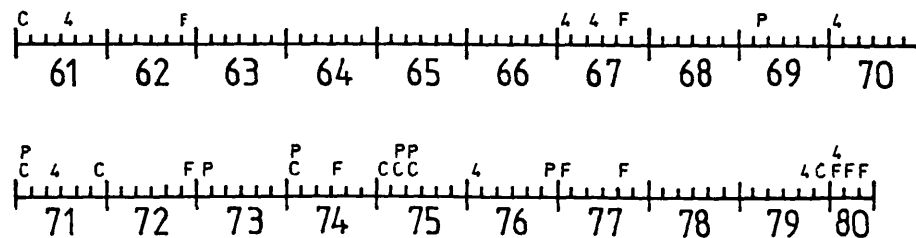
2L



2R



3L



3R

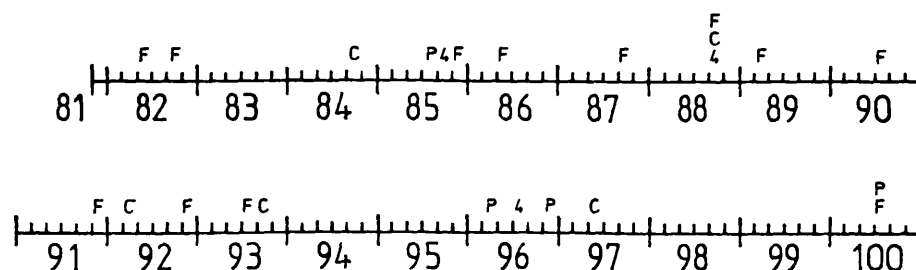
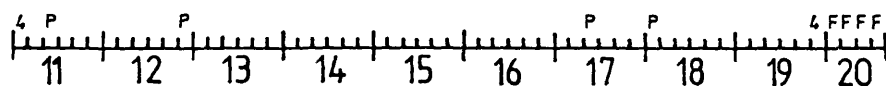
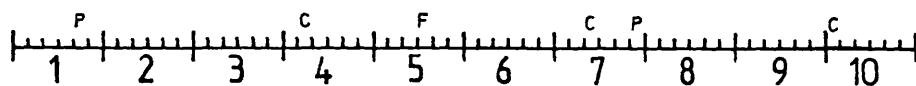


Figure 9

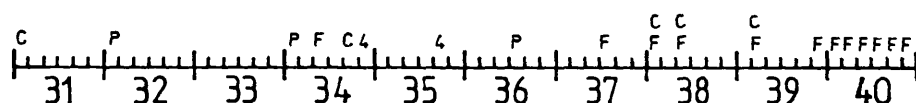
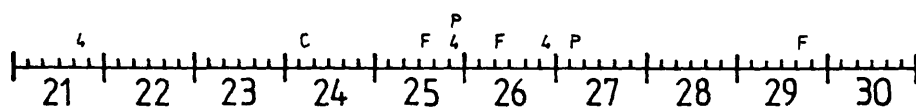
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Aa2

C - copia / F - F element / 4 - 412 / P - P element

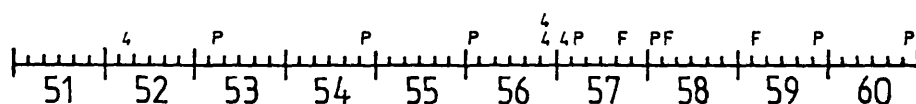
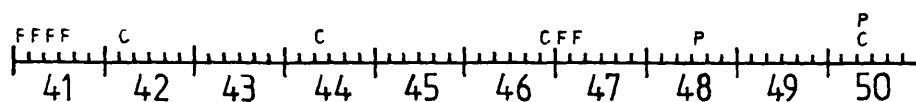
1=X



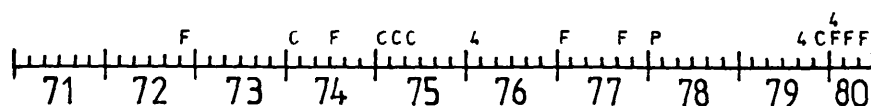
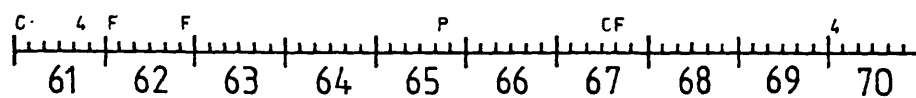
2L



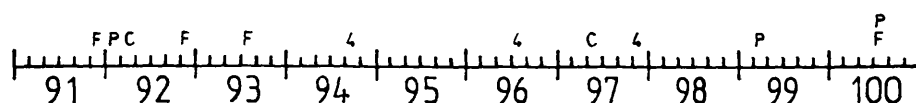
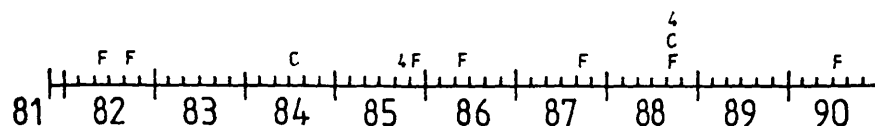
2R



3L



3R



Positions of transposable element insertions in the chromosomes of *D. melanogaster* subline Ab 1(a)I

1=X

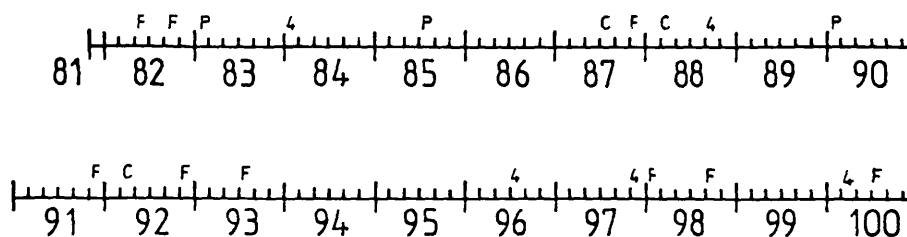
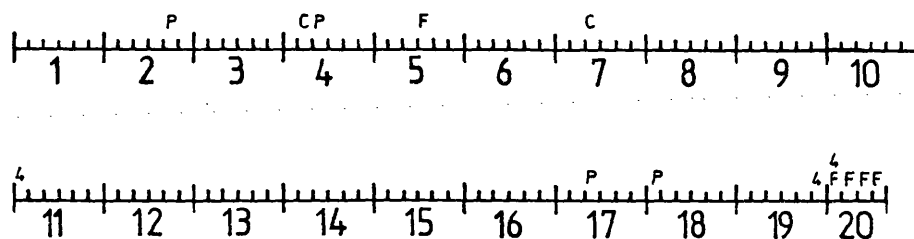


Figure 11

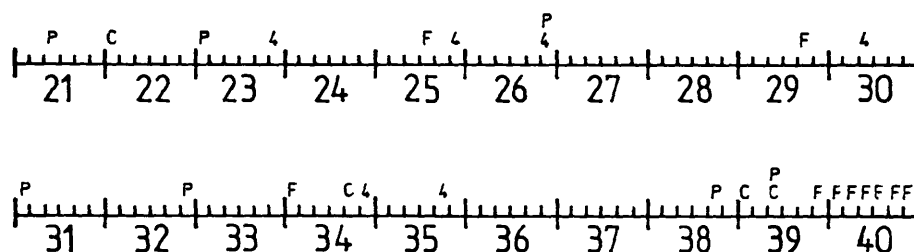
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 1(a)II

C - copia / F - F element / 4 - 412 / P - P element

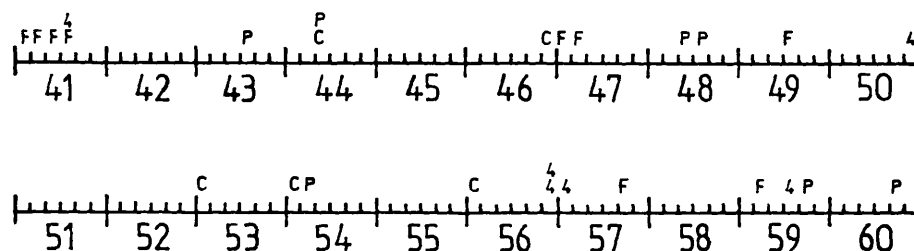
1=X



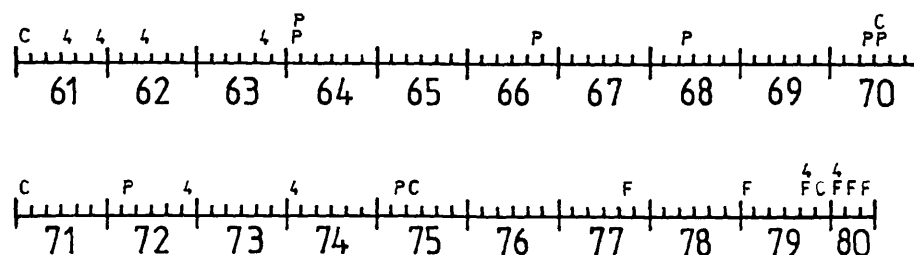
2L



2R



3L



3R

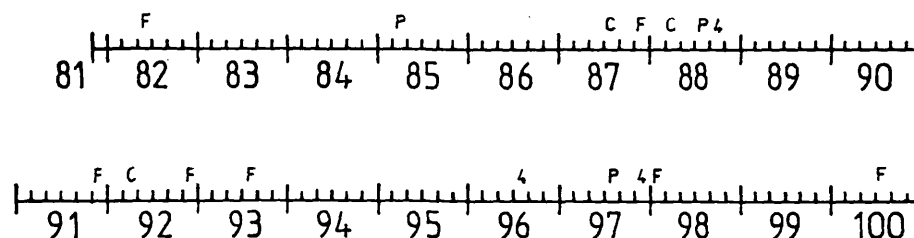
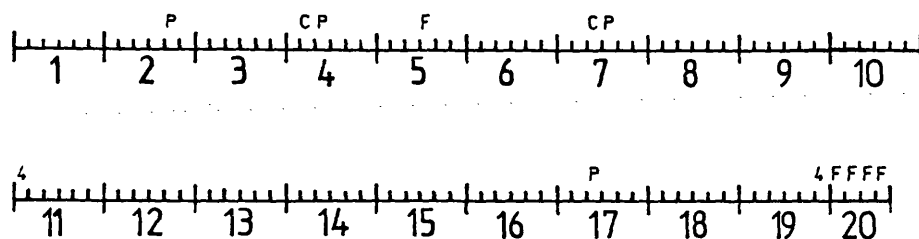


Figure 12

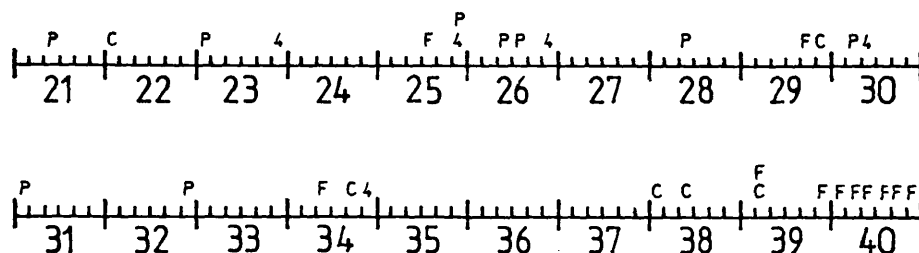
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 1(a)III

C - copia / F - F element / 4 - 412 / P - P element

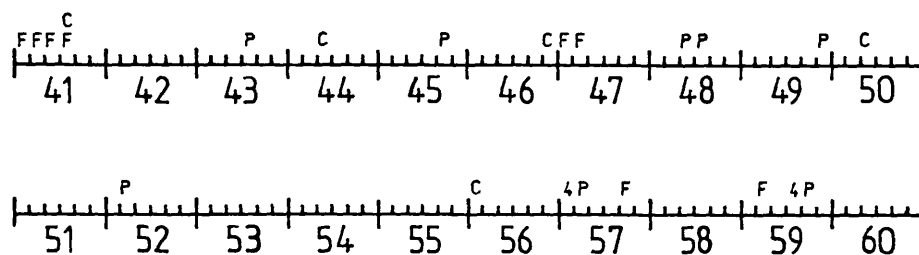
1=X



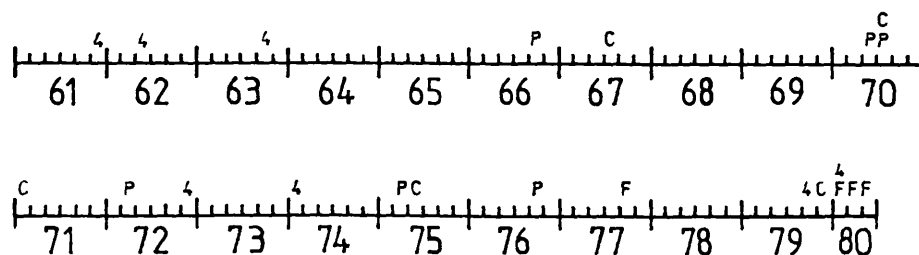
2L



2R



3L



3R

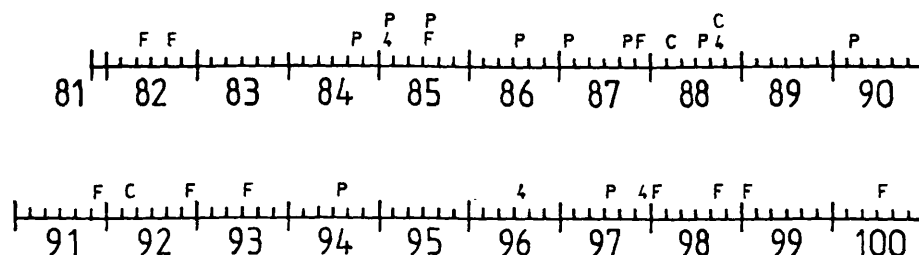
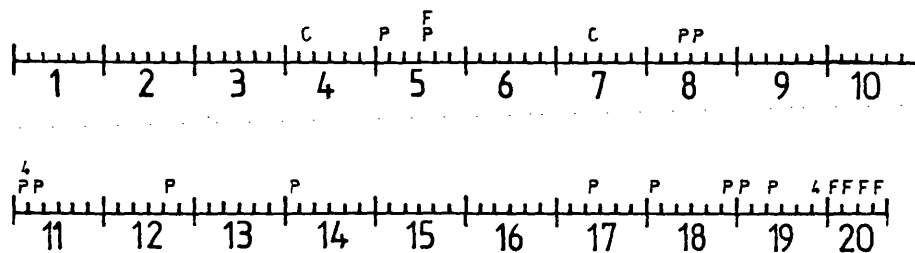


Figure 13

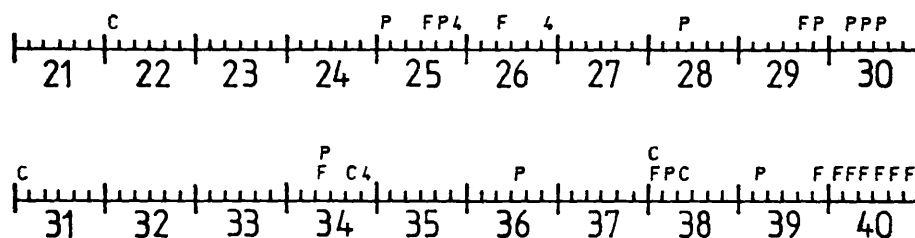
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 1(b)I

C - copia / F - F element / 4 - 412 / P - P element

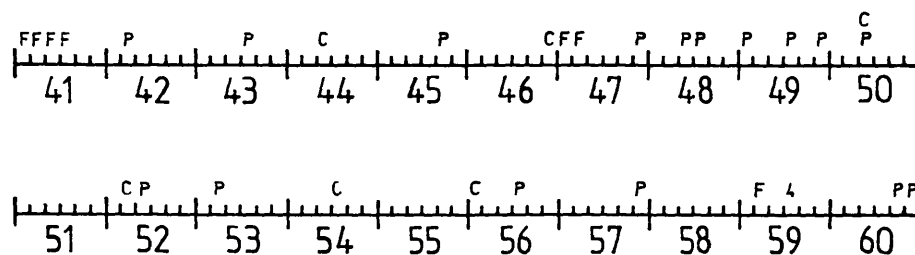
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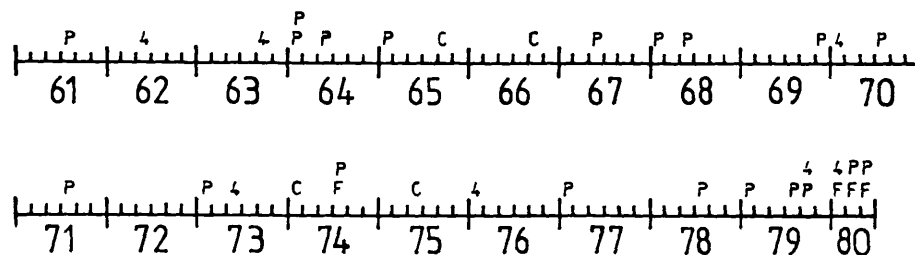
2L



2R



3L



3R

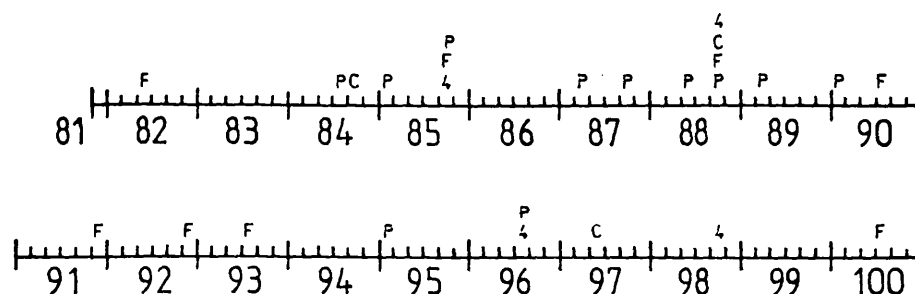
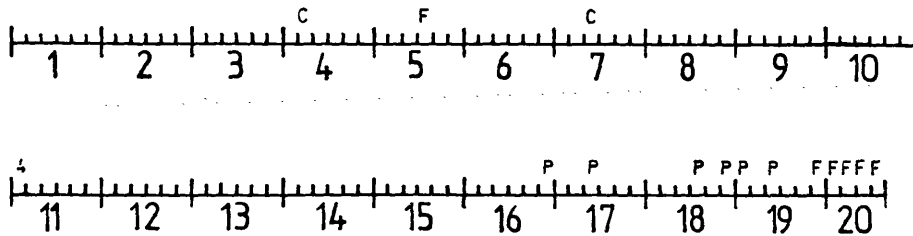


Figure 14

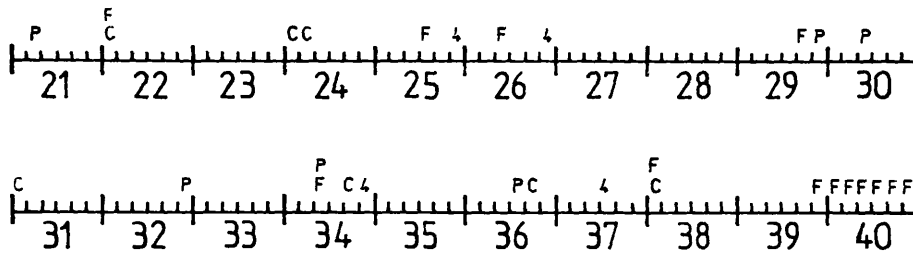
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 1(b)II

C - copia / F - F element / 4 - 412 / P - P element

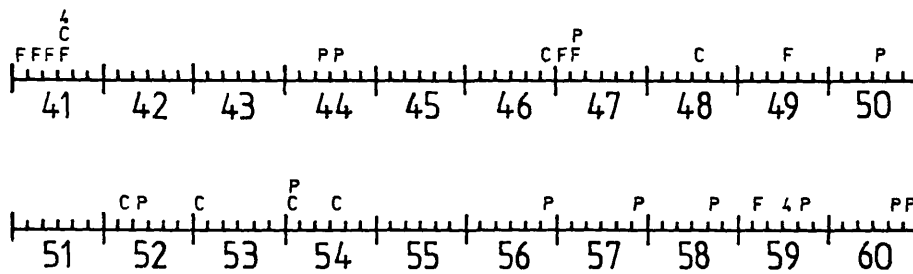
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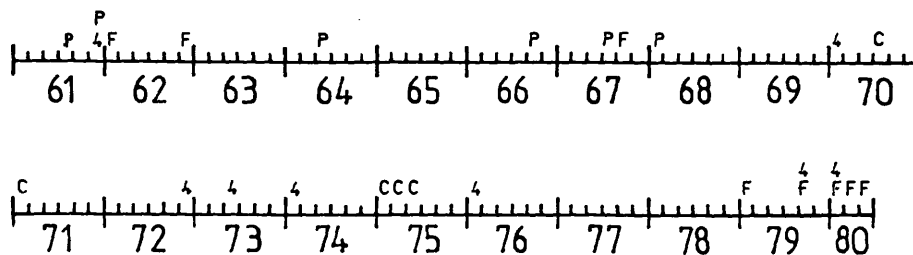
2L



2R



3L



3R

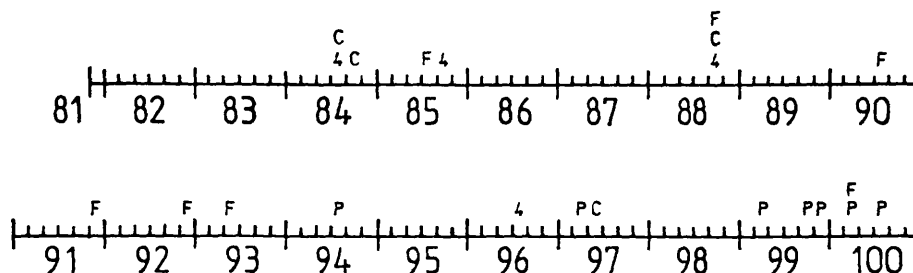
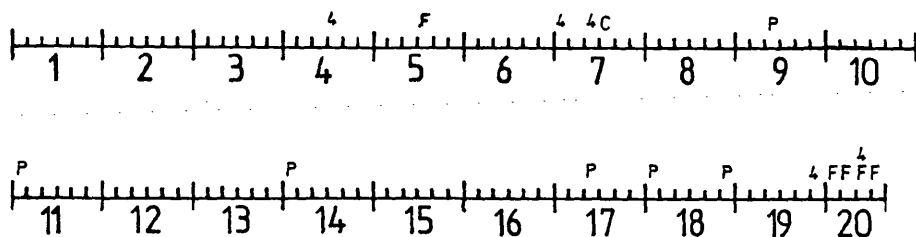


Figure 15

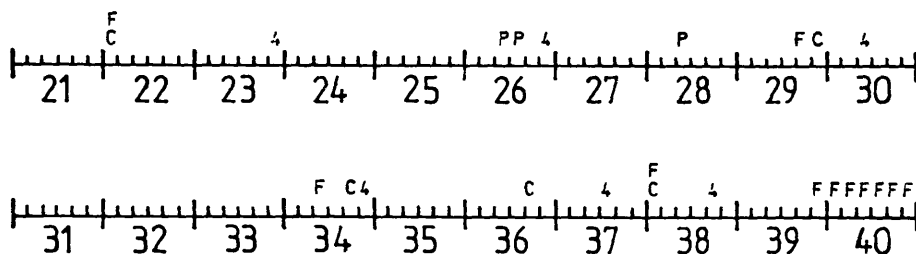
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 2(a)I

C - copia / F - F element / 4 - 412 / P - P element

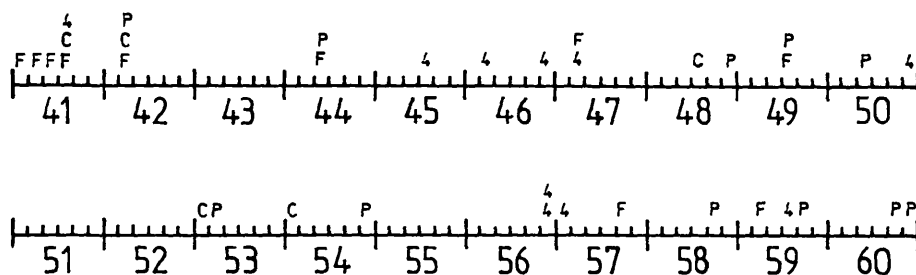
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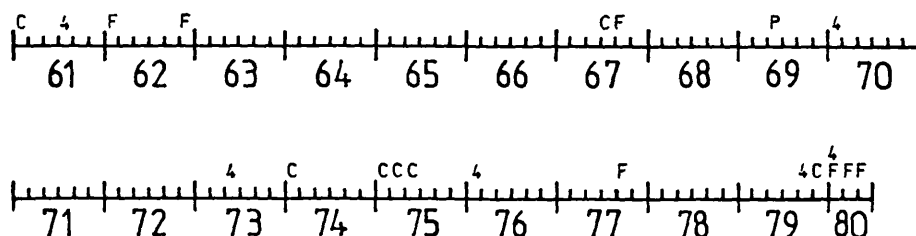
2L



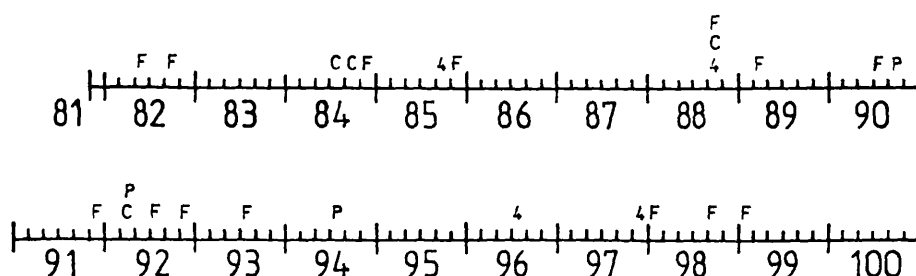
2R



3L

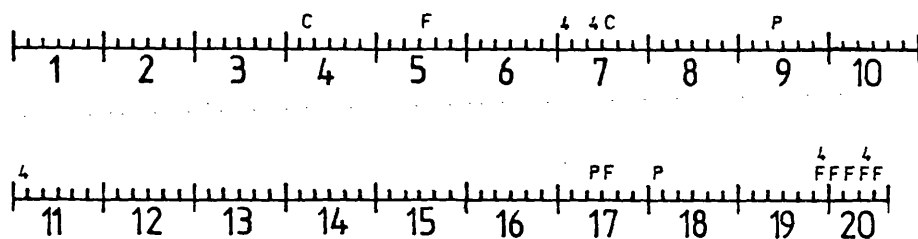


3R



Positions of transposable element insertions in the chromosomes of *D. melanogaster* subline Ab 2(a)II

1=X



The first number line covers integers from 21 to 30. Above the line, the following labels are placed: 'P' above 21, 'P' above 22, 'C' above 23, 'P' above 24, 'P' above 26, 'P' above 27, 'P' above 28, 'FC' above 29, and 'C' above 30.

The second number line covers integers from 31 to 40. Above the line, the following labels are placed: 'F' above 34, 'C' above 34, 'C' above 36, 'C' above 37, 'F' above 39, 'F' above 39, 'F' above 39, 'F' above 39, and 'F' above 39.

The first staff shows measures 61 to 70. Measure 61 has a C note. Measure 62 has a 4-measure rest. Measure 63 has an F note. Measure 64 has a 5-measure rest. Measure 65 has a P note. Measure 66 has a 5-measure rest. Measure 67 has an F note. Measure 68 has a 5-measure rest. Measure 69 has a 5-measure rest. Measure 70 has a 5-measure rest.

The second staff shows measures 71 to 80. Measure 71 has a 5-measure rest. Measure 72 has a 5-measure rest. Measure 73 has a 4-measure rest. Measure 74 has a C note. Measure 75 has an F note. Measure 76 has a 5-measure rest. Measure 77 has a C note. Measure 78 has a 5-measure rest. Measure 79 has a 5-measure rest. Measure 80 has a 5-measure rest.

The image displays two musical staves, each representing a chromatic scale. The first staff covers the range from G1 (81) to G2 (90), and the second staff covers the range from G2 (91) to G3 (100). Each staff has 12 semitones, with notes labeled F, C, and 4F at specific intervals.

Staff 1 (G1 to G2):

- 81: G1
- 82: A1 (F)
- 83: B1 (F)
- 84: C2 (C)
- 85: D2 (C)
- 86: E2 (4F)
- 87: F2
- 88: G2 (C)
- 89: A2 (F)
- 90: B2 (F)

Staff 2 (G2 to G3):

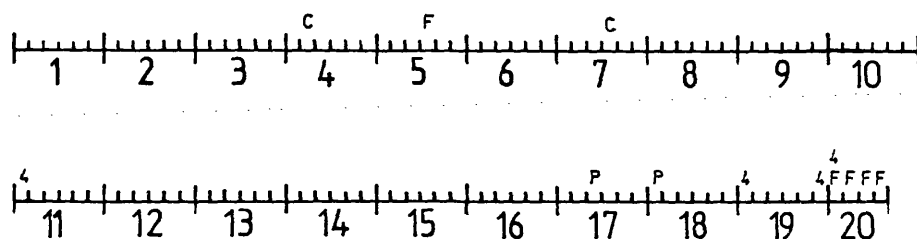
- 91: C3 (F)
- 92: D3 (C)
- 93: E3 (F)
- 94: F3 (F)
- 95: G3 (P)
- 96: A3 (4)
- 97: B3 (4F)
- 98: C4 (F)
- 99: D4 (F)
- 100: E4 (F)

Figure 17

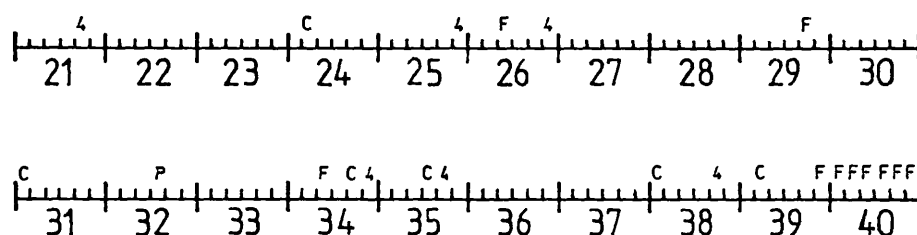
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 2(b)IX

C - copia / F - F element / 4 - 412 / P - P element

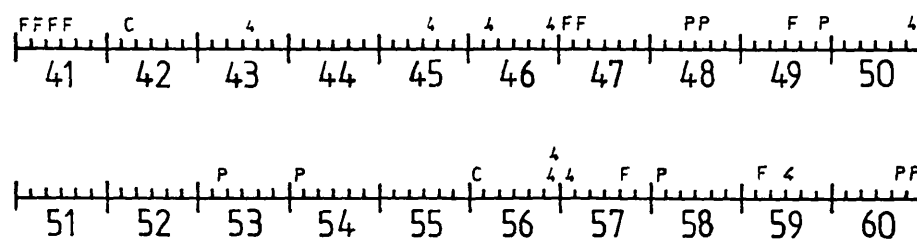
1=X



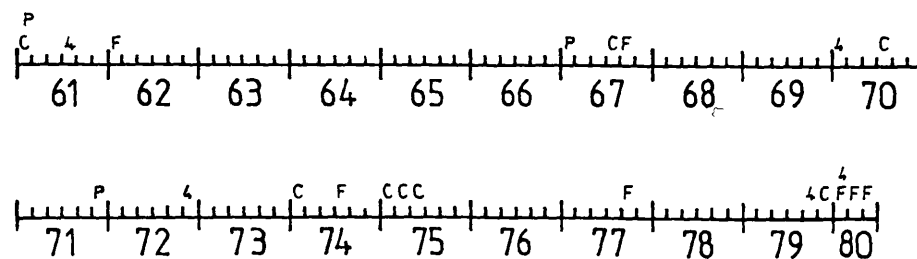
2L



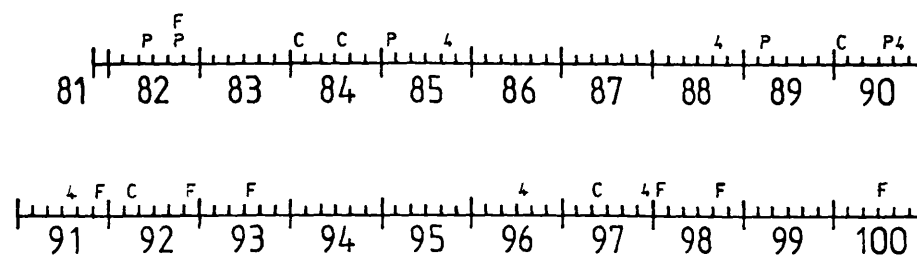
2R



3L



3R



Positions of transposable element insertions in the chromosomes of D. melanogaster subline Ab 2(b)IY

1=X

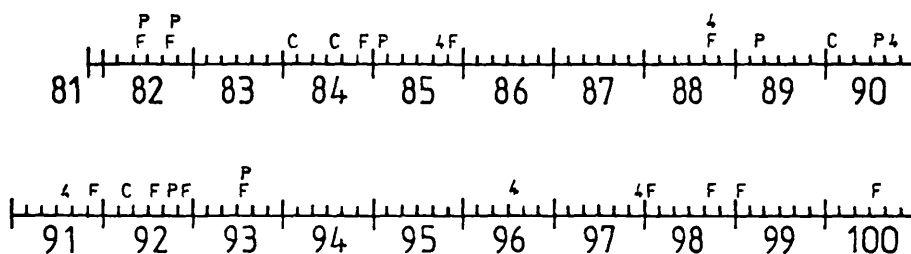
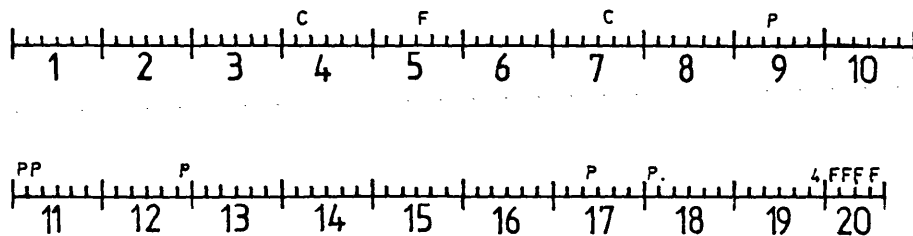


Figure 19

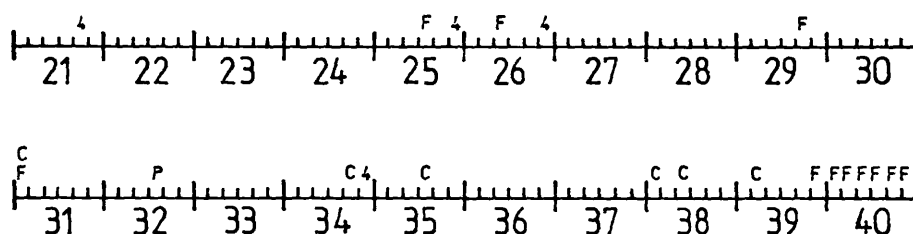
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 2(b)IZ

C - copia / F - F element / 4 - 412 / P - P element

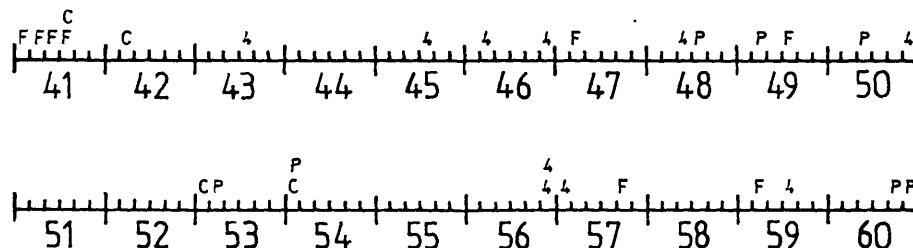
1=X



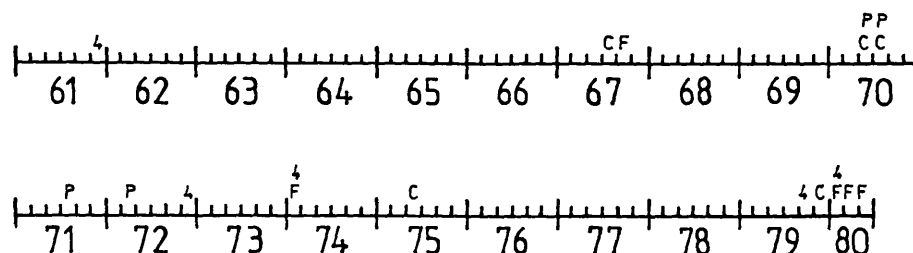
2L



2R



3L



3R

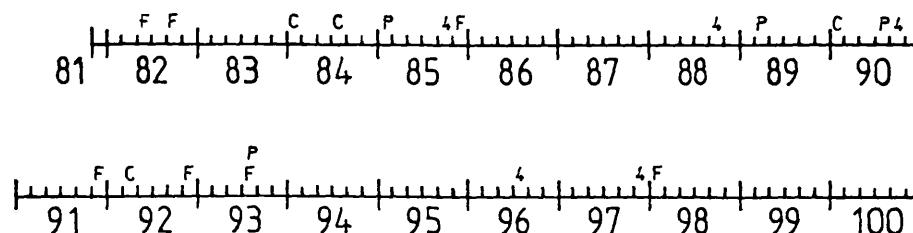
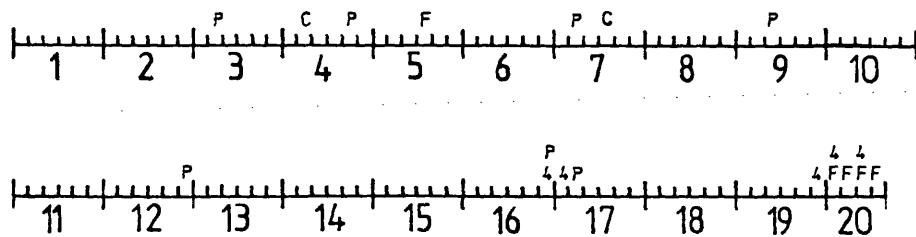


Figure 20

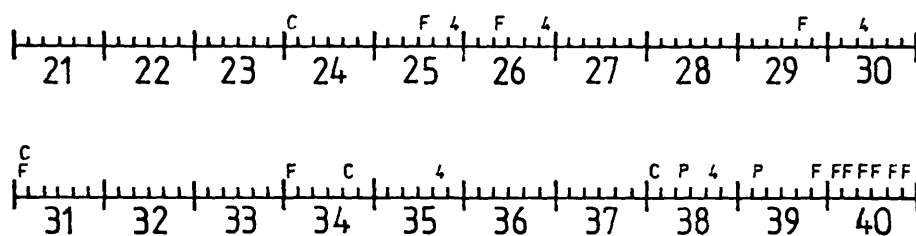
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 2(b)IX

C - copia / F - F element / 4 - 412 / P - P element

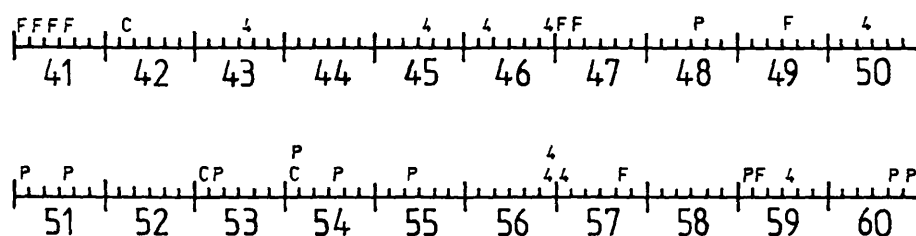
1=X



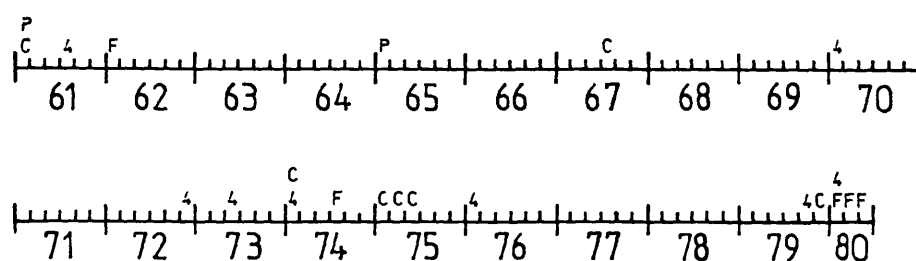
2L



2R



3L



3R

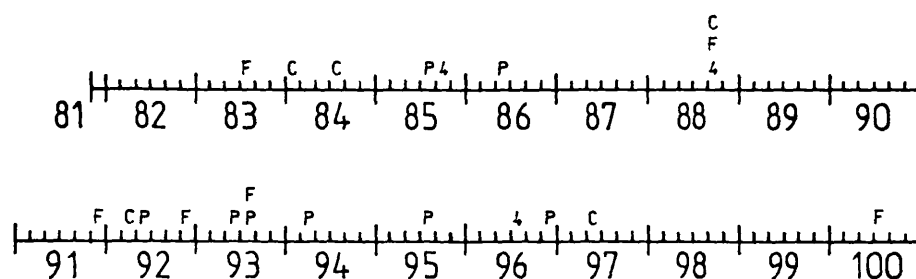
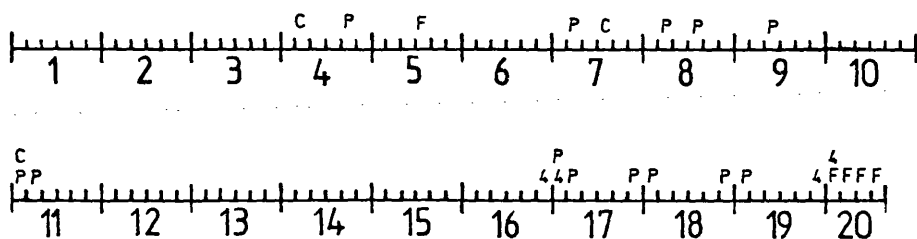


Figure 21

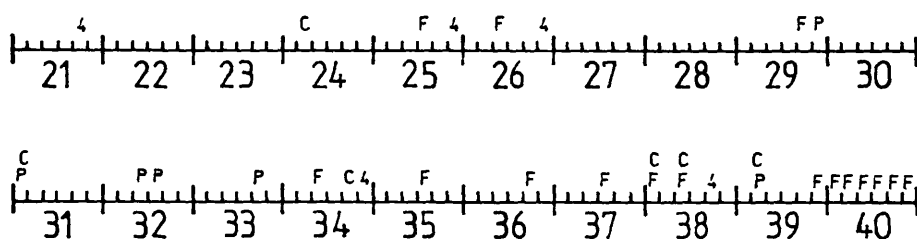
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab 2 (b)IIY

C - copia / F - F element / 4 - 412 / P - P element

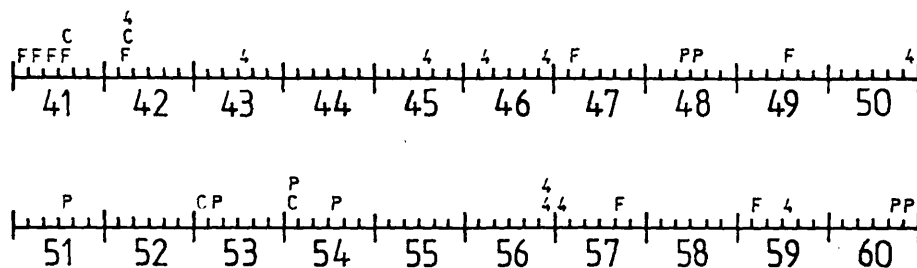
1=X



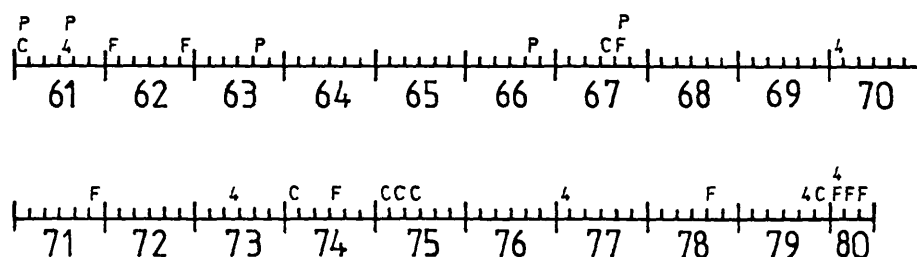
2L



2R



3L



3R

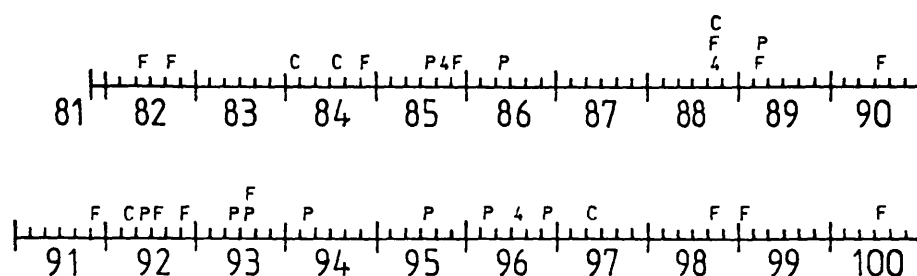
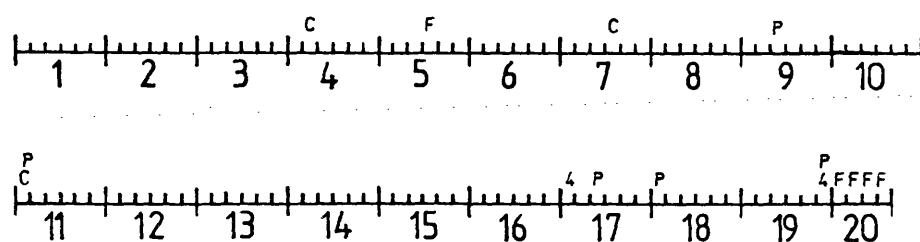


Figure 22

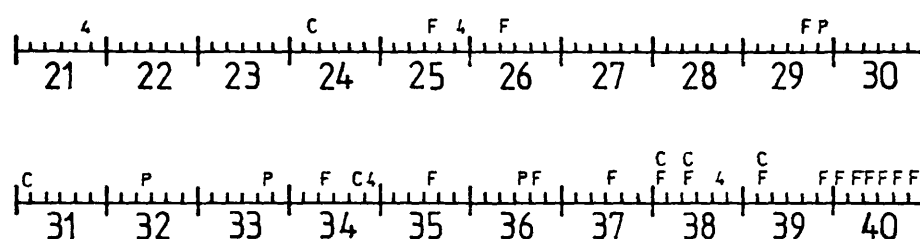
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab2(b)III_X

C - copia / F - F element / 4 - 412 / P - P element

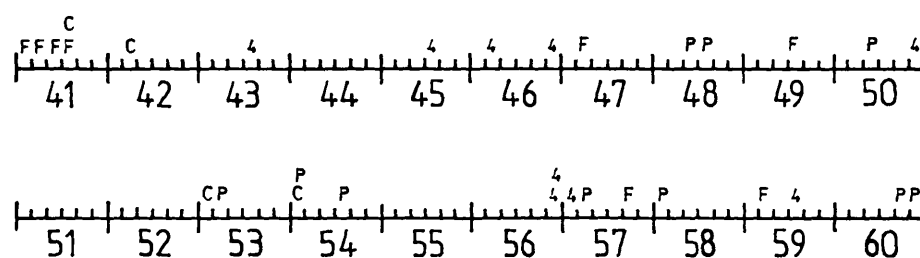
1=X



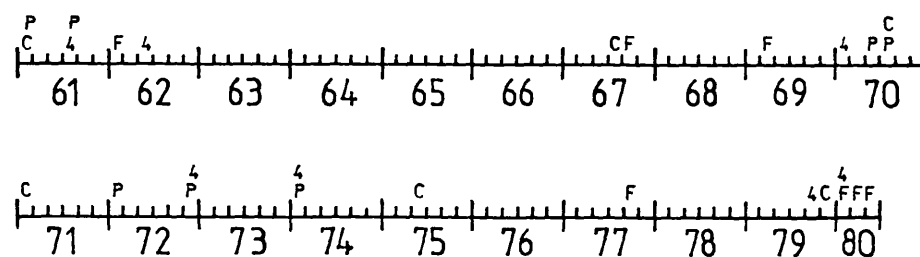
2L



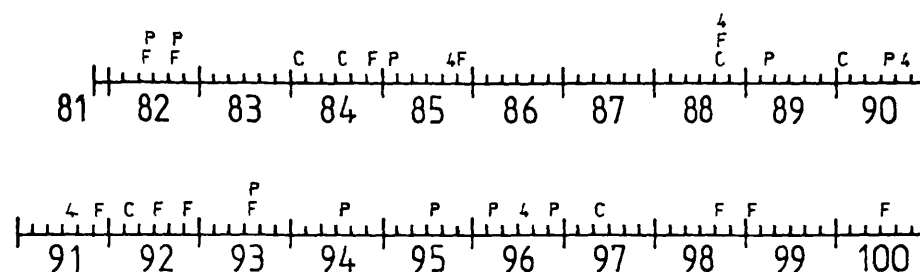
2R



3L



3R



Positions of transposable element insertions in the chromosomes of *D. melanogaster* subline Ab 2(b)IIIY

1=X

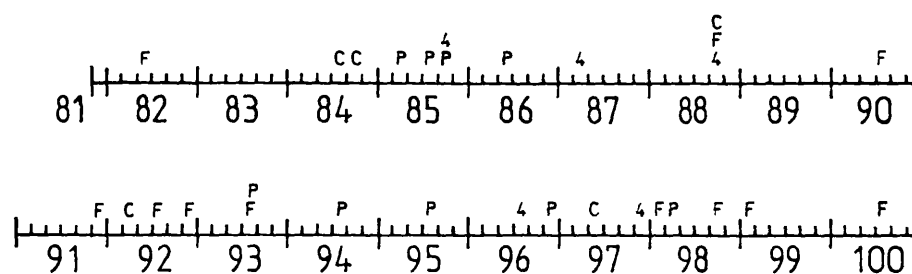
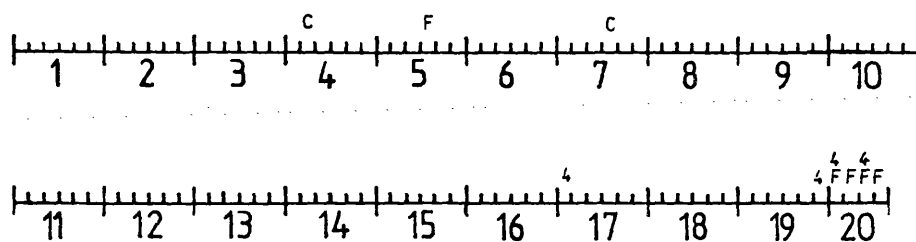


Figure 24

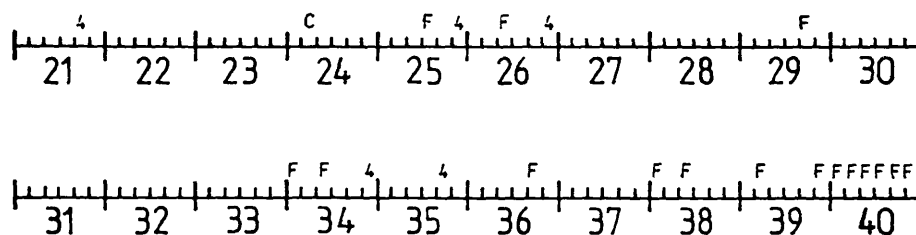
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab2(b)III Z

C - copia / F - F element / 4 - 412

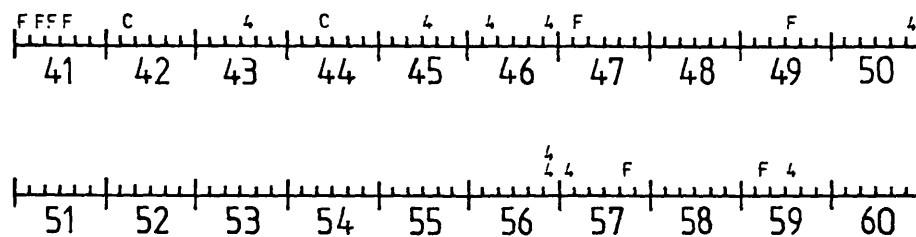
1=X



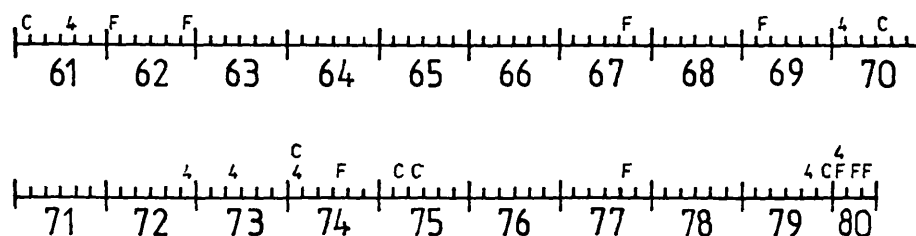
2L



2R



3L



3R

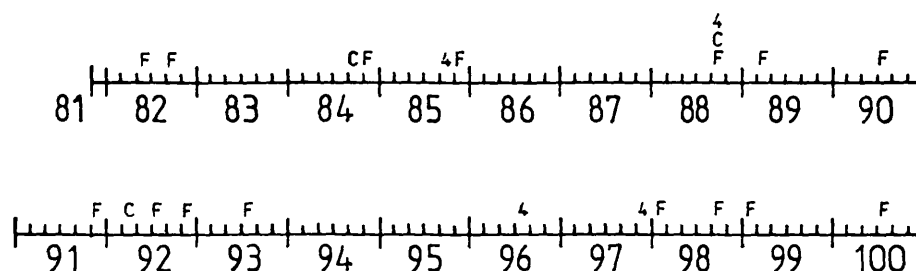
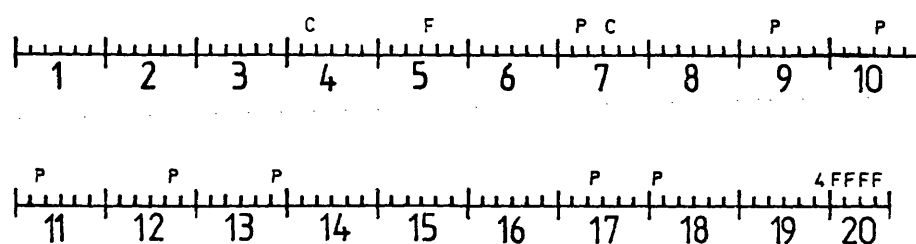


Figure 25

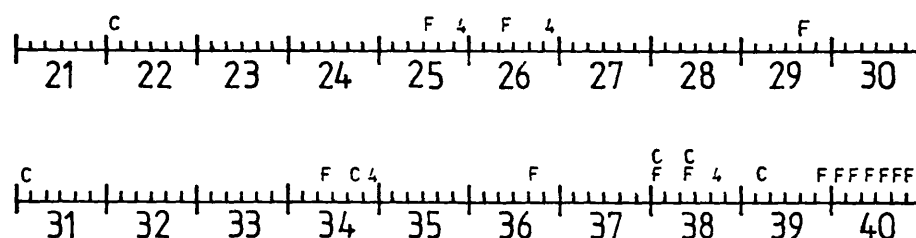
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ab3

C - copia / F - F element / 4 - 412 / P - P element

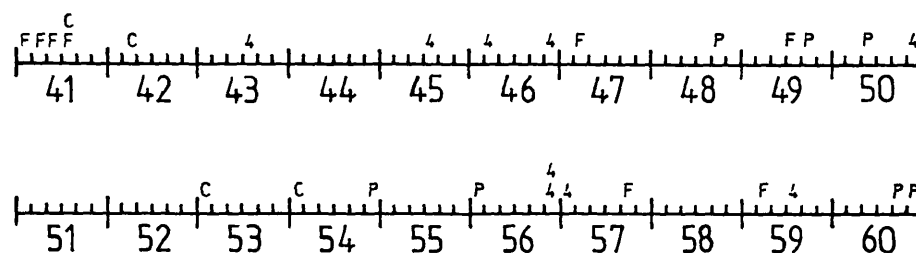
1=X



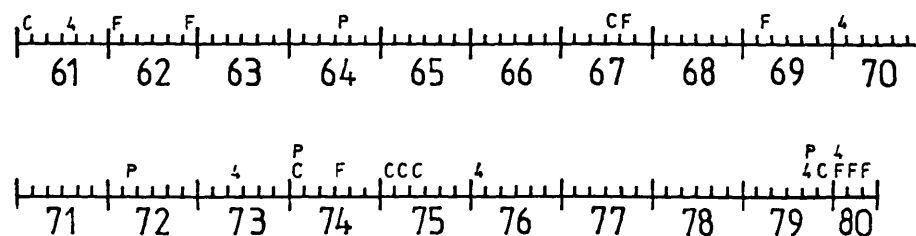
2L



2R



3L



3R

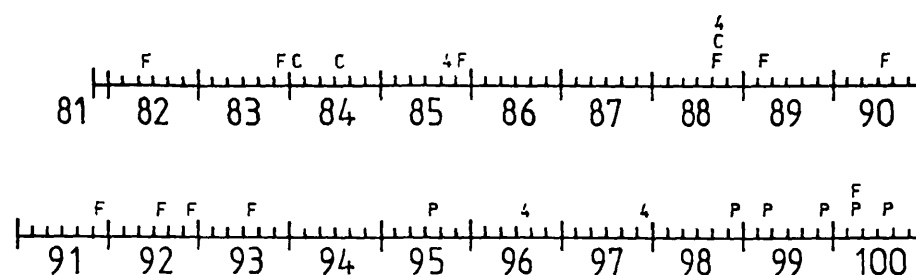
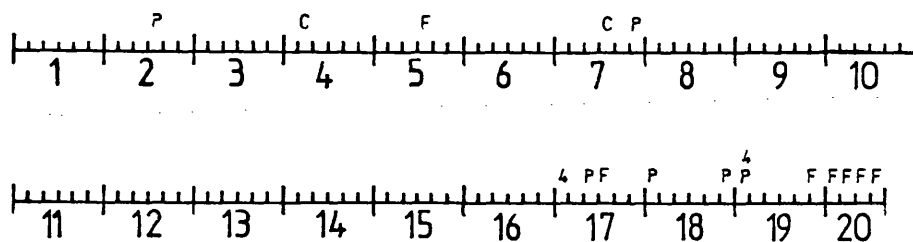


Figure 26

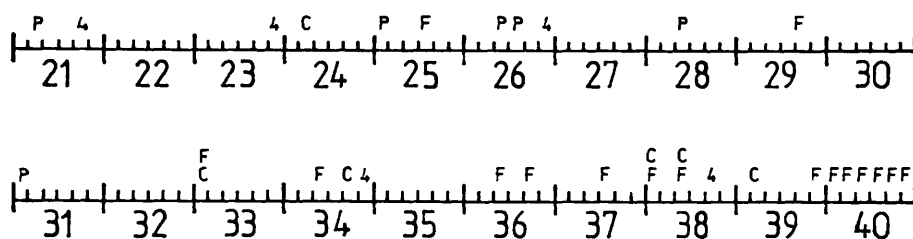
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ac 1(a)

C - copia / F - F element / 4 - 412 / P - P element

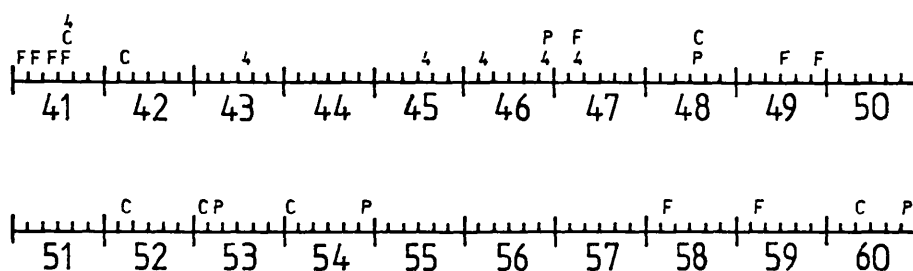
1=X



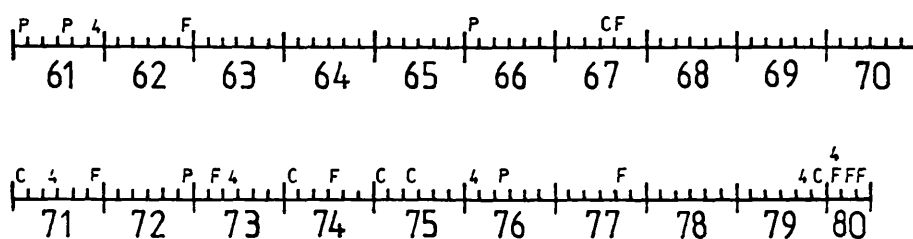
2L



2R



3L



3R

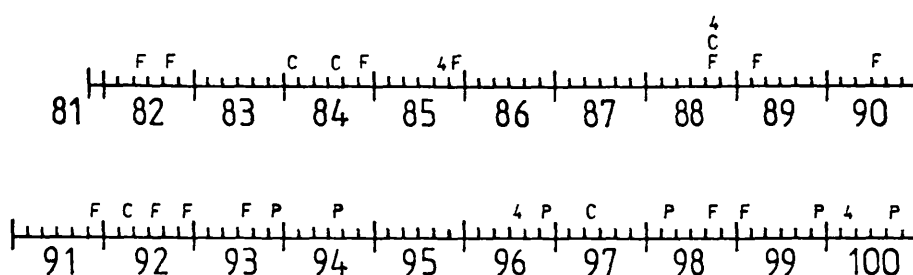
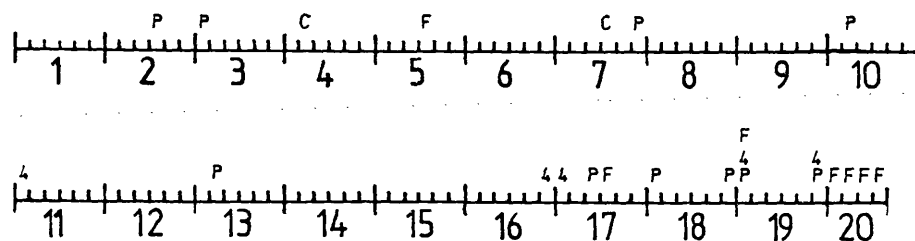


Figure 27

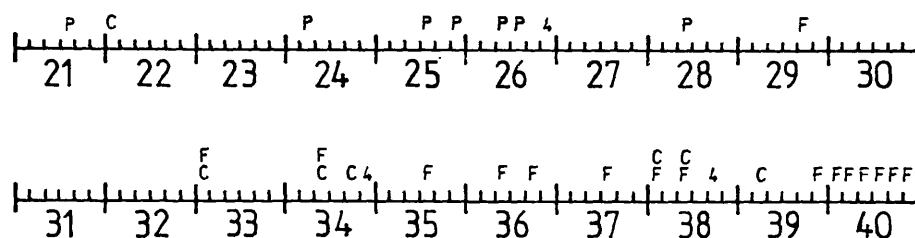
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ac 1(b)

C - copia / F - F element / 4 - 412 / P - P element

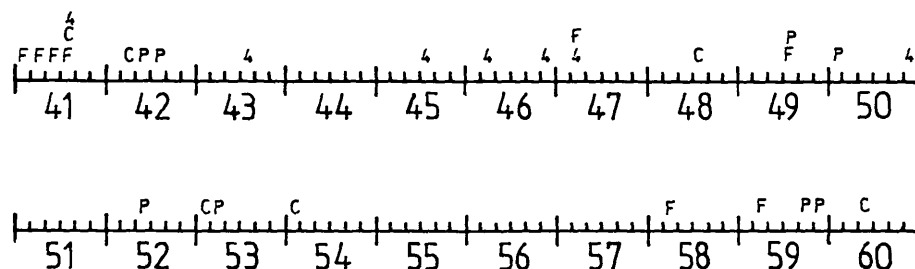
1=X



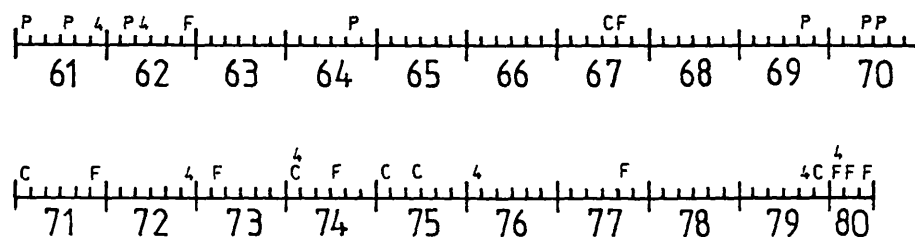
2L



2R



3L



3R

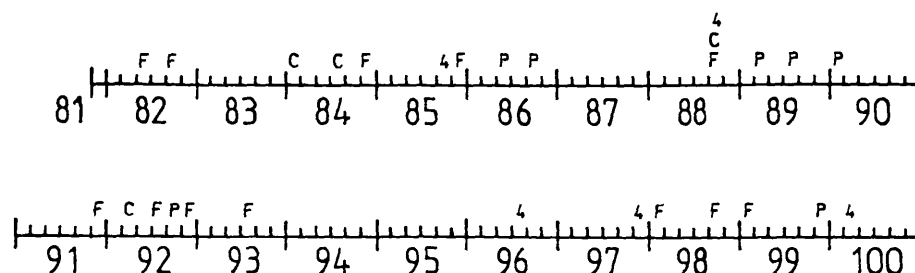


Figure 28

Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ac 2(a)

C - copia / F - F element / 4 - 412 / P - P element

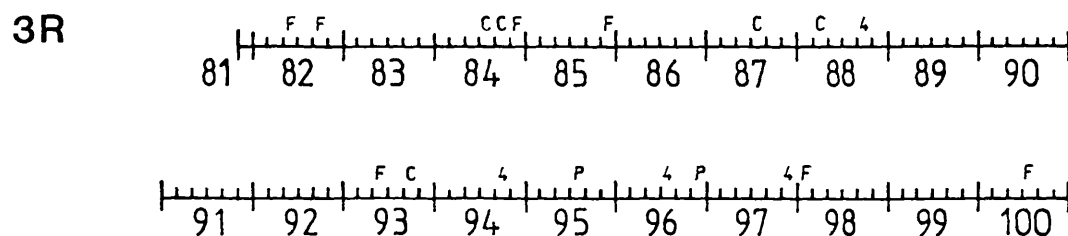
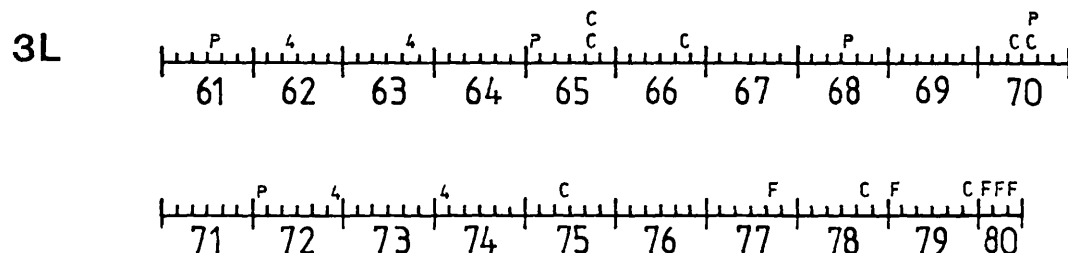
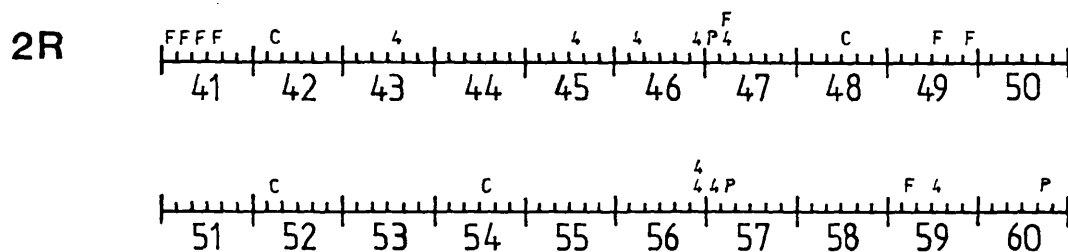
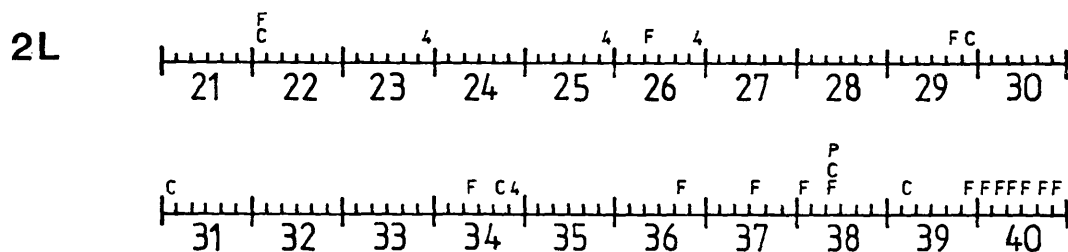
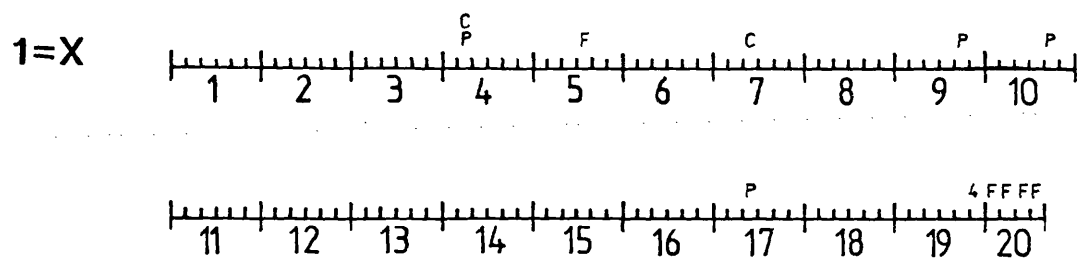
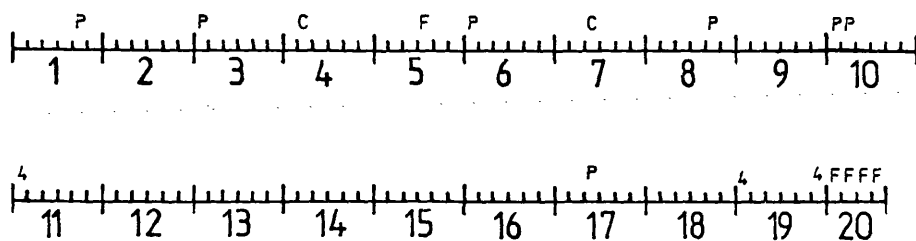


Figure 29

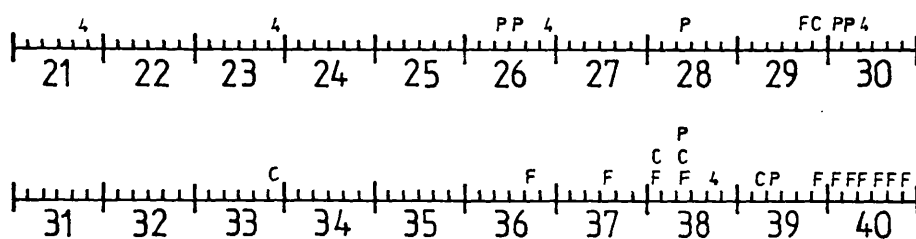
Positions of transposable element insertions in the chromosomes of *D. melanogaster* subline Ac2(b)I

C - copia / F - F element / 4 - 412 / P - P element

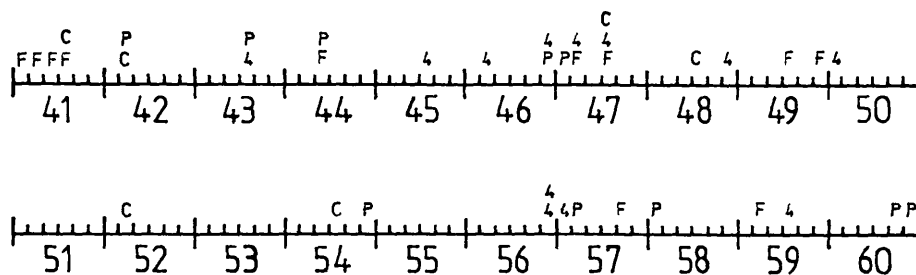
1=X



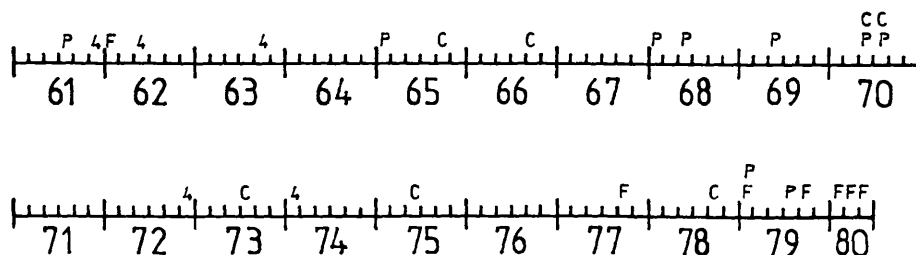
2L



2R



3L



3R

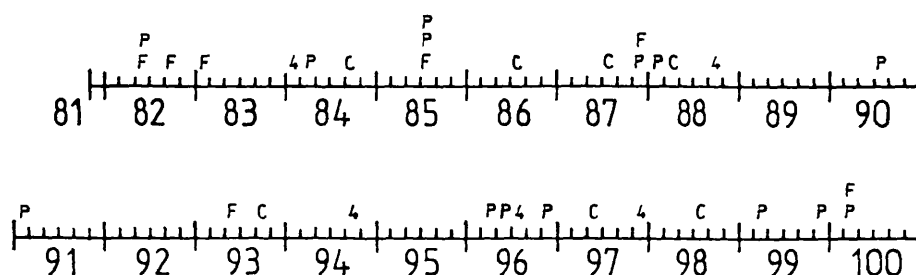
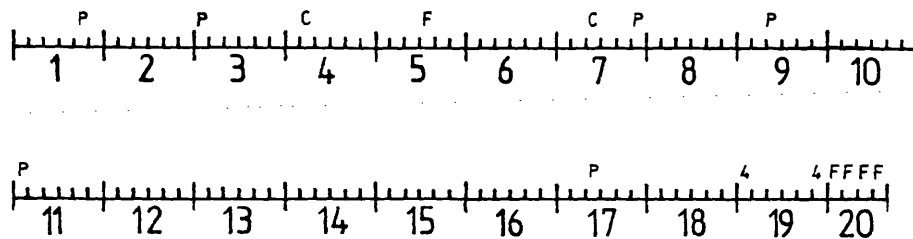


Figure 30

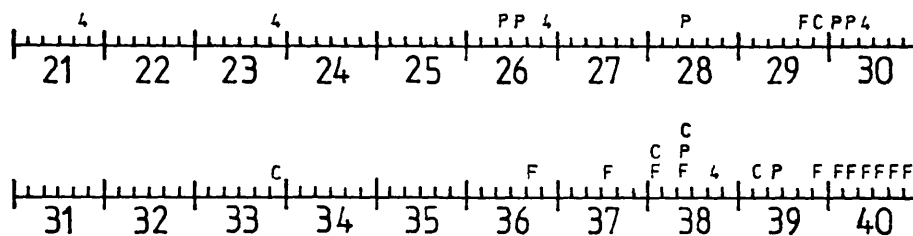
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ac2(b)II

C - copia / F - F element / 4 - 412 / P - P element

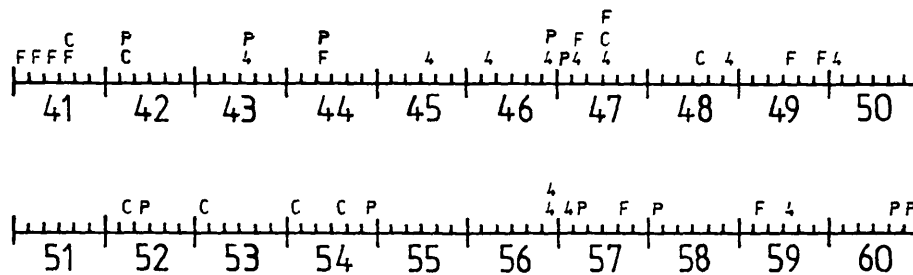
1=X



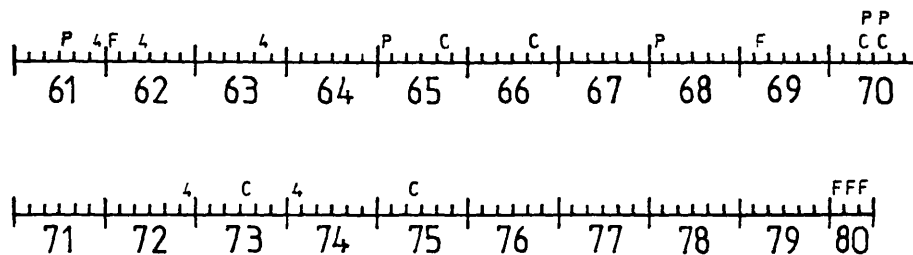
2L



2R



3L



3R

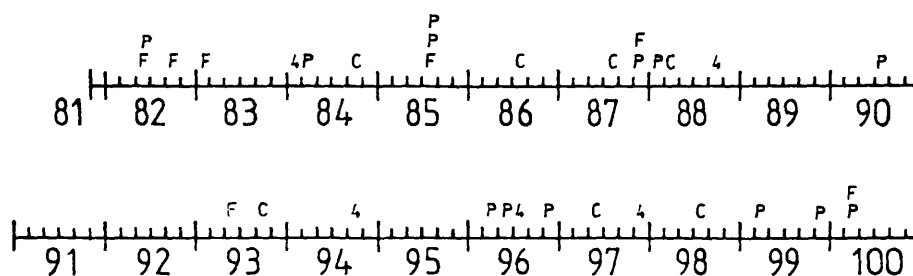
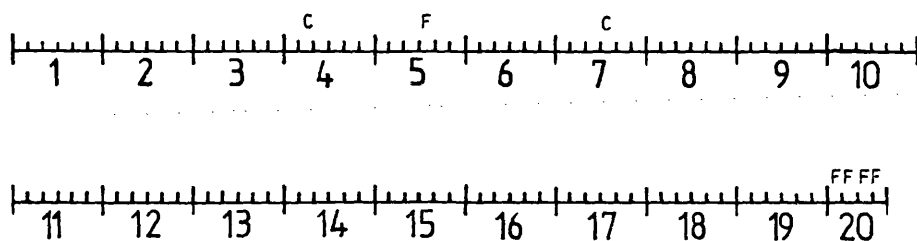


Figure 31

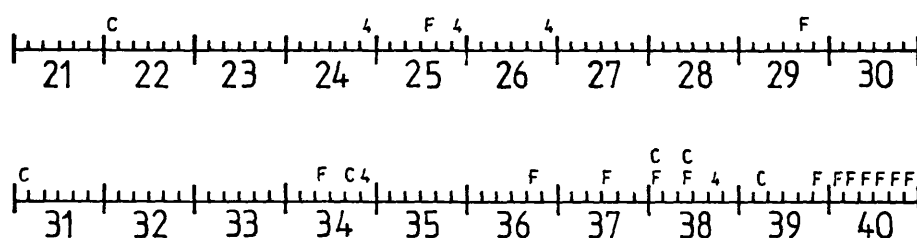
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline A c 3 (a)

C - copia / F - F element / 4 - 412

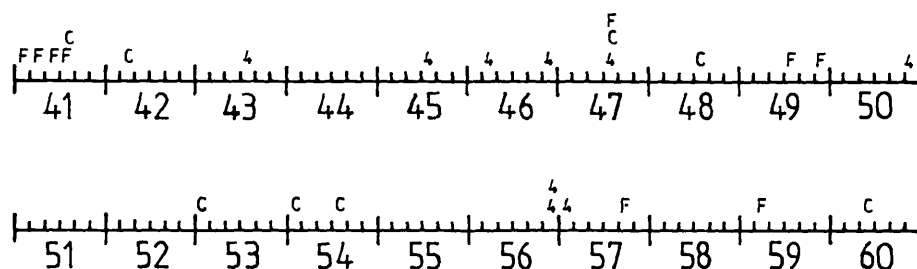
1=X



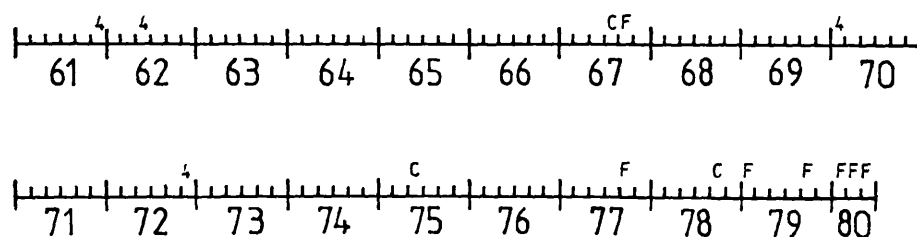
2L



2R



3L



3R

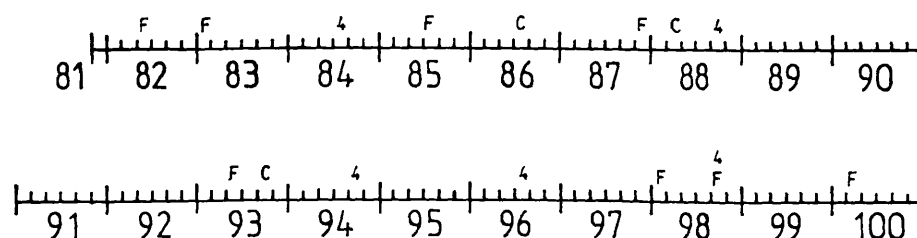
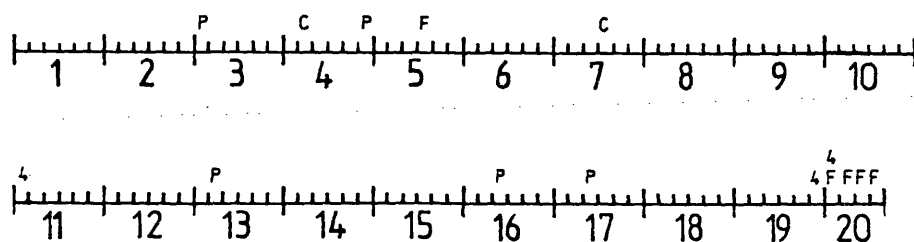


Figure 32

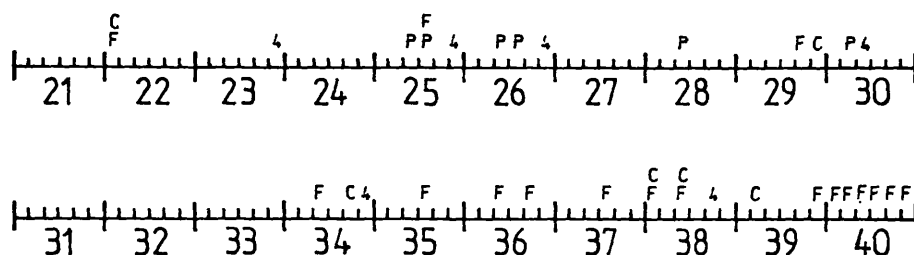
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ac3(b)

C - copia / F - F element / 4 - 412 / P - P element

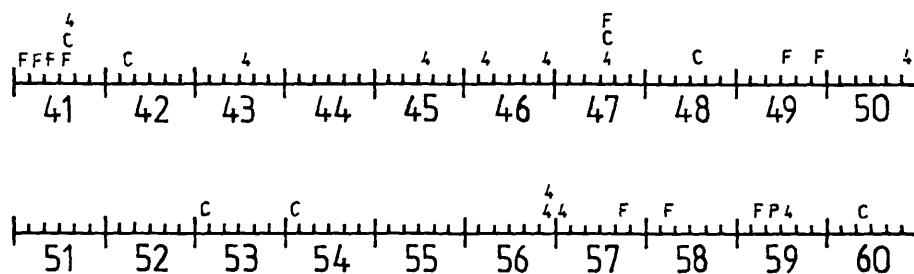
1=X



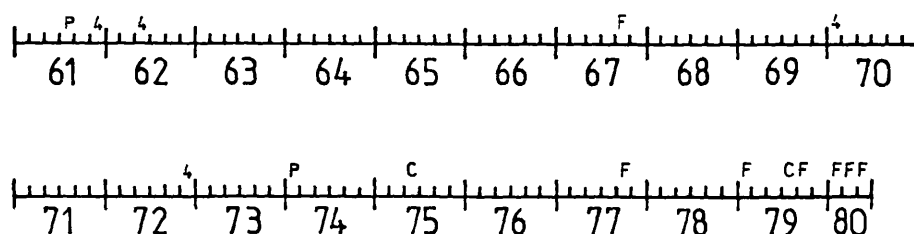
2L



2R



3L



3R

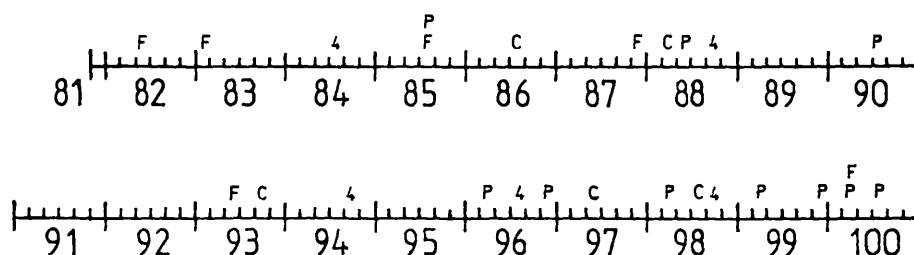
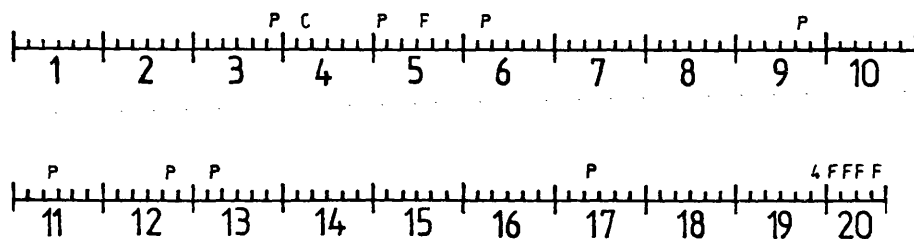


Figure 33

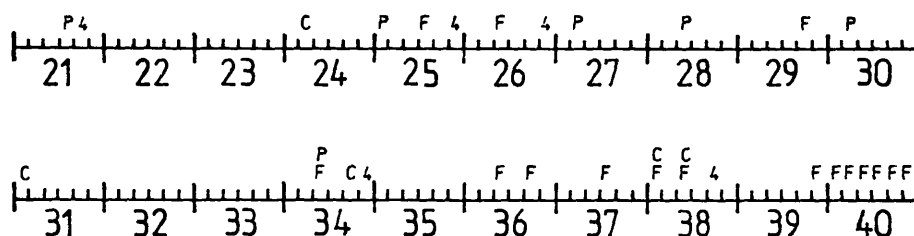
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ac4(a)

C - copia / F - F element / 4 - 412 / P - P element

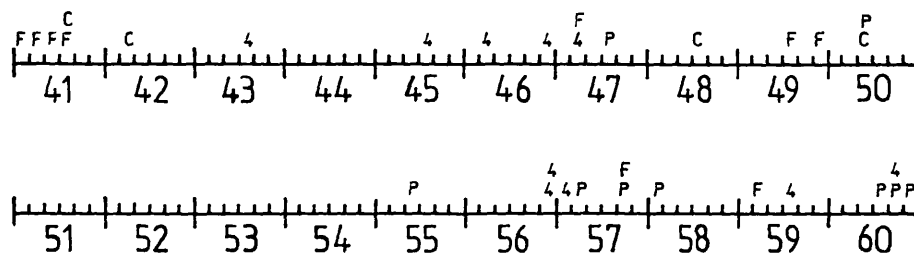
1=X



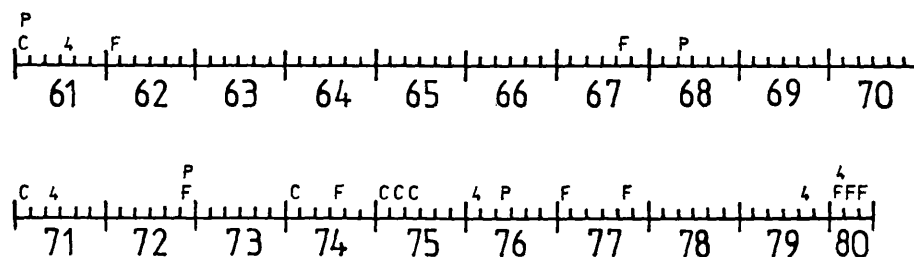
2L



2R



3L



3R

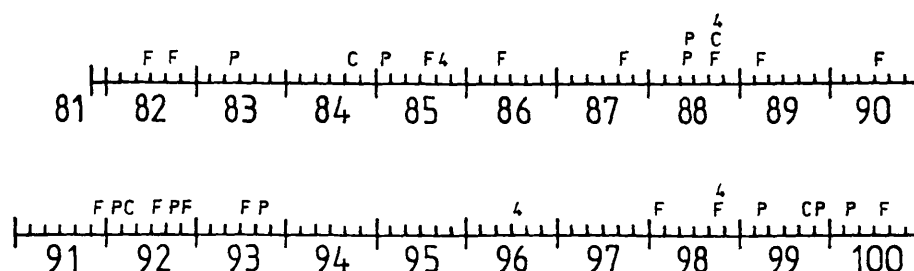
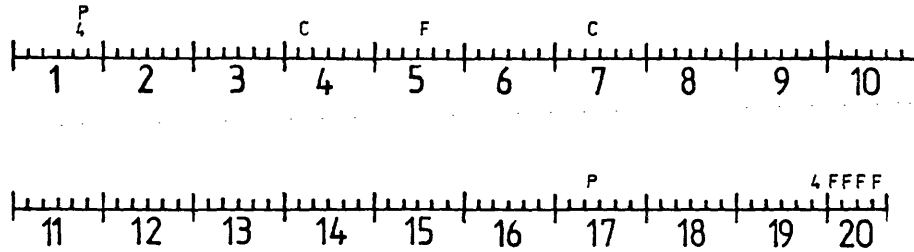


Figure 34

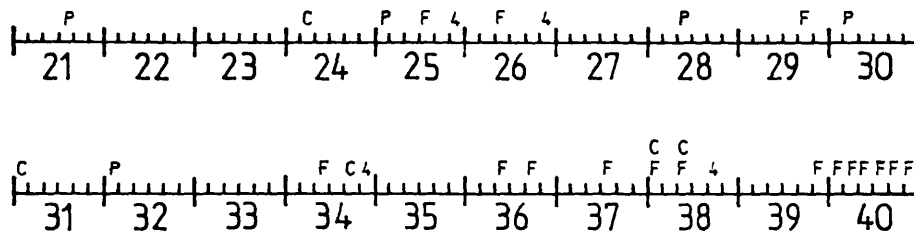
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ac 4 (b)

C - copia / F - F element / 4 - 412 / P - P element

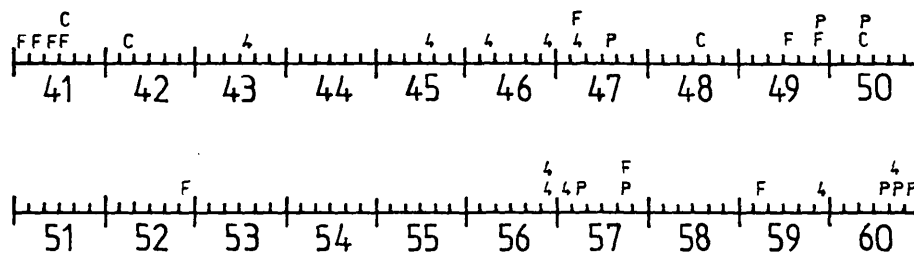
1=X



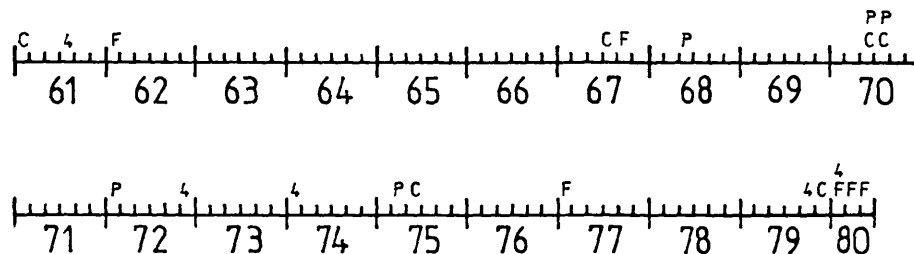
2L



2R



3L



3R

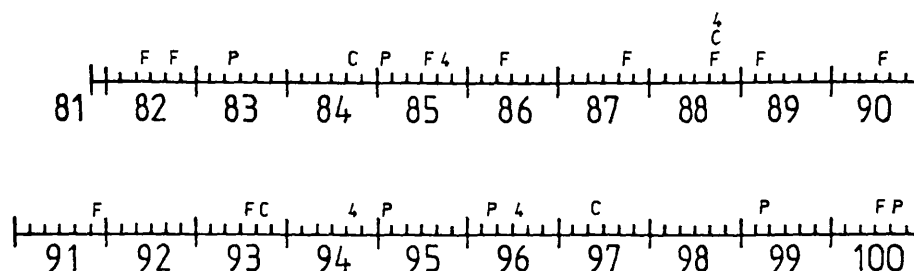
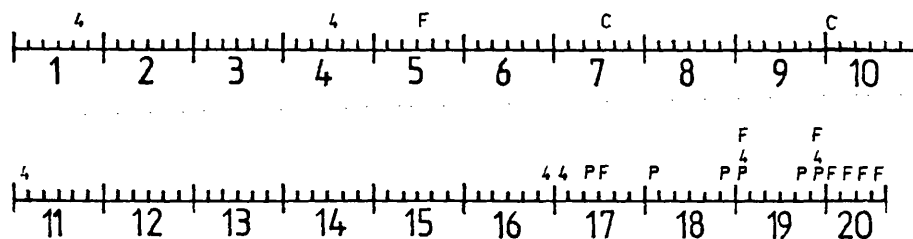


Figure 35

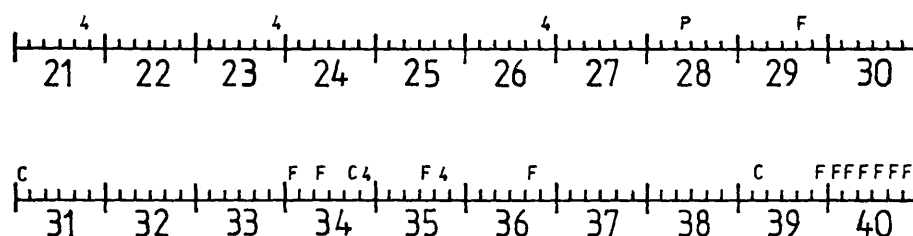
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ba 1

C - copia / F - F element / 4 - 412 / P - P element

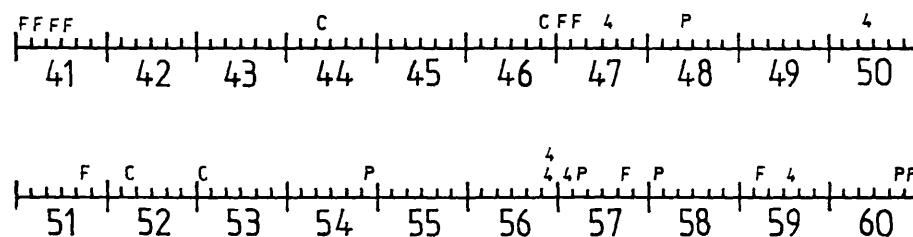
1=X



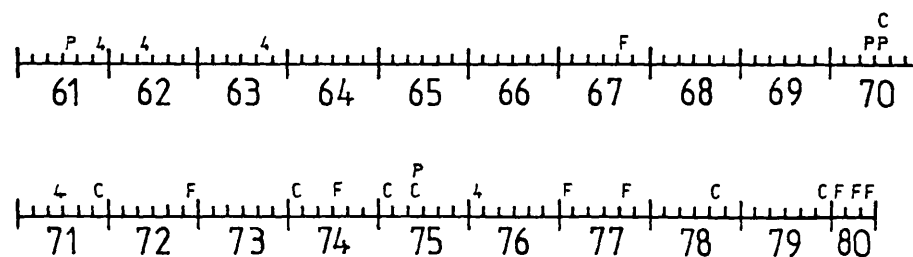
2L



2R



3L



3R

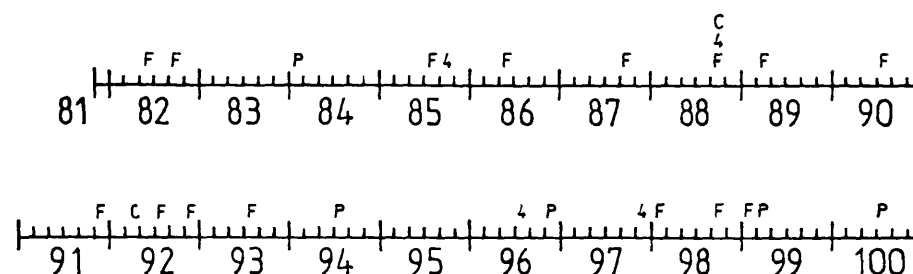
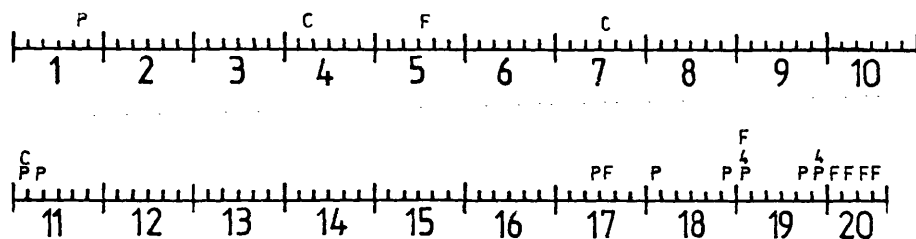


Figure 36

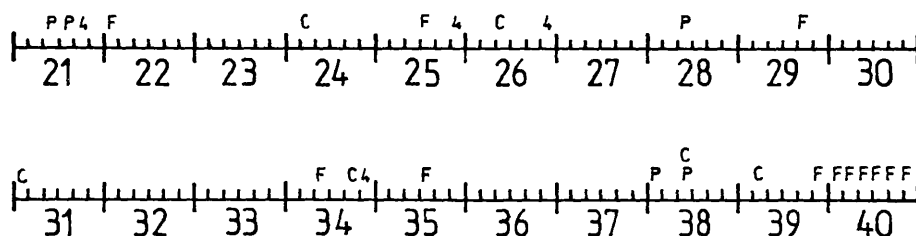
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Ba2

C - copia / F - F element / 4 - 412 / P - P element

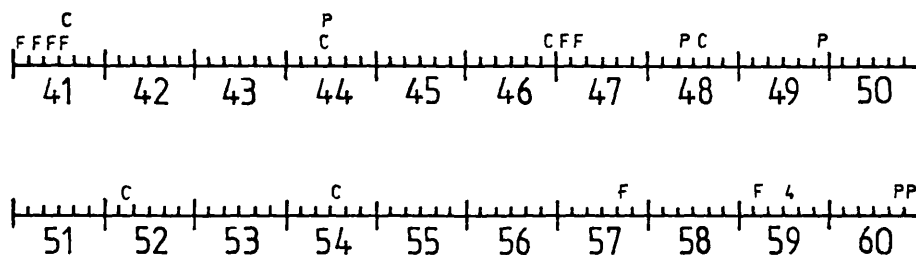
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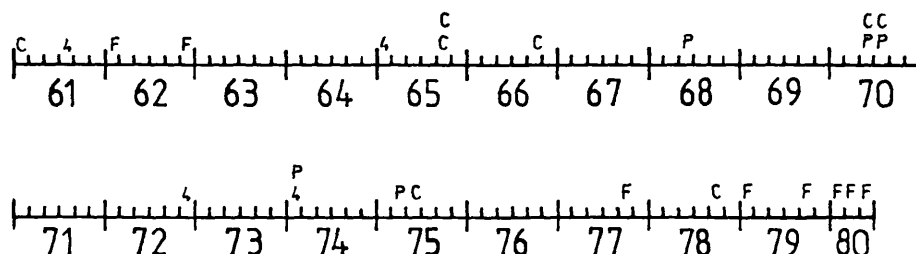
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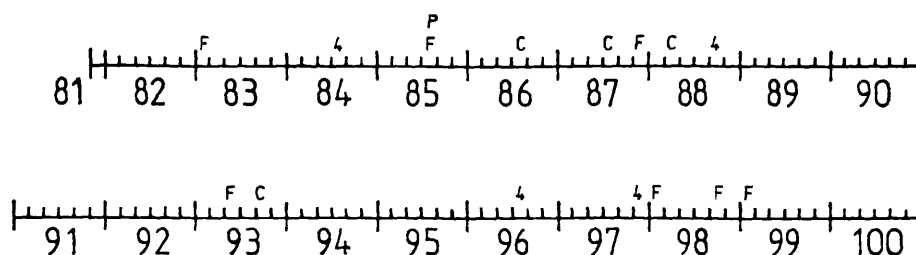
2R



3L

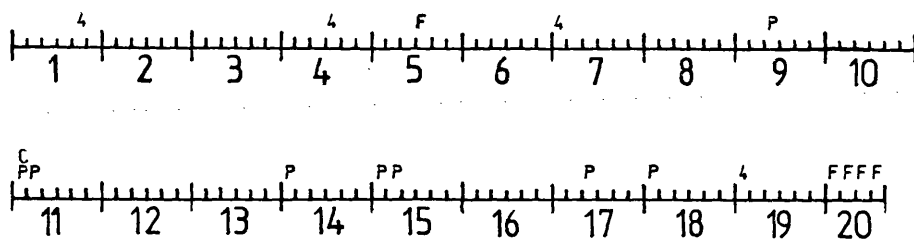


3R

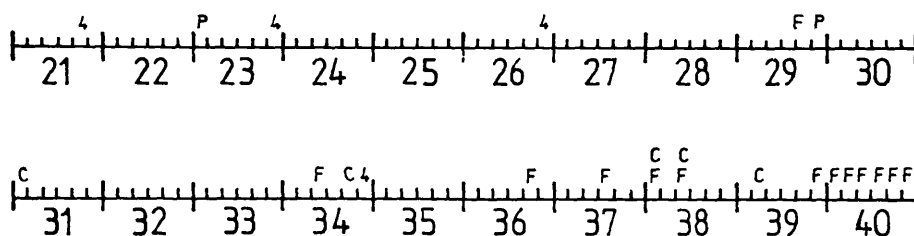


Positions of transposable element insertions in the chromosomes of *D. melanogaster* subline B b 1

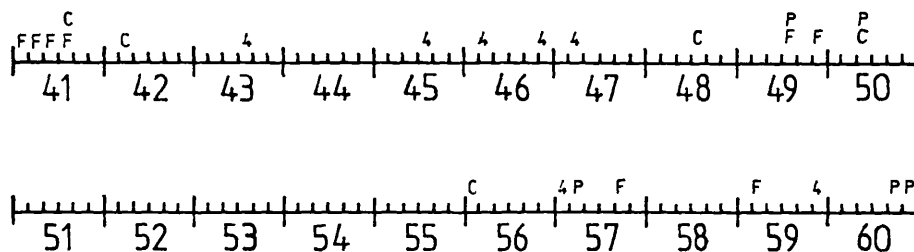
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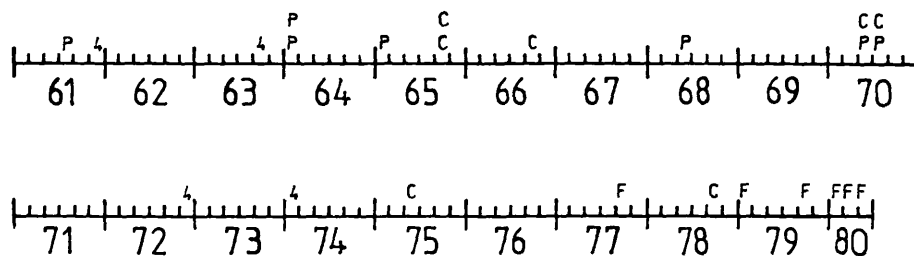
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2R



3L



3R

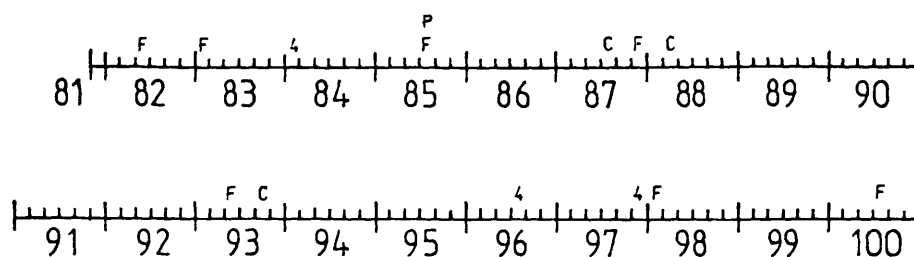
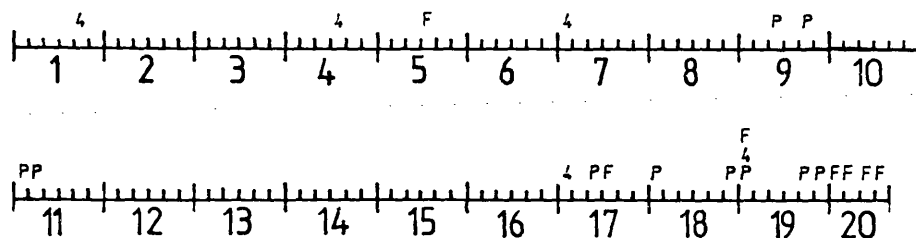


Figure 38

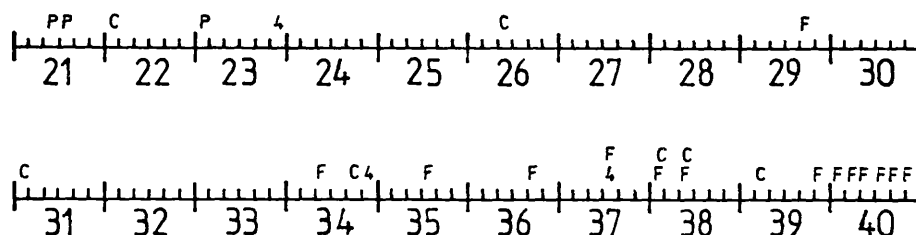
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Bb2

C - copia / F - F element / 4 - 412 / P - P element

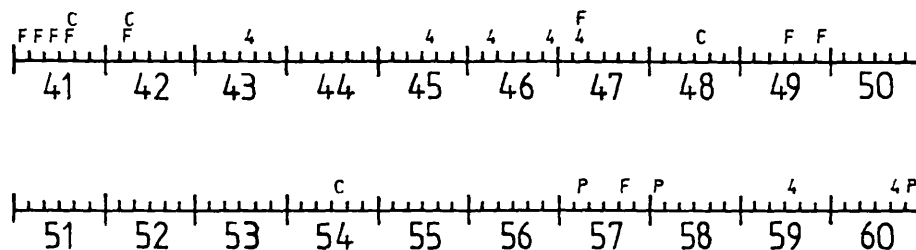
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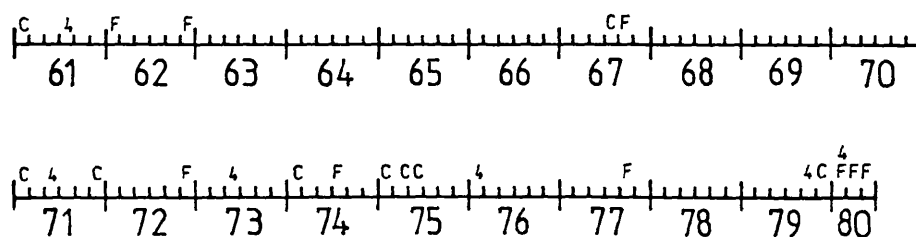
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2R



3L



3R

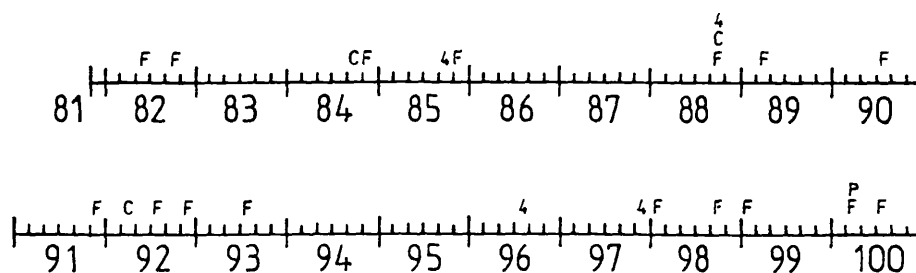
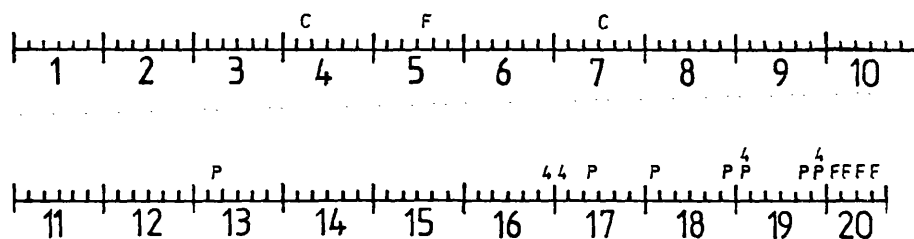


Figure 39

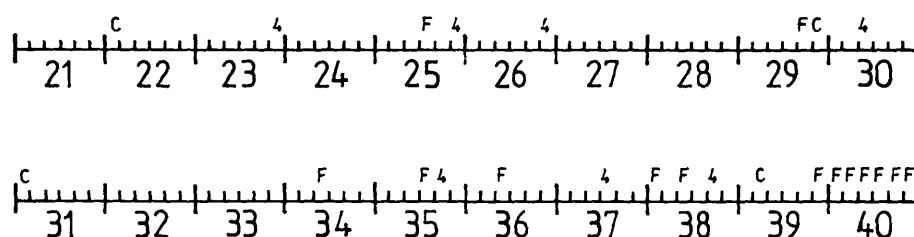
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Bc 1

C - copia / F - F element / 4 - 412 / P - P element

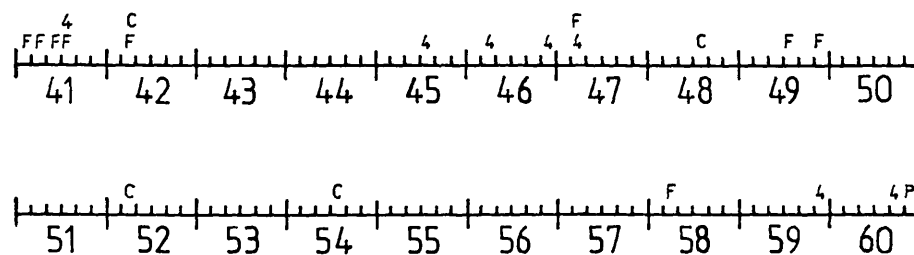
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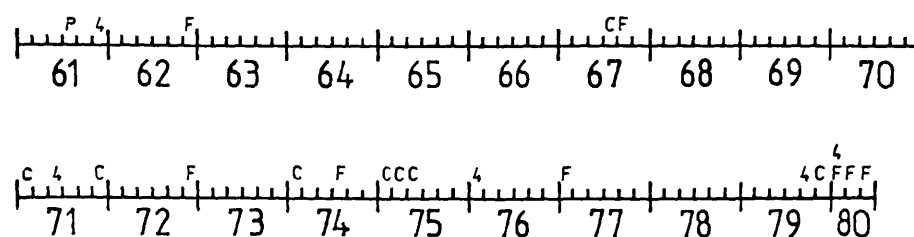
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2R



3L



3R

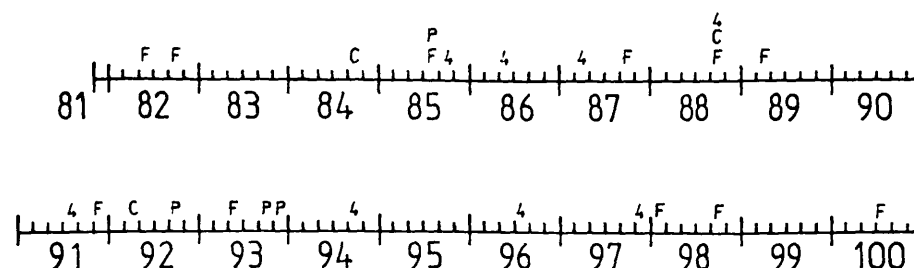
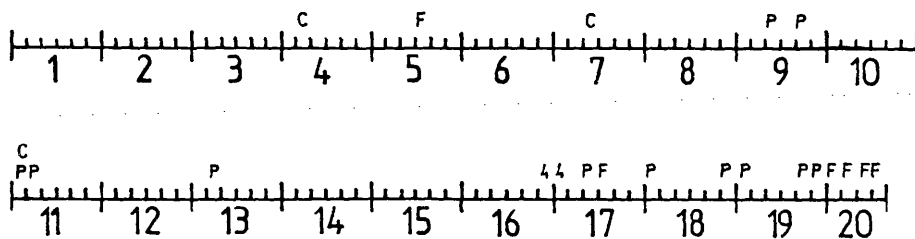


Figure 40

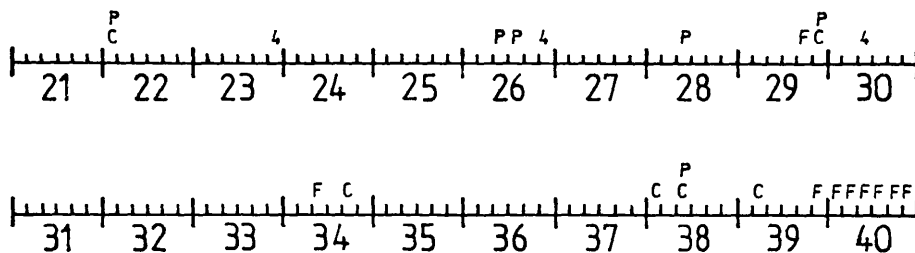
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline B c 2

C - copia / F - F element / 4 - 412 / P - P element

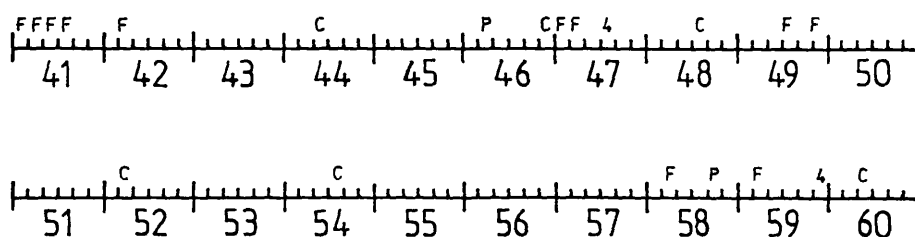
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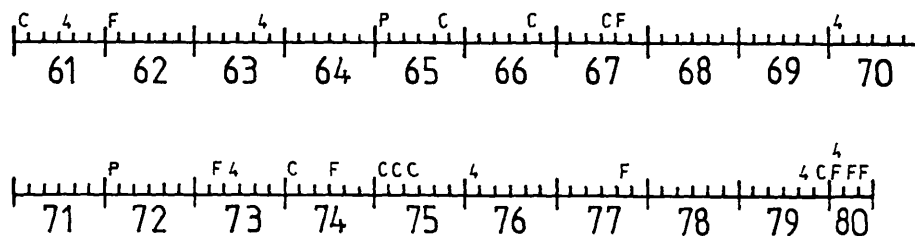
2L



2R



3L



3R

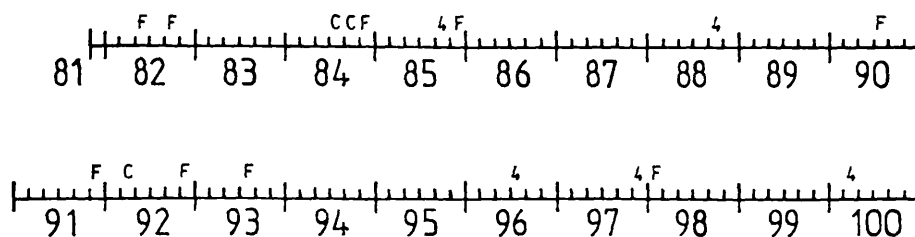
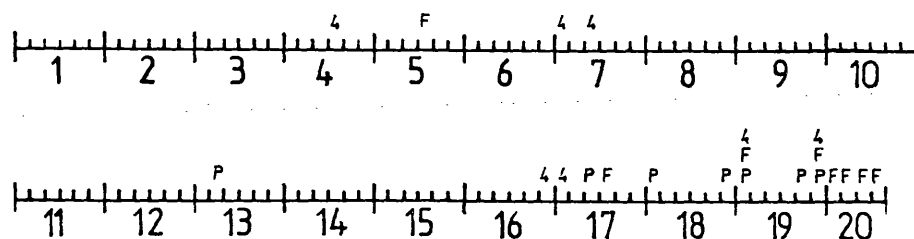


Figure 41

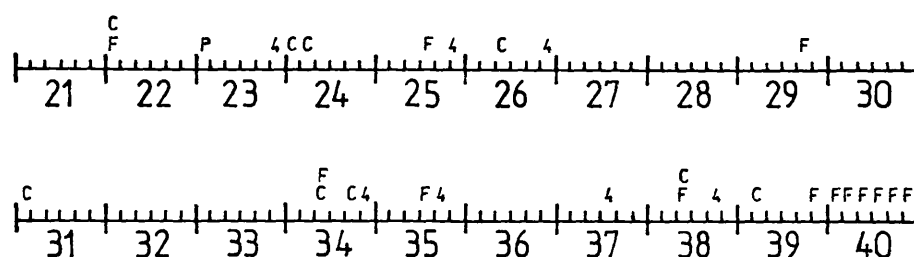
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Bd 1

C - copia / F - F element / 4 - 412 / P - P element

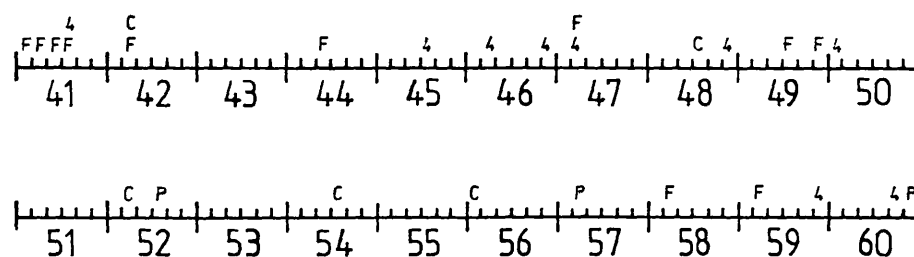
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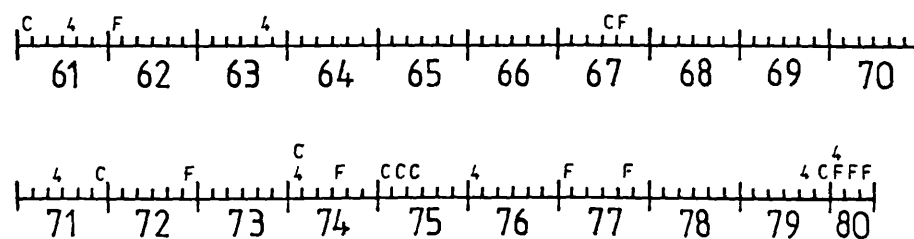
2L



2R



3L



3R

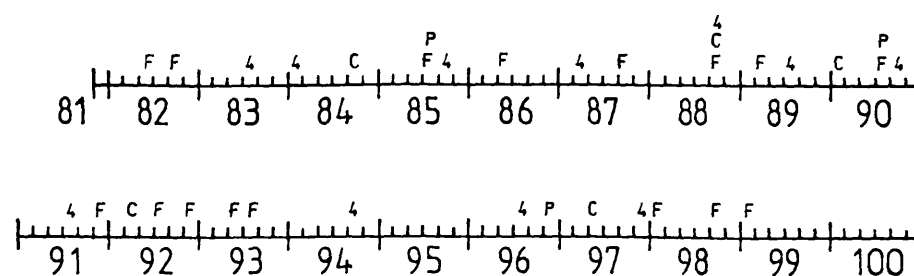
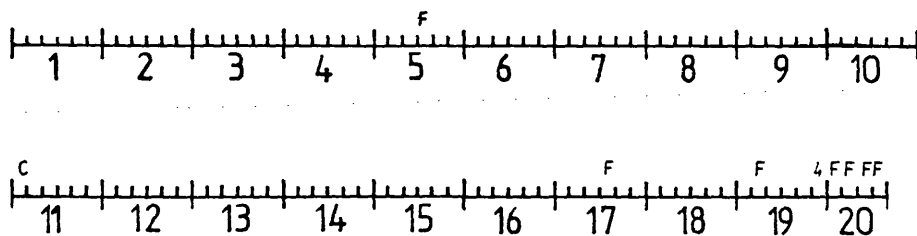


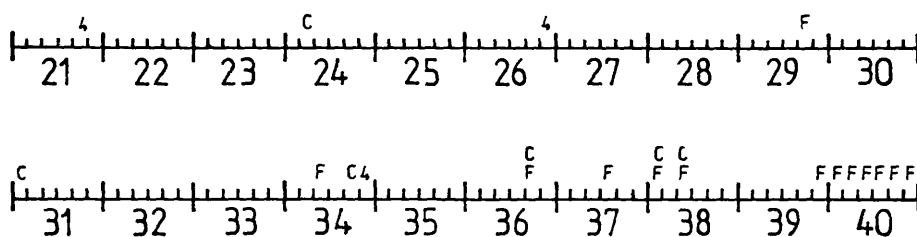
Figure 42
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Bd2

C - copia / F - F element / 4 - 412

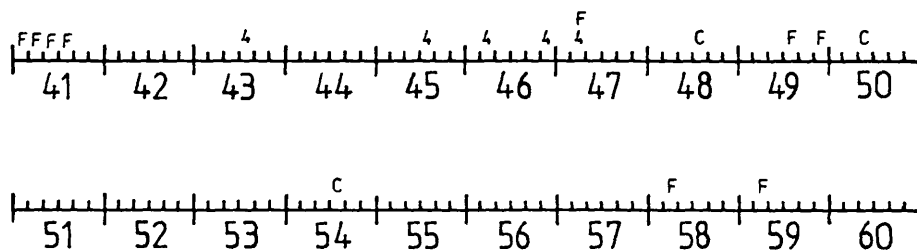
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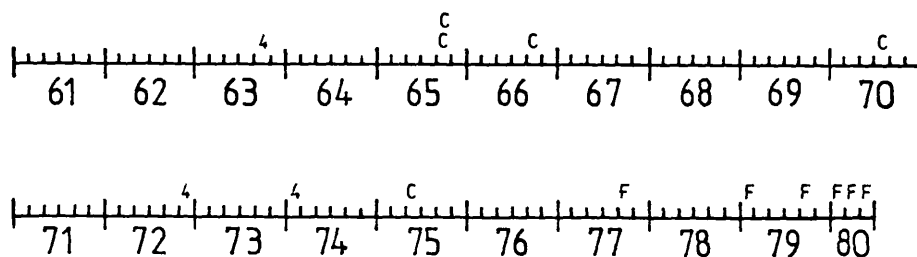
2L



2R



3L



3R

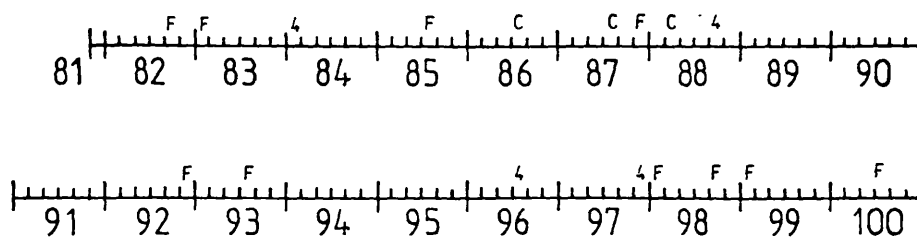
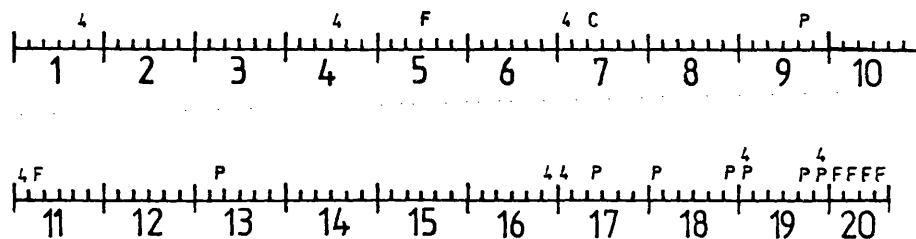


Figure 43

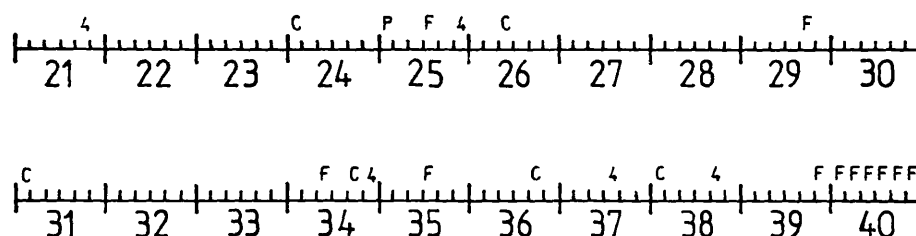
Positions of transposable element insertions in the chromosomes
of D. melanogaster subline B d 3

C - copia / F - F element / 4 - 412 / P - P element

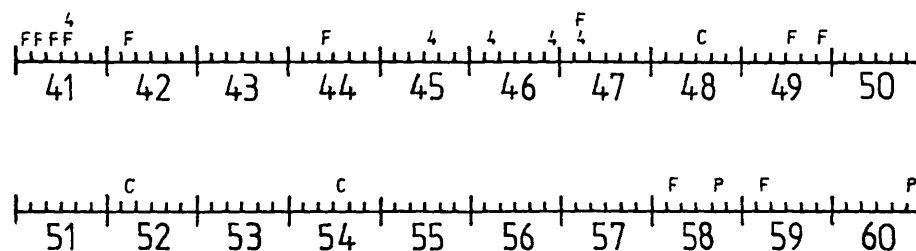
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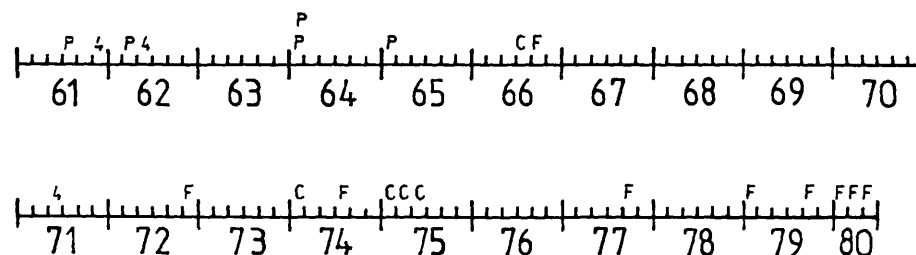
2L



2R



3L



3R

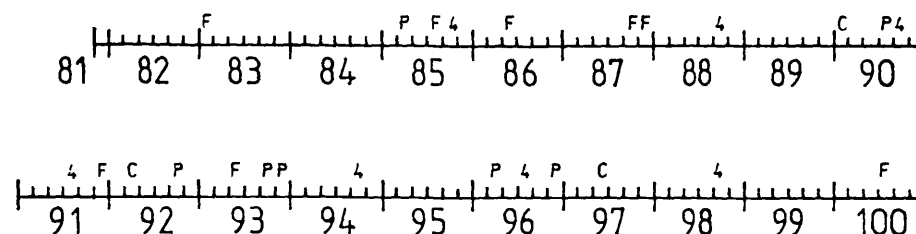
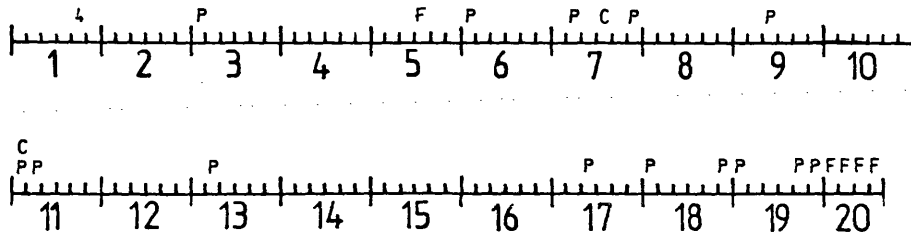


Figure 44

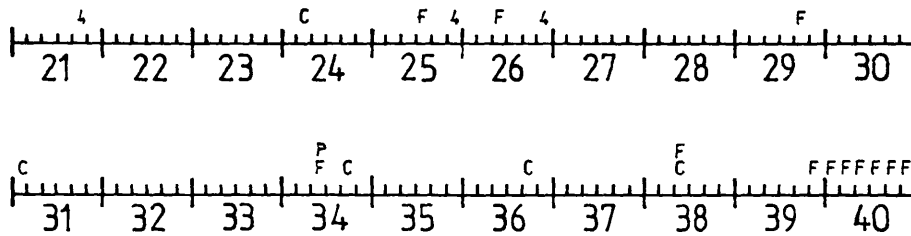
**Positions of transposable element insertions in the chromosomes
of D. melanogaster subline Be**

C – copia / F – F element / 4 – 412 / P – P element

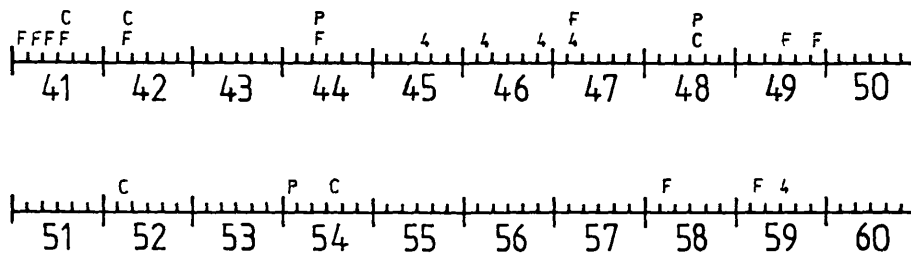
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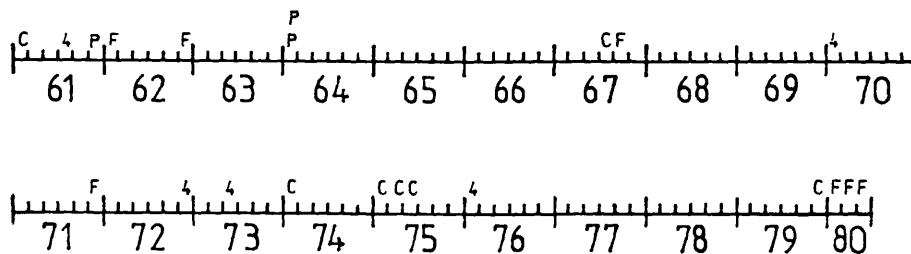
2L



2R



3L



3R

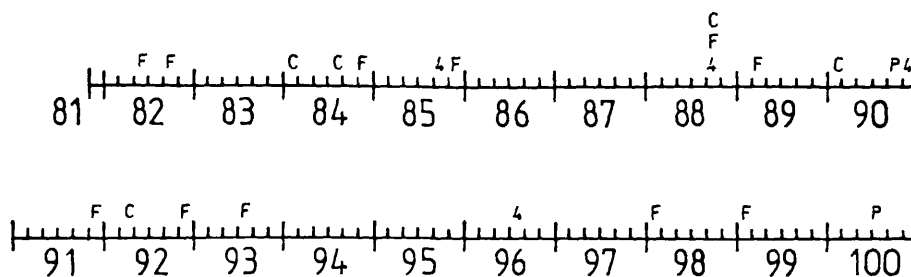
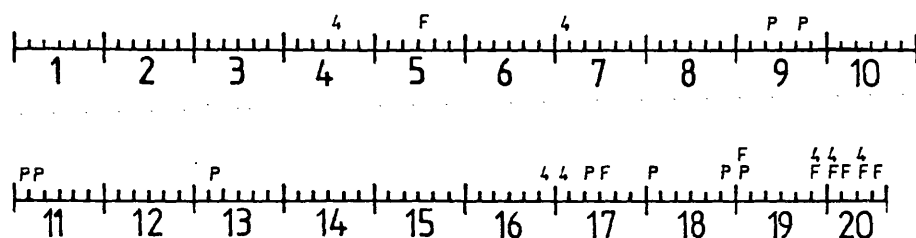


Figure 45

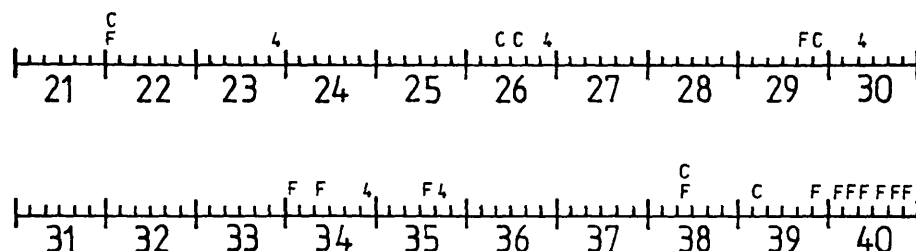
Positions of transposable element insertions in the chromosomes of *D. melanogaster* subline Bf

C - copia / F - F element / 4 - 412 / P - P element

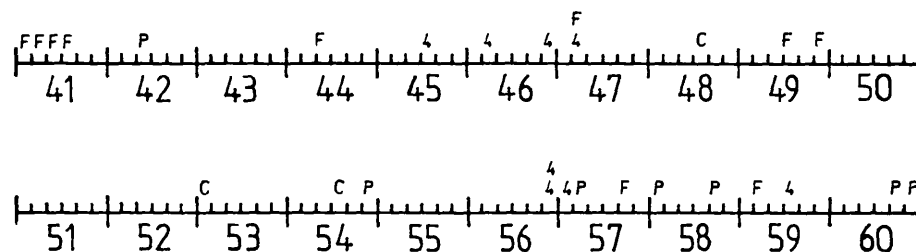
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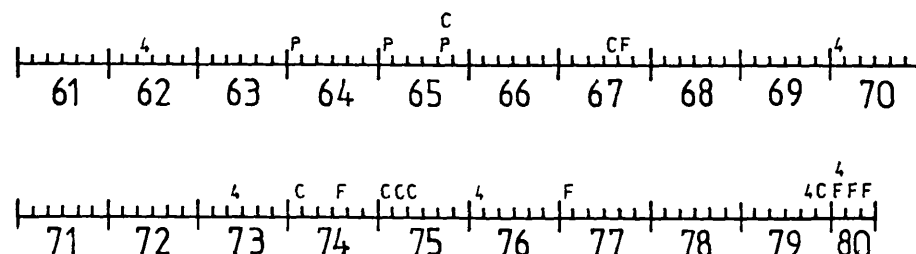
2L



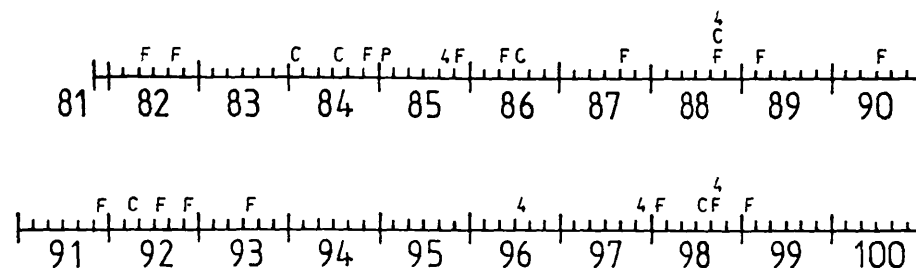
2R



3L



3R



New sites shared only by sublines derived from a single F3 generation line, however, probably represent transposition events occurring during the course of the experiment.

3.6 Has P-M Hybrid Dysgenesis Occurred In The Dysgenic Lines?

Lines were scored for P element insertion sites to see whether P-M hybrid dysgenesis had taken place in the dysgenic cross. 121 new sites were observed in the 25 dysgenic lines analysed (figure 4 legend) whilst only 5 new sites were scored for the 10 control lines (figure 5 legend). The former value is probably an underestimate since the finite number of scorable sites on the chromosomes using this method would result in repeated transpositions to indistinguishable sites in different lines. Such sites would then be discounted in the data analysis.

Since sublines taken early on are likely to become fixed for different sites of transposable elements created early in the experiment, the number of F1 gametes examined will be greater than the number of F2 vials. This number will, however, be less than the 25 sublines finally examined for the P element. The expected number of different F1 gametes scored in the 25 lines can be calculated to be 14.60 in the dysgenic experiment (see Appendix). In the control the number is simply 10, the number of lines examined. These numbers (14.60 and 10) are used to calculate transposition rates for the elements and also the expected proportions of new sites in the two sets of lines in a χ^2 test. One can estimate transposition rates of approximately 2/5th of a copy per original copy for the dysgenic cross and 1/40th of a copy per copy for the controls

(figure 46), over the whole 15 generations (based upon an assumption of selective neutrality of new transposable element sites), thus giving a 17-fold increase in P element transposition rate from the control to the dysgenic cross. A χ^2 test, based upon a ratio of expectation of 14.60 to 10, revealed that the 121 new sites observed in the dysgenic lines were significantly different at the 1% level from the 5 sites of the controls (figure 47), indicating that hybrid dysgenesis had indeed taken place.

One can also calculate the average number of P transposable element sites in the dysgenic and nondysgenic lines. On crossing an M and P strain the number of P elements present in the resulting fly would be expected to be approximately half of those present in the P strain. If transposition occurs at a high rate one might expect the number of P elements to increase back to a level close to the original number present in the parental P strain. The P strain, Harwich, used in the experiment was estimated to have 36 P transposable elements per fly, and thus one may expect the progeny of a cross to an M strain to have approximately 18 P elements. On analysis, it was calculated that the average nondysgenic subline possessed 20 P element sites, whilst the dysgenic lines were found to have, on average, 32 elements (figure 48). Thus again, hybrid dysgenesis has been seen to have occurred in the experimental lines.

3.7 Have copia, 412 And F Transposable Elements Been Mobilised In The Hybrid Dysgenic Cross?

The lines were scored for copia, 412 and F transposable

Figure 46: Estimation of P element transposition rates. For convenience the nondysgenic control lines were used to calculate the approximate number of original sites in the lines. The assumption was made that no new insertion sites occurred in the chromosome or chromosome 4. The total number of sites refers to a summation of the number of sites in each subline, and not the number of sites present at different locations in all the sublines.

$$\begin{aligned} \text{Number of original sites} &= \frac{\text{Total number of sites} - \text{Total number of new sites}}{\text{Number of lines examined}} \\ &= \frac{202 - 5}{10} = 19.7 \end{aligned}$$

$$\text{Transposition rate} = \frac{\text{Number of new sites}}{\text{Expected number of genomes in the lines examined}} \times \frac{1}{\text{Number of original sites}}$$

DYSGENIC CROSS

$$\text{Transposition rate} = \frac{121}{14.6} \times \frac{1}{19.7} = 0.421 \text{ copy per original copy}$$

NONDYSGENIC CROSS

$$\text{Transposition rate} = \frac{5}{10} \times \frac{1}{19.7} = 0.025 \text{ copy per original copy}$$

Figure 47: χ^2 test to determine the significance between the number of new P element sites in the dysgenic lines and those in the controls.

$$E_d = \frac{\text{Total number of new sites} \times \text{Expected number of genomes in the dysgenic lines}}{\text{Total expected number of genomes}}$$

$$E_c = \frac{\text{Total number of new sites} \times \text{Expected number of genomes in the control lines}}{\text{Total expected number of genomes}}$$

O_d = Observed number of new sites in the dysgenic lines

O_c = Observed number of new sites in the control lines

$$\chi^2 = \frac{(E_d - O_d)^2}{E_d} + \frac{(E_c - O_c)^2}{E_c}$$

$$E_d = \frac{126 \times 14.6}{24.63} = 74.69$$

$$E_c = \frac{126 \times 10}{24.63} = 51.16$$

$$O_d = 121$$

$$O_c = 5$$

$$\chi^2 = \frac{(74.69 - 121)^2}{74.69} + \frac{(51.16 - 5)^2}{51.16} = 28.71 + 41.65 = 70.36$$

For 1 degree of freedom at the 1% level this value is significant. Thus the observed number of new dysgenic and control line P element sites are significantly different.

.....

Figure 48: Estimation of the average number of P element sites in the dysgenic and nondysgenic lines examined.

.....

$$\text{Average number of sites} = \frac{\text{Total number of sites}}{\text{Number of lines examined}}$$

DYSGENIC LINES

$$\text{Average number of sites} = \frac{802}{25} = 32.1$$

NONDYSGENIC LINES

$$\text{Average number of sites} = \frac{202}{10} = 20.2$$

element sites, 27 dysgenic lines being analysed (figure 4) and 11 control lines (figure 5). In total, 14 new insertion sites were observed in the experimental lines, of which 5 were seen for each of the elements copia and 412, and 4 for the F elements (table 1 and figures 49-66). No new sites, however, were observed in the controls. The expected number of F1 gametes scored in the 27 lines of the hybrid dysgenic crosses was calculated to be 14.91 (see Appendix), and this value was used in a χ^2 test based on a ratio of expectation of 14.91 to 11 (the number of nondysgenic lines). Taking the different transposable element families individually, the rates of movement between the dysgenic and control lines were not significantly different. However, the difference between the total number of new sites in the dysgenic lines and the controls was found to be significantly different at the 1% level (figure 67), indicating that altogether, the transposition rate of the elements was elevated under the hybrid dysgenic conditions. Transposition rates of approximately 1/65th of a copy per original copy for copia, 1/73rd for 412, and 1/186th for the F element were calculated over the 15 generations (figure 68).

By examining the new insertion sites shared by sublines one can obtain some idea of when the transposition events occurred. The F element in region 31A of lines Ab2(b)IZ and Ab2(b)IIX can be determined to have transposed in the germ cells of an F3 generation fly or earlier, whilst the F and copia elements in region 33A of lines Ac1(a) and Ac1(b) transposed in the germ cells of an F1 fly. The copia elements in regions 33F and 73D of lines Ac2(b)I and Ac2(b)II underwent transposition at generation F10 or earlier, and the copia

Table 1: New insertion sites obtained for the transposable elements F, copia and 412. The characters given refer to the chromosome regions in which the transposable element sites were found. 4 new sites were observed for the F element, 5 for copia and 5 for 412.

Element	<u>Drosophila melanogaster</u> line											
	Aa1	Aa2	Ab2(b)IX	Ab2(b)IZ	Ab2(b)IIX	Ab2(b)IIY	Ac1(a)	Ac1(b)	Ac2(b)I	Ac2(b)II	Ac4(b)	
F	-	-	-	31A 74A	31A	-	33A	33A	-	-	52F	
<u>copia</u>	30C	-	35D	35D	-	-	33A	33A	33F 73D	33F 73D	-	
<u>412</u>	67A	52B	-	48C	-	42B	-	-	-	-	-	
67D												

Figure 49: In situ hybridisation of the F transposable element probe to Ab2(b)IZ dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 31A.



Figure 50: In situ hybridisation of the F transposable element probe to Ab2(b)IZ dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 74A.

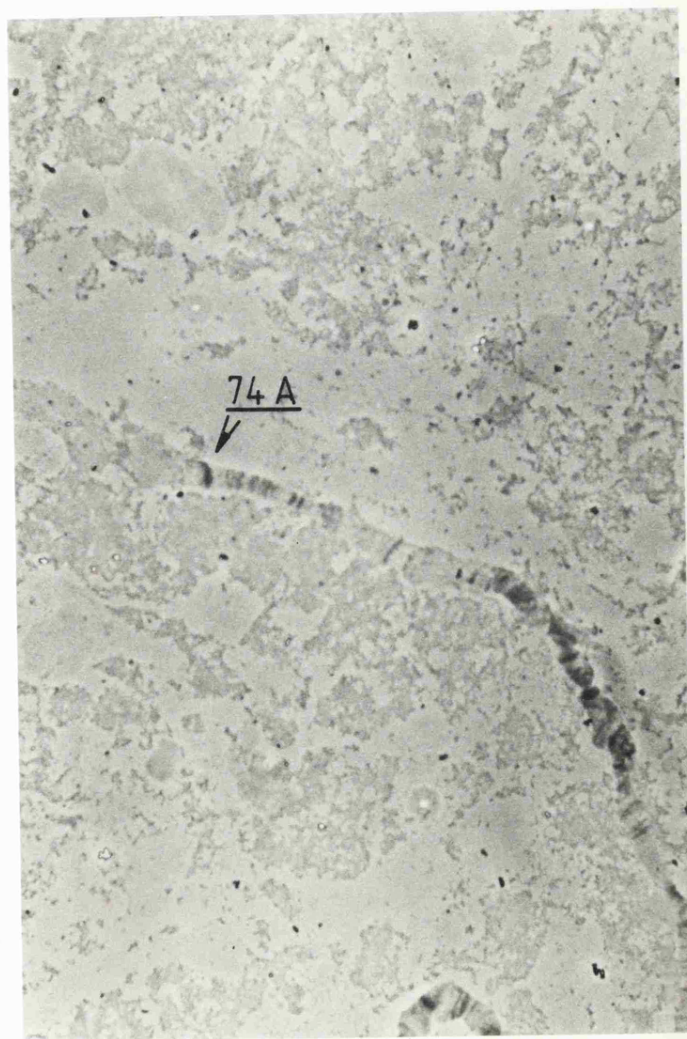


Figure 51: In situ hybridisation of the F transposable element probe to Ab2(b)IIX dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 31A.

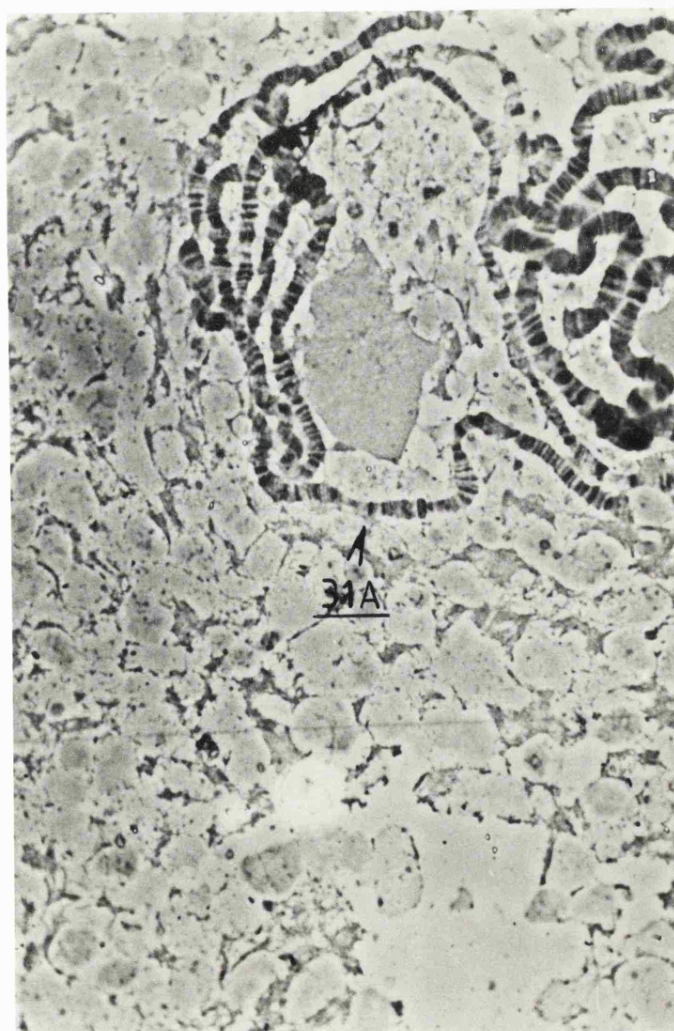


Figure 52: In situ hybridisation of the F transposable element probe to Acl(a) dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 33A.

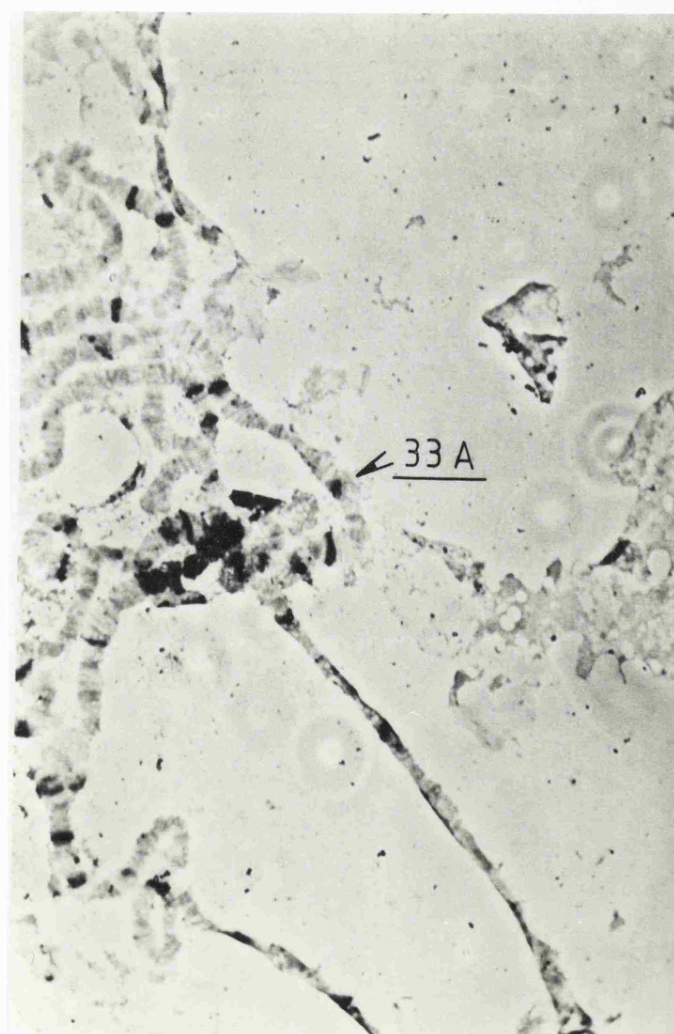


Figure 53: In situ hybridisation of the F transposable element probe to Ac1(b) dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 33A.

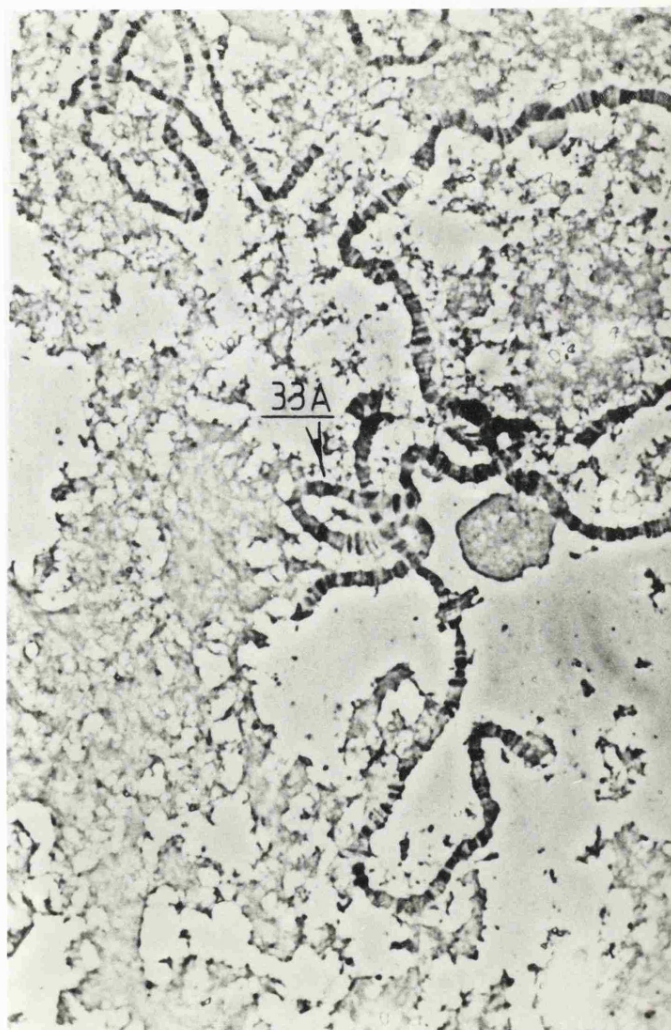


Figure 54: In situ hybridisation of the F transposable element probe to Ac4(b) dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 52F.

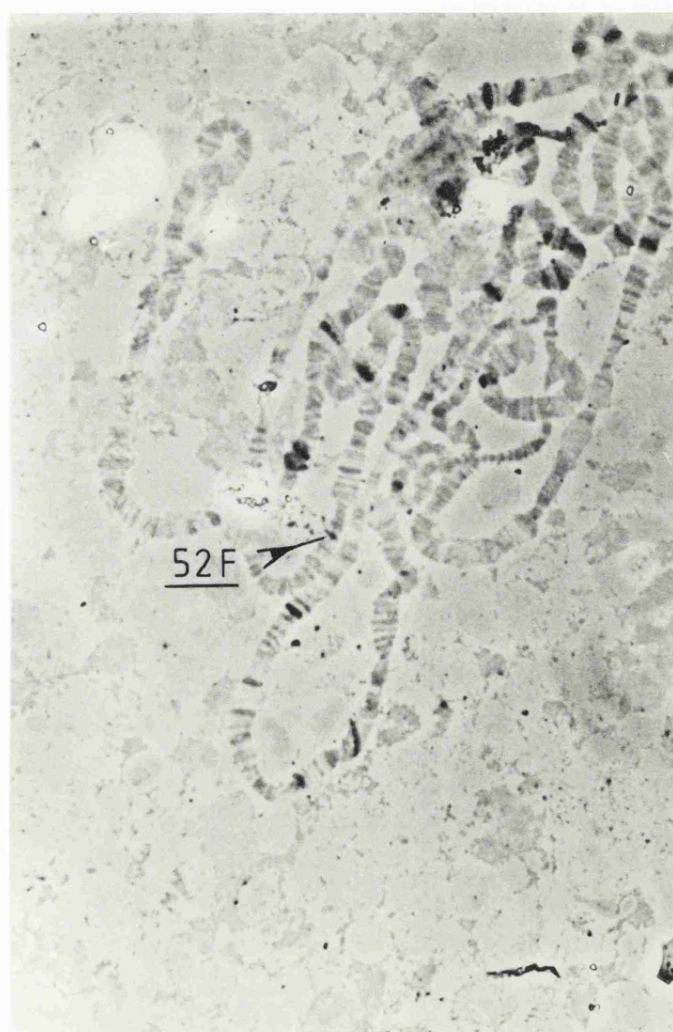


Figure 55: In situ hybridisation of the copia transposable element probe to Aa1 dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 30C.

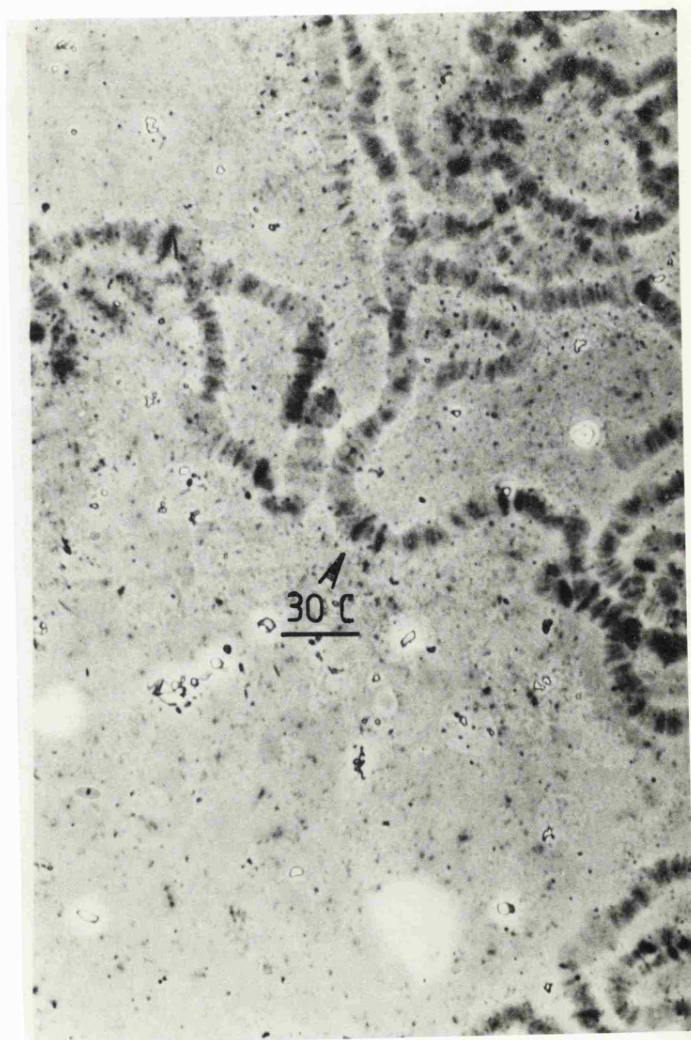


Figure 56: In situ hybridisation of the copia transposable element probe to Ab2(b)IX dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 35D.

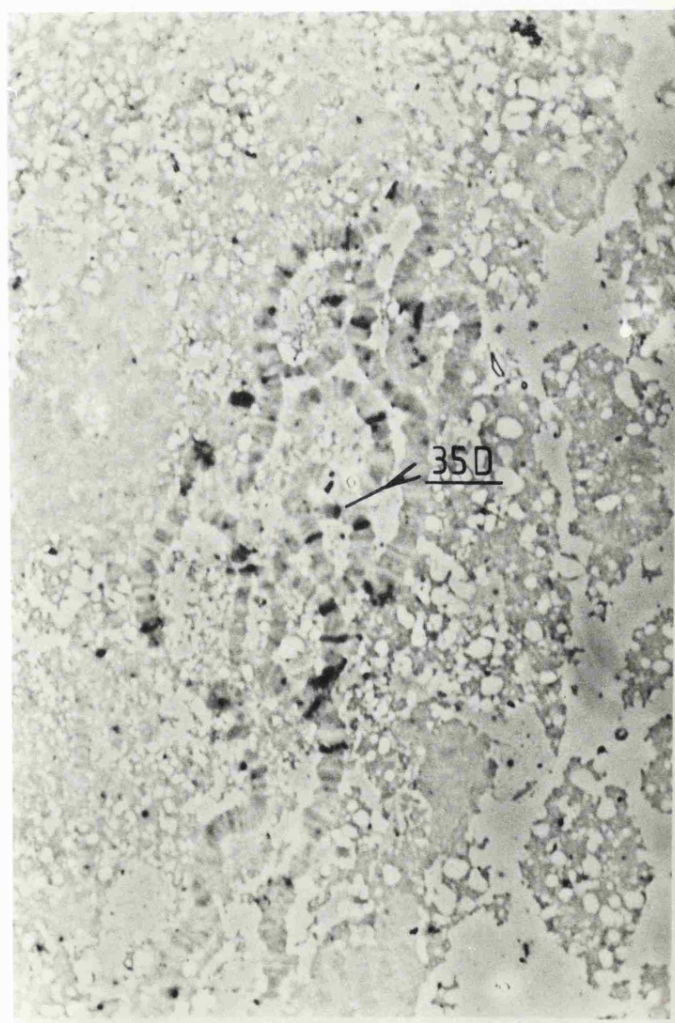


Figure 57: In situ hybridisation of the copia transposable element probe to Ab2(b)IZ dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 35D.

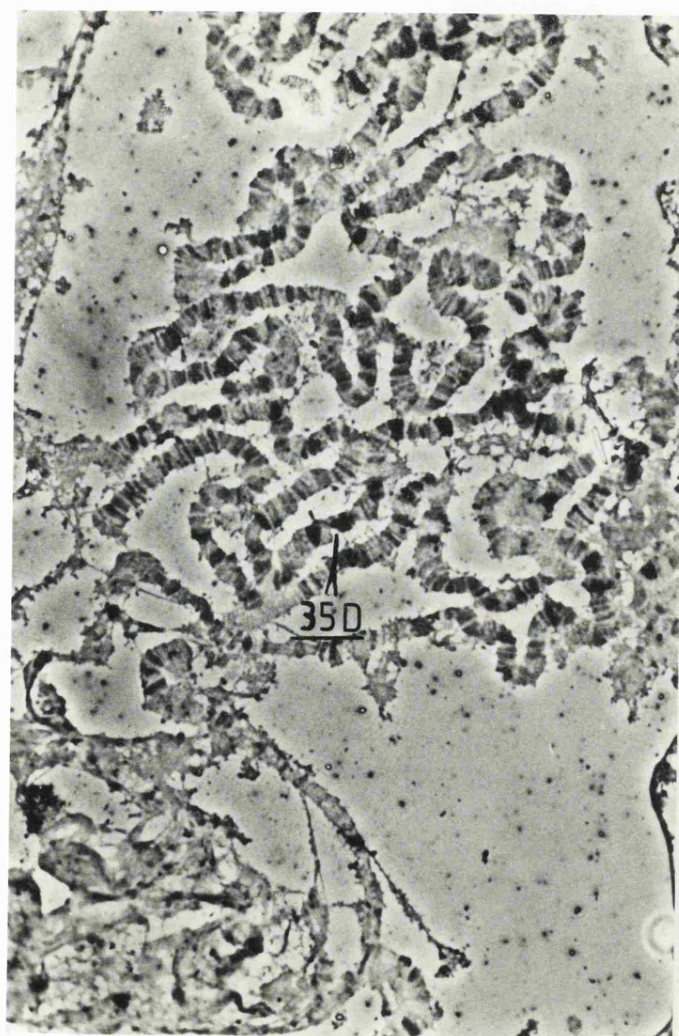


Figure 58: In situ hybridisation of the copia transposable element probe to Acl(a) dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 33A.

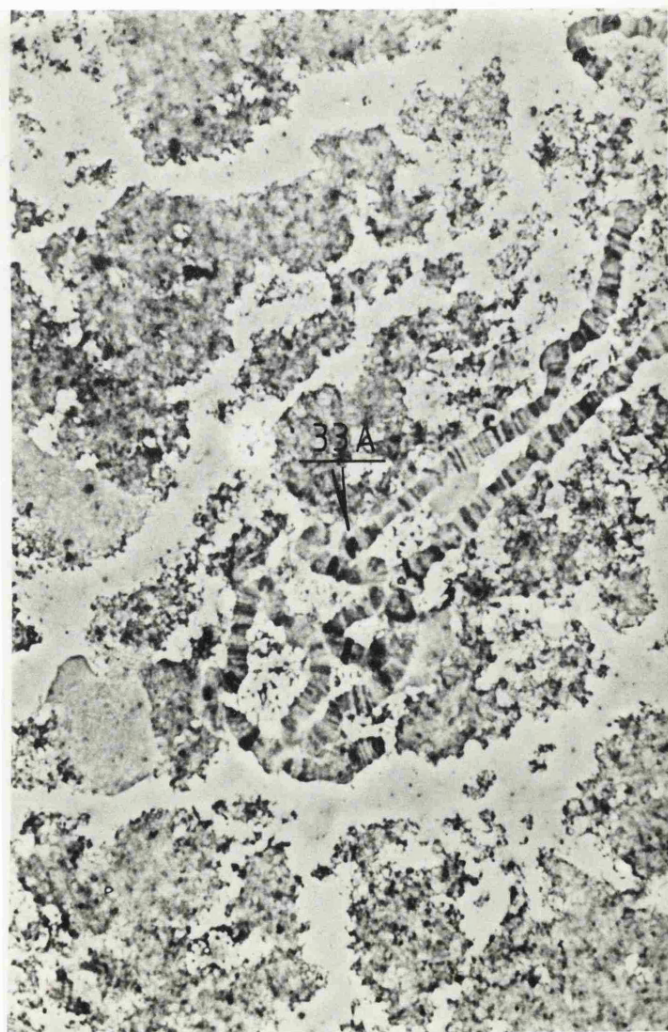


Figure 59: In situ hybridisation of the copia transposable element probe to Ac1(b) dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 33A.

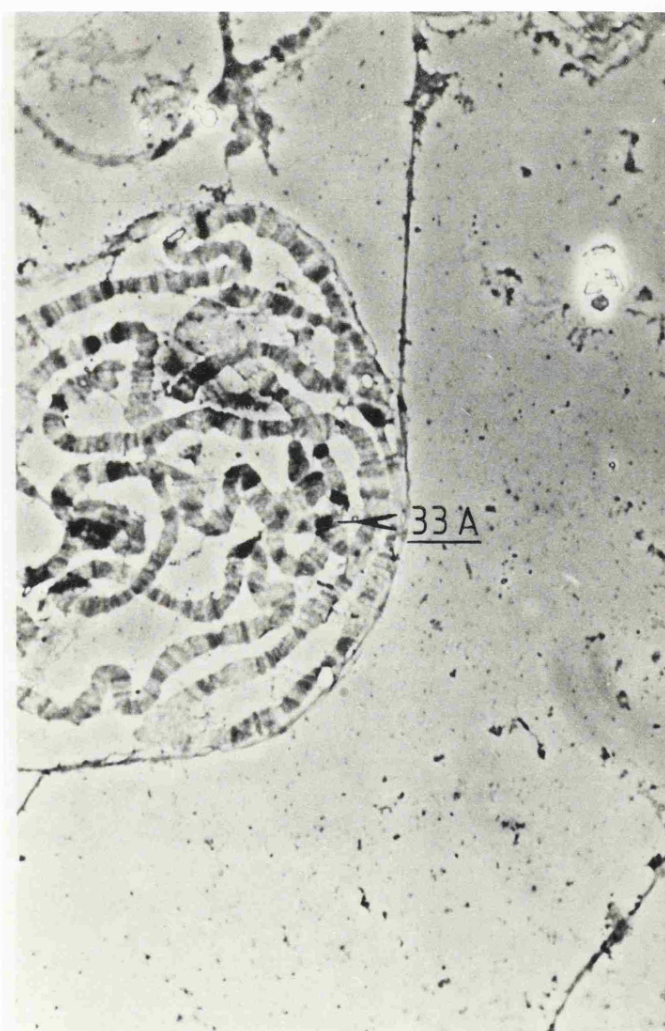


Figure 60: In situ hybridisation of the copia transposable element probe to Ac2(b)I dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 33F.

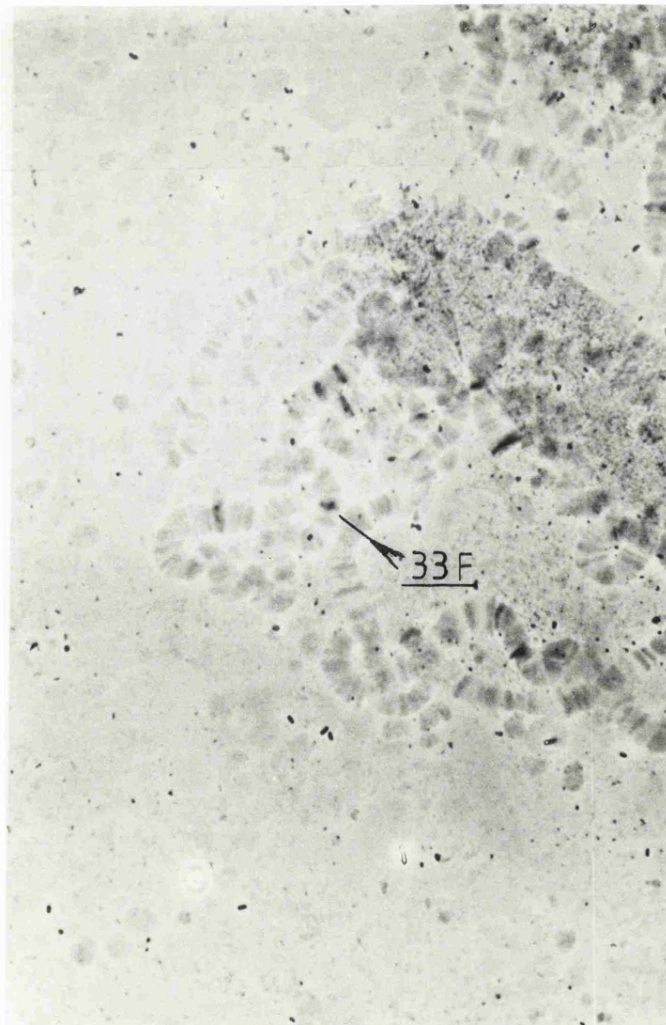


Figure 61: In situ hybridisation of the copia transposable element probe to Ac2(b)I dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 73D.

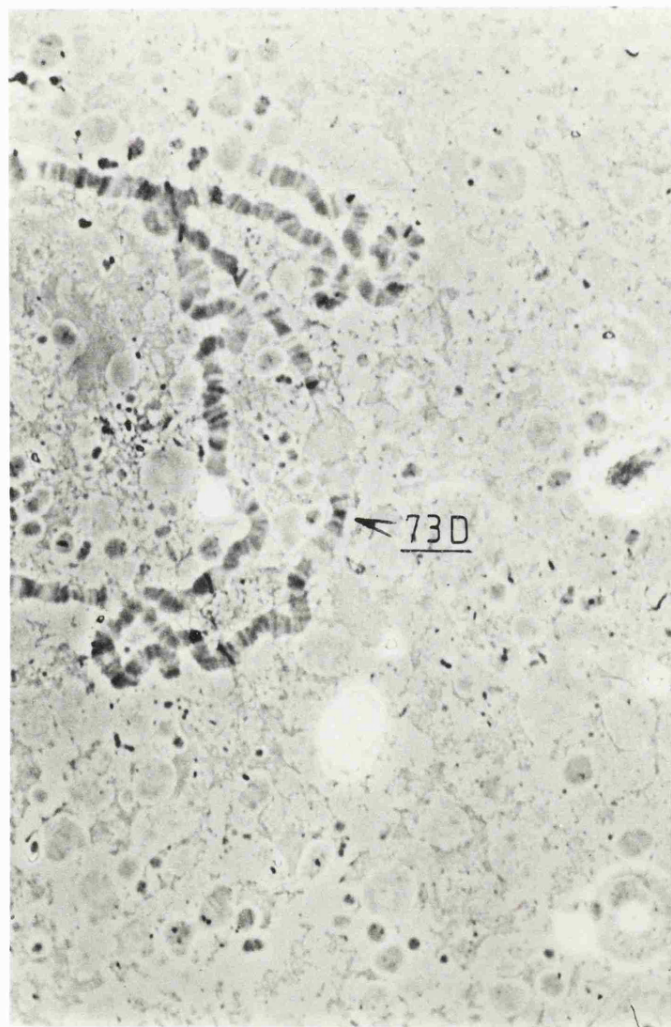


Figure 62: In situ hybridisation of the copia transposable element probe to Ac2(b)II dysgenic subline polytene chromosomes. The arrows indicate the new sites of insertion at 33F and 73D.

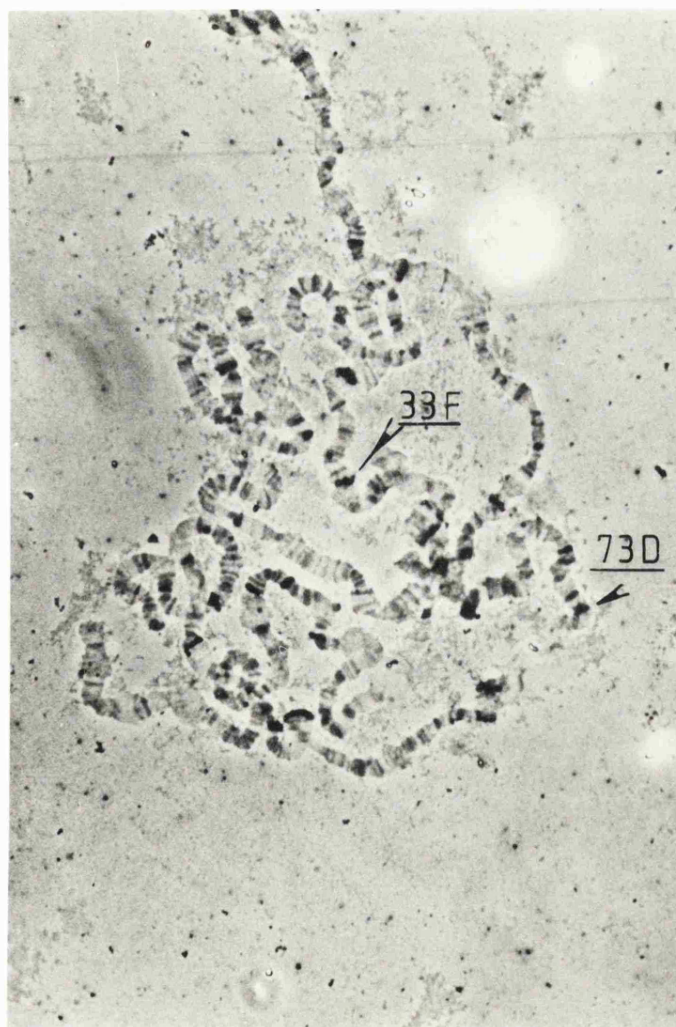


Figure 63: In situ hybridisation of the 412 transposable element probe to Aal dysgenic subline polytene chromosomes. The arrows indicate the new sites of insertion at 67A and 67D.

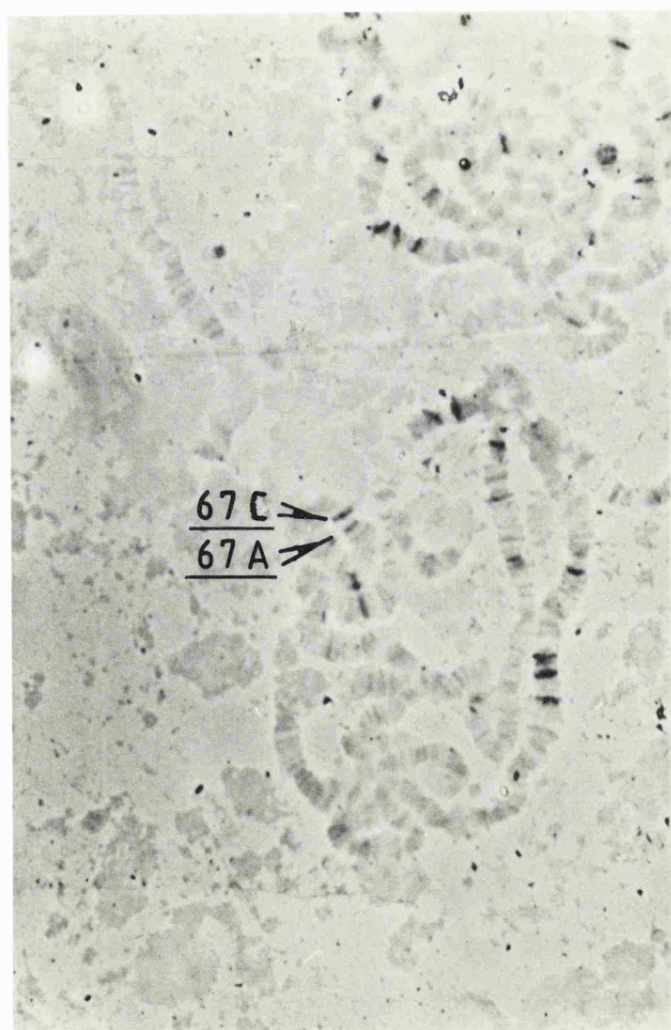


Figure 64: In situ hybridisation of the 412 transposable element probe to Aa2 dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 52B.

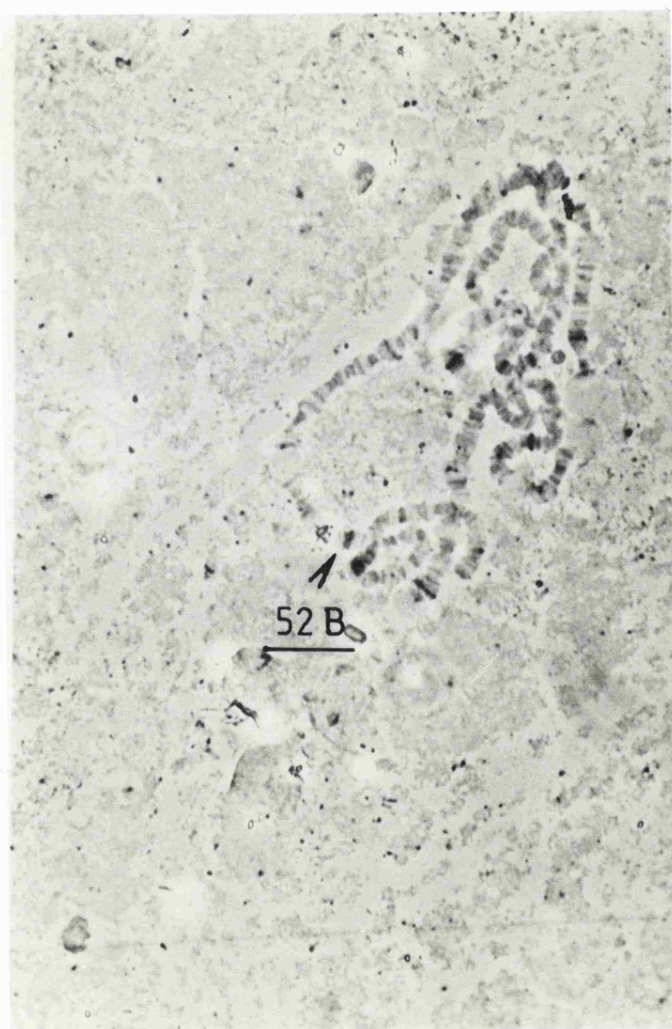


Figure 65: In situ hybridisation of the 412 transposable element probe to Ab2(b)IZ dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 48C.



Figure 66: In situ hybridisation of the 412 transposable element probe to Ab2(b)IIY dysgenic subline polytene chromosomes. The arrow indicates the new site of insertion at 42B.

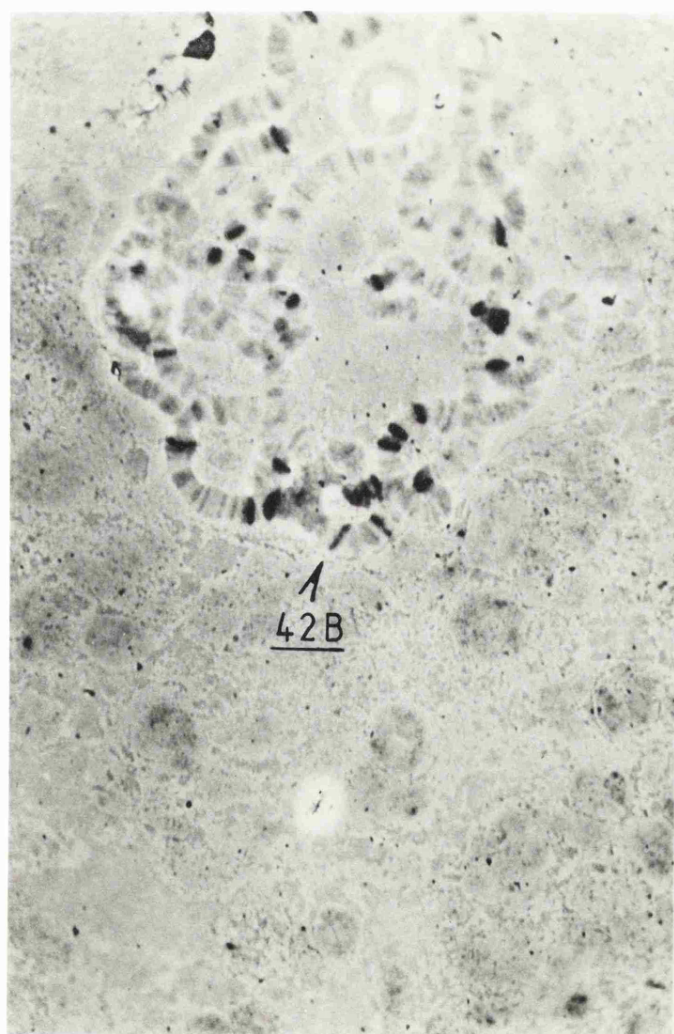


Figure 67: χ^2 test to determine the significance between the number of new copia, 412 and F element sites in the dysgenic lines and those in the controls.

$$\chi^2 = \frac{(E_d - O_d)^2}{E_d} + \frac{(E_c - O_c)^2}{E_c}$$

$$E_e = \frac{14 \times 14.91}{25.91} = 8.06 \quad E_c = \frac{14 \times 11}{25.91} = 5.94$$

$$O_e = 14 \quad O_c = 0$$

$$\chi^2 = \frac{(8.06 - 14)^2}{8.06} + \frac{(5.94 - 0)^2}{5.94} = 4.38 + 5.94 = 10.32$$

For 1 degree of freedom at the 1% level this value is significant. Thus the observed number of new dysgenic and control line copia, 412 and F element sites are significantly different.

Figure 68: Estimation of copia, 412 and F element transposition rates. The assumption is made that no new insertion sites occur in the chromocentre or chromosome 4.

$$\text{Transposition rate} = \frac{\text{Number of new sites}}{\text{Expected number of genomes in the lines examined}} \times \frac{1}{\text{Number of original sites}}$$

COPIA

$$\text{Number of original sites} = \frac{922 - 9}{42} = 21.7$$

$$\text{Transposition rate} = \frac{5}{14.91} \times \frac{1}{21.7} = 0.0155 \text{ copy per original copy}$$

412

$$\text{Number of original sites} = \frac{1035 - 5}{42} = 24.5$$

$$\text{Transposition rate} = \frac{5}{14.91} \times \frac{1}{24.5} = 0.0137 \text{ copy per original copy}$$

F ELEMENT

It is difficult to calculate an accurate figure for the number of original sites as one can not determine, from the given data, the total number of sites in the lines, due to the presence of elements in the chromocentre. One can, however, use an estimated figure of 50 elements per genome (DiNocera et al. 1983) to obtain a transposition rate.

$$\text{Transposition rate} = \frac{4}{14.91} \times \frac{1}{50} = 0.0054 \text{ copy per original copy}$$

.....

element in 35D of lines Ab2(b)IX and Ab2(b)IZ transposed at
the latest in an F5 fly.

.....

Chapter 4 : The Characterisation Of P Element-Induced singed Mutations

4.1 P-M Hybrid Dysgenesis-Induced singed Mutations

In P-M hybrid dysgenesis the sex-linked singed (sn) locus seems to be a "hot spot" for P element insertions (Green 1977), the locus being particularly mutable in hybrid dysgenesis. Such an example can be seen in dysgenic $\pi 2$ hybrids where P insertions occur within sn at a frequency of as high as 1% (Engels 1979). The sn phenotype is that of twisted, shortened bristles and wavy hairs (Bender 1960).

A number of sn mutations have been induced by hybrid dysgenesis (Brookfield and Mitchell 1985) and maintained for over 40 generations, either as males in a stock with C(1)DXy^f females, or as strains in which both sexes possess the mutation. $\pi 2$ and *loua* refer to the P strains used in the hybrid dysgenic cross. The mutations have been found to differ in strengths, varying from a weak phenotype, as can be seen in the scanning electron micrograph of figure 69, to the stronger phenotype of the bristles in figure 70. They have been classified as either weak or strong in table 2.

Many of the sn mutations have been found to be unstable in these stocks reverting either to a wild-type or to a weaker sn phenotype, the revertants produced being dominant over the sn alleles from which derived. Reversion rates have been measured for a number of the stocks under dysgenic conditions (table 2).

P-M hybrid dysgenesis is tissue specific due to the differential splicing of the P factor mRNA, active

Figure 69: Scanning electron micrograph of the mesonotum and scutellum of a $\pi 2$ sn³ male exhibiting a weak sn bristle phenotype.

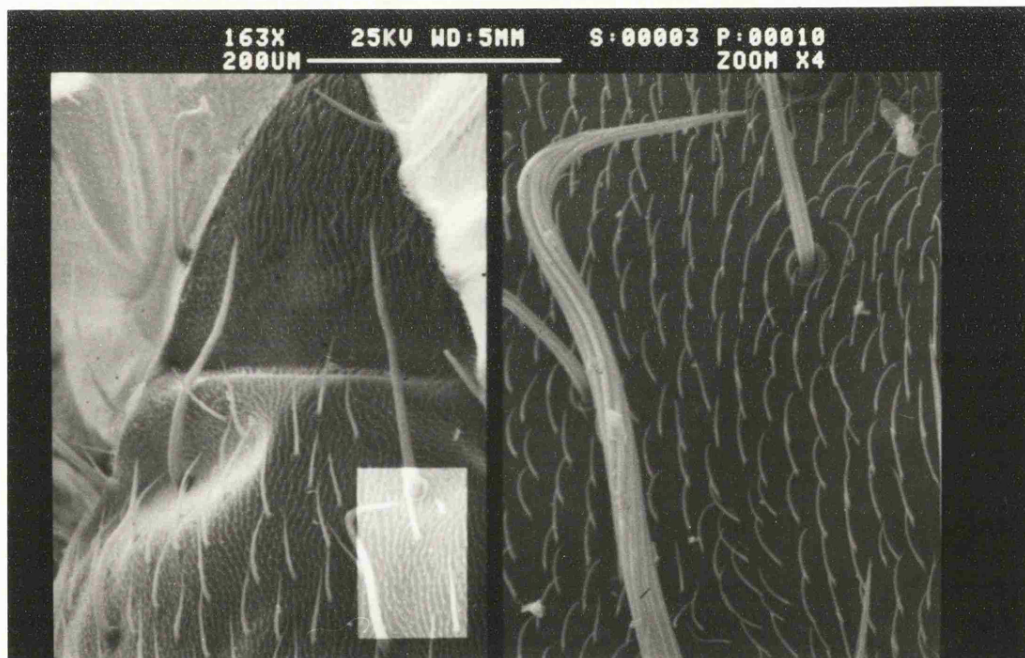


Figure 70: Scanning electron micrograph of the mesonotum and scutellum of a loua sn⁵ male exhibiting a strong sn bristle phenotype.

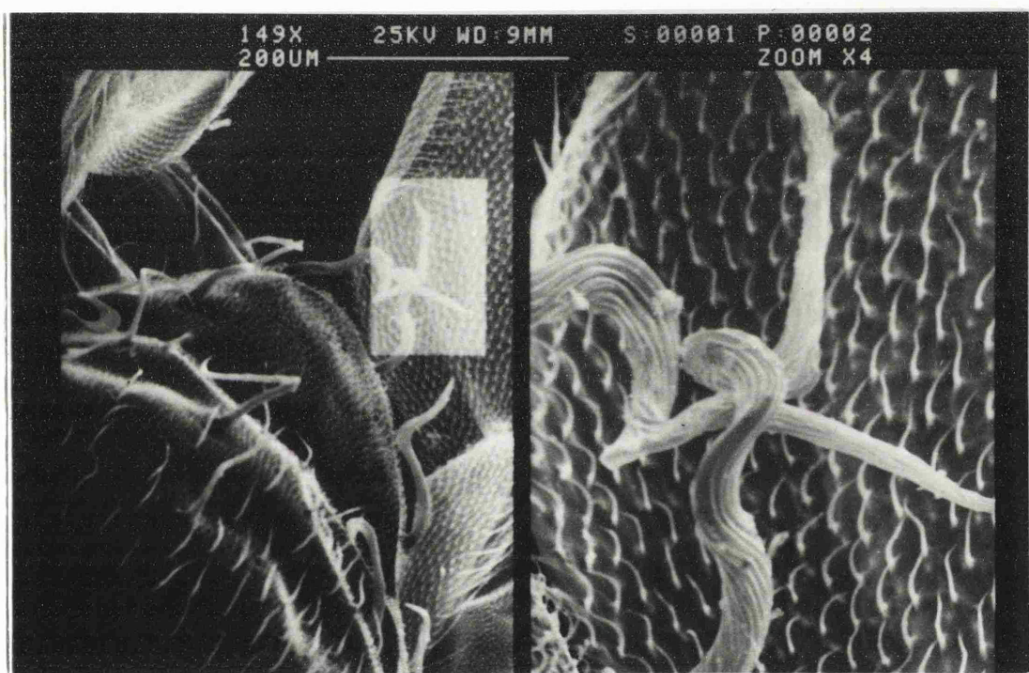


Table 2: Properties of P-M hybrid dysgenesis-induced singed mutations. Phenotypic strengths have been classed as either weak or strong (very weak for sn^{vw}). Reversion rates were calculated from measurements of proportions of revertants in the first 10 generations following isolation of the mutations. Somatic destabilisation rates are the frequency of mosaic males in the first 3 generations of a cross between C(1)DXy^f females from mosaic lines and the sn mutation males. Values preceding and following the reversion and somatic destabilisation rate estimates are 95% confidence limits.

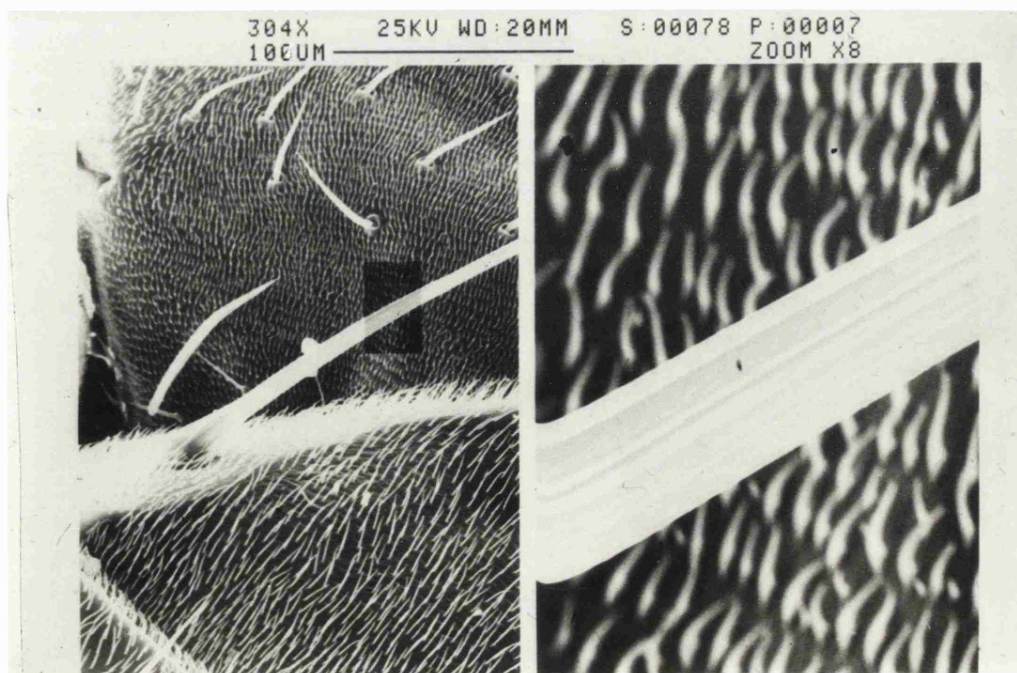
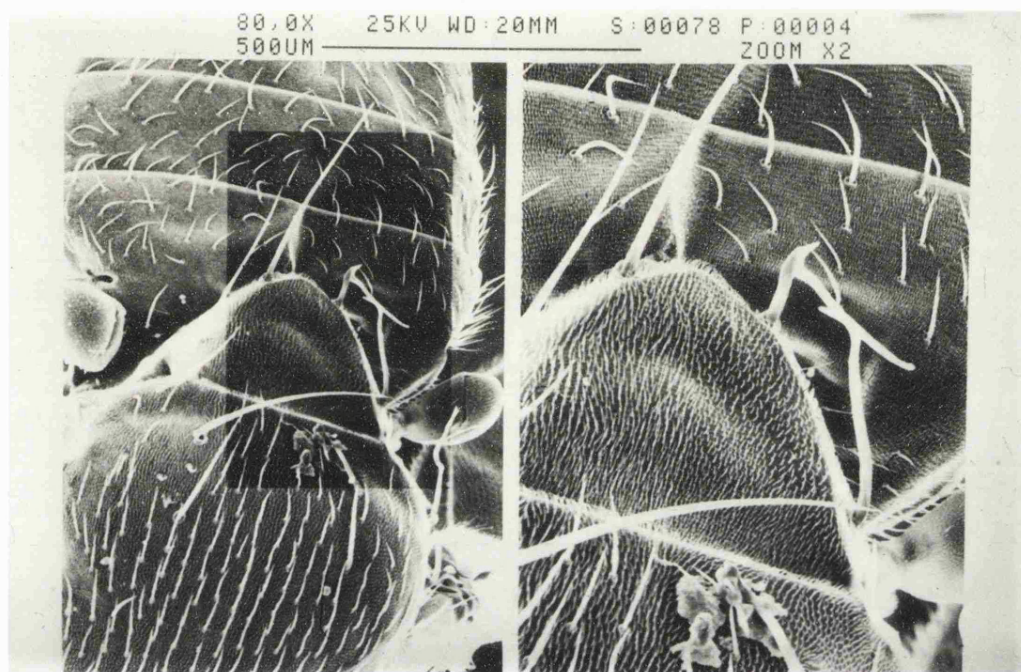
Mutant Strain	<u>Singed</u> Phenotypic Strength	Reversion Rate	Somatic Destabilisation Rate
<u>π2 sn</u> ¹	Weak	0.26-0.51%-0.76	0.00%-0.38
<u>π2 sn</u> ²	Weak	0.00-0.02%-0.13	0.00%-0.33
<u>loua sn</u> ⁵	Strong	0.00-0.03%-0.16	0.00%-0.49
<u>π2 sn</u> ³	Weak	1.14-1.44%-1.74	0.13-0.27%-0.41
<u>loua sn</u> ¹⁰	Strong	Not calculated, does revert	0.00%-0.27
<u>loua sn</u> ⁴	Strong	0.44-0.92%-1.91	1.34-1.85%-2.36
<u>loua sn</u> ⁶	Strong	0.5%-1%	0.28-0.56%-0.84
<u>π2 sn</u> ⁸	Weak	0.5%-1%	0.26-0.68%-1.48
<u>π2 sn</u> ⁵	Weak	Not calculated, does revert	0.21-0.49%-1.00
<u>loua sn</u> ⁹	Weak	Not calculated, does revert	1.26-2.18%-3.10
<u>sn</u> ^{vw}	V. Weak	High reversion	6%-10%
<u>sn</u> ^w	Weak	50%	0.01-0.06%-0.32

transposase, and thus P element transposition, only being produced in germ cells (Laski et al. 1986; Rio et al. 1986). The mutant strain loua sn⁴, however, (maintained as sn males and C(1)DXy^f females) was found to yield occasional mosaic male flies with areas of cuticle wild-type for sn (Brookfield and Lewis, submitted). The sn mutation was reverting in somatic cells. Figure 71 shows such an area of tissue. The mosaicism phenotype has been maintained for more than 50 generations by crossing mosaic males to M strain C(1)DXy^f females, and experiments carried out to try and characterise the system (Brookfield and Lewis, submitted).

Mosaic males were initially crossed with C(1)DXy^f females from non-mosaic lines. F1 sn males were produced which exhibited no mosaicism, but the somatic instability was found to reappear in some F2 males. If C(1)DXy^f females were taken from a mosaic line and crossed with loua sn⁴ males from lines with no history of mosaicism, then the mosaic phenotype was found to occur in a number of the F1 males. Such results demonstrate that the somatic instability mutation is not on the X chromosome, and is not simply dominant or recessive in its effects. The data is consistent with it being a maternal effect or Y chromosomal mutation, or having both these properties.

C(1)DXy^f females from mosaic lines were crossed with the males of the various hybrid dysgenesis-induced sn mutations, to test the destabilisation of these alleles. The mutations were found to vary in their capacity for producing mosaics (table 2), and the somatic reversion rates calculated were not well correlated to the germline reversion rates. sn^w is a good example of this observation, as although it reverts in

Figure 71: Scanning electron micrographs of a region of wild-type macrochaetae on the mesonotum and scutellum of a loua sn⁴ mosaic male.

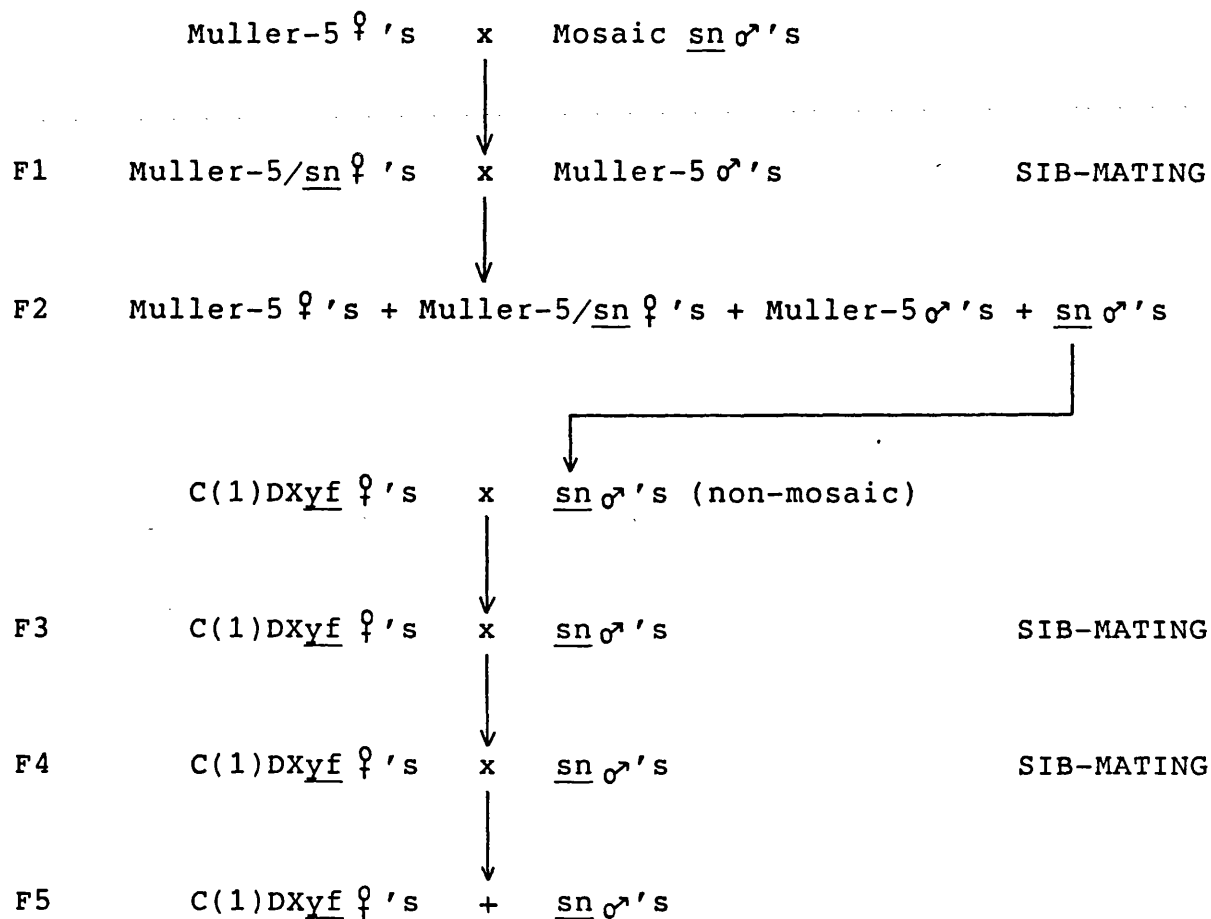


dysgenic conditions at a rate of approximately 50%, its frequency of somatic instability was only 0.06%. The phenomenon is thus not simply an extension of the normal P factor transposase activity to somatic cells. Hybrid dysgenesis-induced white, scalloped and yellow mutations were also tested for instability, but with negative results.

A cross involving M strain Muller-5 females was initially attempted to try and map the trans-acting component required for somatic movements (figure 72). Mosaic males were crossed to Muller-5 females, and the F1 progeny mated. F2 non-mosaic sn males were crossed to C(1)DXy^f females from non-mosaic lines and the F1 of this cross (F3 in figure 72) sib-mated. The F4 generation flies were also sib-mated. It was found that there were only a few mosaics among the F2 sn males, and yet non-mosaic males from this generation, when crossed to C(1)DXy^f females, produced a greater number of mosaics in their grand- and great grandchildren. Such results could have been explained by a Y chromosomal maternal effect mutation, except that the F2 generation did show some mosaics (only a few, but too many to be explained by Y chromosomal non-disjunction), and also the mosaicism of the F4 generation was also present in the F5. Thus there seems to be an increased mosaicism in the C(1)DXy^f female cross progeny.

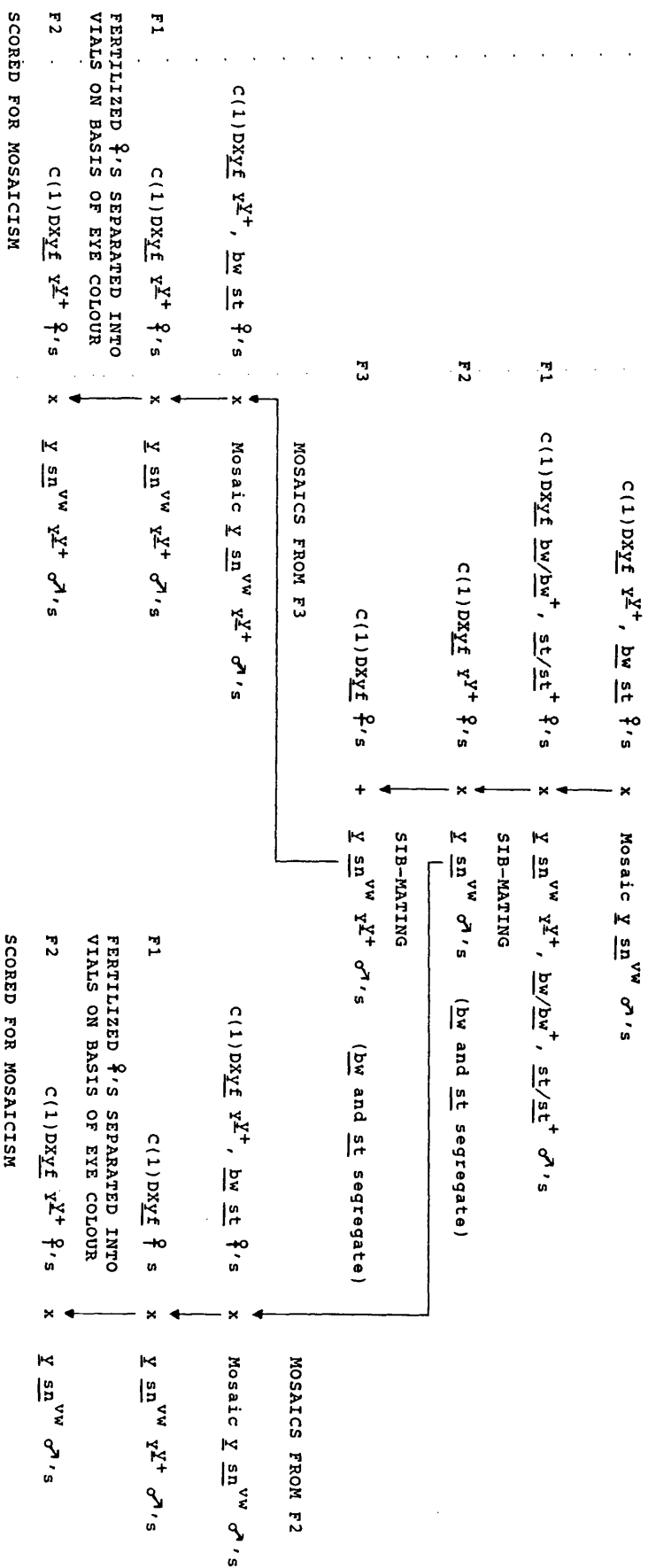
The cross in figure 73 was carried out to try and resolve the question of whether the trans-acting component was Y chromosomal. C(1)DXy^f y^{Y+}, bw st females (possessing a translocated y⁺ allele on their Y chromosome, and brown (bw) and scarlet (st) markers on their 2nd and 3rd chromosomes respectively) were crossed with mosaic sn males possessing a stable yellow (y) mutation on their X chromosomes. In such a

Figure 72: A cross demonstrating that singed mosaicism occurs preferentially in the male offspring of attached-X crosses.



The sn ♂'s in the F2, F3, F4 and F5 generations were scored for mosaicism.

Figure 73: A cross demonstrating that the trans-acting mosaicism component is not Y-chromosomal, but may be a dominant 2nd-chromosomal maternal effect mutation.



cross the Y chromosome from the mosaic males will be associated with the y phenotype. The mosaic sn males used were derived from a strain singed-(very weak) (sn^{vw}). The flies were originally isolated as a germline revertant of loua sn⁶ possessing a very weak sn bristle phenotype. They were found to revert somatically to a more extreme sn phenotype, but with a higher penetrance than that seen for "sn to wild-type" mosaic strains. The requirements in trans for this somatic reversion process, however, were the same as those in the normal "sn to wild-type" reversion. The sn^{vw} strain is described in more detail in Chapter 5.

Mosaic sn males were produced in both the F2 and F3 generations of the figure 73 cross, and these males (y in F2 and y⁺ in F3) were crossed again to the females of the original C(1)DXyf y^{Y+}, bw st strain. It was found that in 30 out of 64 lines established from the F2 generation the mosaicism reappeared in the F2 of this cross, and also in 6 out of 10 lines from the F3 generation it reappeared in these F2's. Thus, although the lines constructed from the F3 generation had had the Y chromosome from the initial mosaic male removed, mosaicism was still inherited. The trans-acting component is not therefore on the Y chromosome. In the F2 generation of the original cross the bw and st markers were found to segregate at the frequencies as expected from Mendelian segregation. When these F2 generation were then crossed to the C(1)DXyf y^{Y+}, bw st females the results suggested that the trans-acting component may perhaps be a dominant maternal effect mutation on the 2nd chromosome. The st genotype was not associated with the ability to pass on mosaicism, but it was found that the number of bw⁺ alleles

that an F2 male possessed was positively correlated (significant at $P < 0.05$) to his ability to pass on the mosaicism trait. Also, on dividing the F1 generation of such a cross into sublines in which the females possessed different eye colours, the sublines produced from $\underline{bw}^+/\underline{bw}$ genotype F1 males often produced a greater number of mosaics in the F2's than those from $\underline{bw}/\underline{bw}$ females (significant at $P < 0.01$). No such effect was seen with the st marker. Thus the mosaicism trait appears to be linked to the \underline{bw}^+ allele of the 2nd chromosome in the above experiment. Further evidence for a maternal effect was seen in that the $\underline{bw}/\underline{bw}$ F2 males of the $\underline{bw}^+/\underline{bw}$ sublines had just as high a mosaicism rate as their \underline{bw}^+ siblings.

The size distribution of the mosaic patches was used to generate fate maps of the mesonotal macrochaetal bristles (Brookfield and Lewis, submitted). These showed that the elements were mobilised after the first few nuclear divisions following fertilization, but within the first 24 hours after egg laying. A reversion process ending this early is consistent with being due to a maternal effect.

The P element-induced sn mutations were analysed at their molecular level by Southern and Northern blotting in an attempt to correlate any variations determined, with their observed phenotypic strengths, reversion rates, and somatic destabilisation rates. This may perhaps, at the DNA level, be reflected in the P element insertion sizes, positions and orientations (assuming that the mutations are due to the insertion of P elements), and at the RNA level, by the sizes and abundances of the sn mRNA's produced.

4.2 DNA Molecular Organisation Of The singed Mutations

The sn mutations were characterised at the DNA level by Southern hybridisation to genomic DNA using a probe from the sn region. The probe was CSSn9 which contains an EcoRI fragment from the sn region of Canton-S cloned into pBR322 (Roiha et al. 1987). A restriction endonuclease map of the area including this fragment (Roiha et al. 1987), and also a map of the 2.9 kb P factor (O'Hare and Rubin 1983), are shown in figure 74. In strains which were maintained as sn males with C(1)DXyf females, only the males were collected for DNA analysis. The labelling and hybridisation protocols used for each strain are stated in the appropriate autoradiograph figure legends.

Genomic DNA was initially isolated from adults of the strains Canton-S, π 2 sn¹, π 2 sn², loua sn⁵, π 2 sn³, loua sn⁴ and loua sn⁶, and single and double restriction endonuclease digested with EcoRI, HindIII, and SalI. The gels were Southern blotted and hybridised with α -[³²P]-dCTP labelled CSSn9 probe. HindIII digested bacteriophage λ length standards (BRL) were used for sizing the DNA. The autoradiographs produced can be seen in figures 75-81.

Attempts were made at constructing restriction endonuclease maps for the strains, from the autoradiographs, using both the map of the sn region and that of the P factor (figure 74). It was found, however, that each of the sn strains appeared to possess P element insertions with deletions removing both the SalI and EcoRI sites, and some of the strains were also deleted for the HindIII site at bp +877.

Figure 74 Restriction endonuclease maps of the Canton-S sn region and of the P factor

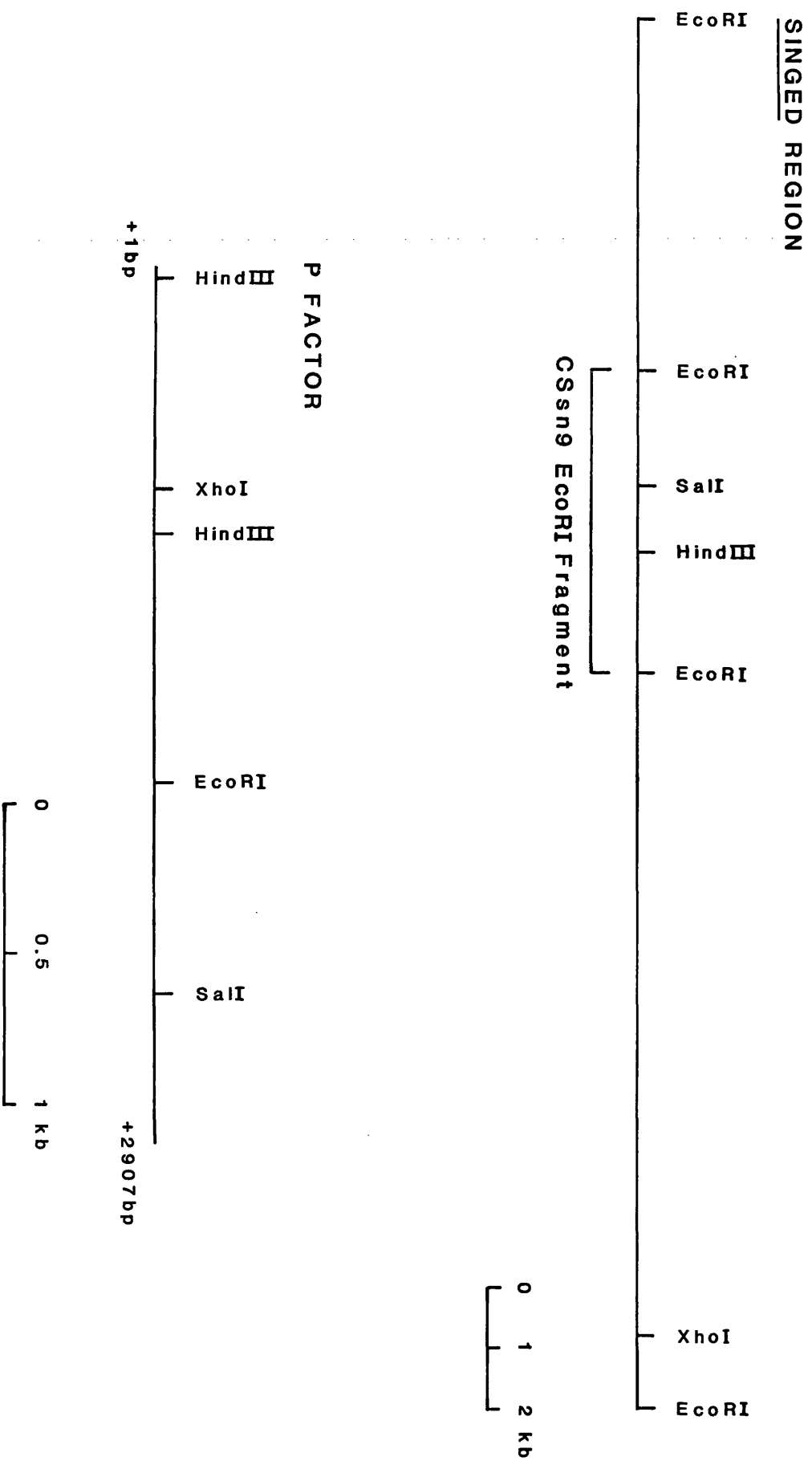


Figure 75: Blot hybridisation of genomic DNA from the strain Canton-S with the probe CSsn9. The probe was nick translated, and hybridised to the restriction endonuclease digested genomic DNA using a nitrocellulose filter and protocol I. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI. Fragment lengths are in kb.



Figure 76: Blot hybridisation of genomic DNA from the strain $\pi 2$ sn^1 with the probe CSsn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol III. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- EcoRI, SalI; 4- EcoRI, HindIII; 5- HindIII, SalI. Fragment lengths are in kb.

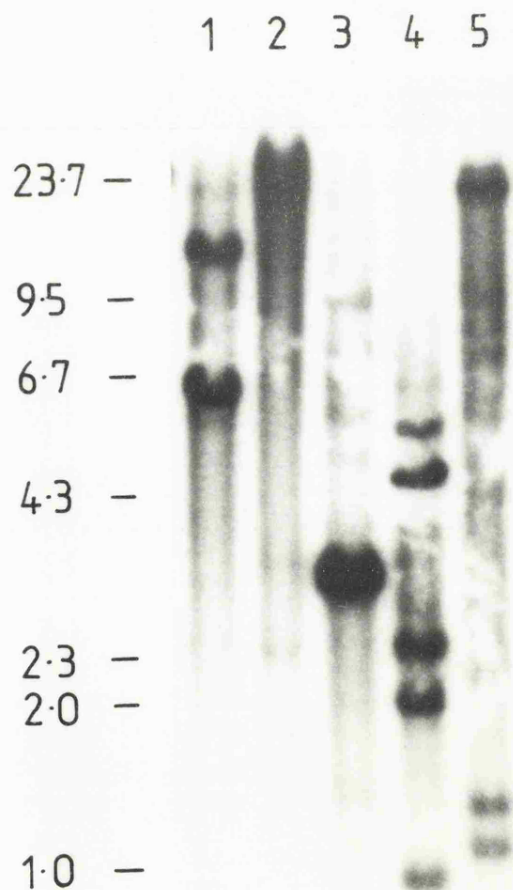


Figure 77: Blot hybridisation of genomic DNA from the strain $\pi 2$ sn² with the probe CSSn9. The probe was nick translated, and hybridised to the restriction endonuclease digested genomic DNA using a nitrocellulose filter and protocol I. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI. Fragment lengths are in kb.

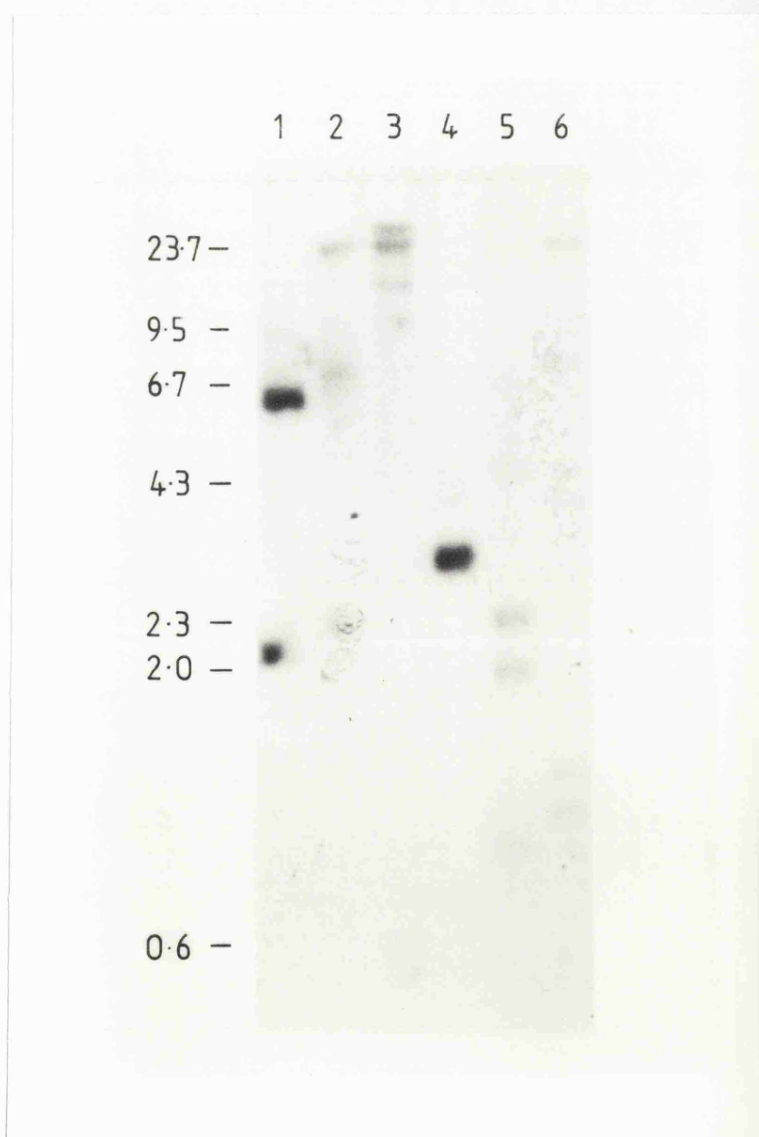


Figure 78: Blot hybridisation of genomic DNA from the strain loua sn⁵ with the probe CSsn9. The probe was nick translated, and hybridised to the restriction endonuclease digested genomic DNA using a nitrocellulose filter and protocol I. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI. Fragment lengths are in kb.

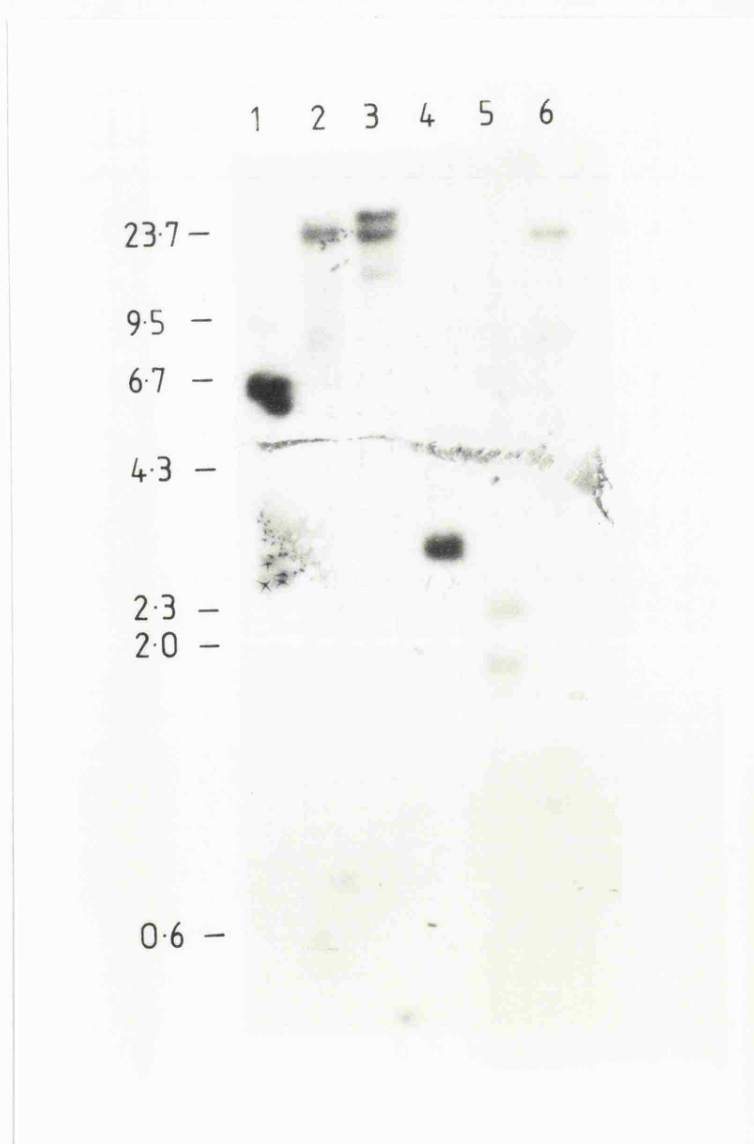


Figure 79: Blot hybridisation of genomic DNA from the strain $\pi 2$ sn³ with the probe CSSn9. The probe was nick translated, and hybridised to the restriction endonuclease digested genomic DNA using a nitrocellulose filter and protocol I. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI. Fragment lengths are in kb.

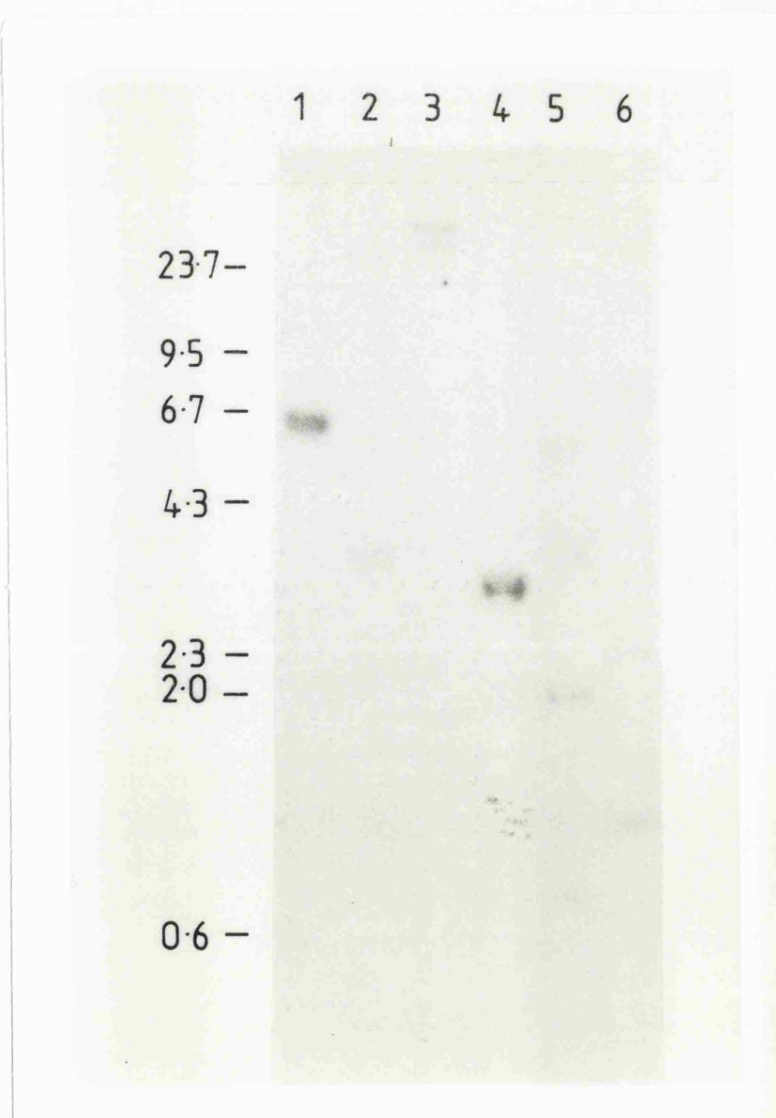


Figure 80: Blot hybridisation of genomic DNA from the strain loua sn⁴ with the probe CSsn9. The probe was nick translated, and hybridised to the restriction endonuclease digested genomic DNA using a nitrocellulose filter and protocol I. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI. Fragment lengths are in kb.

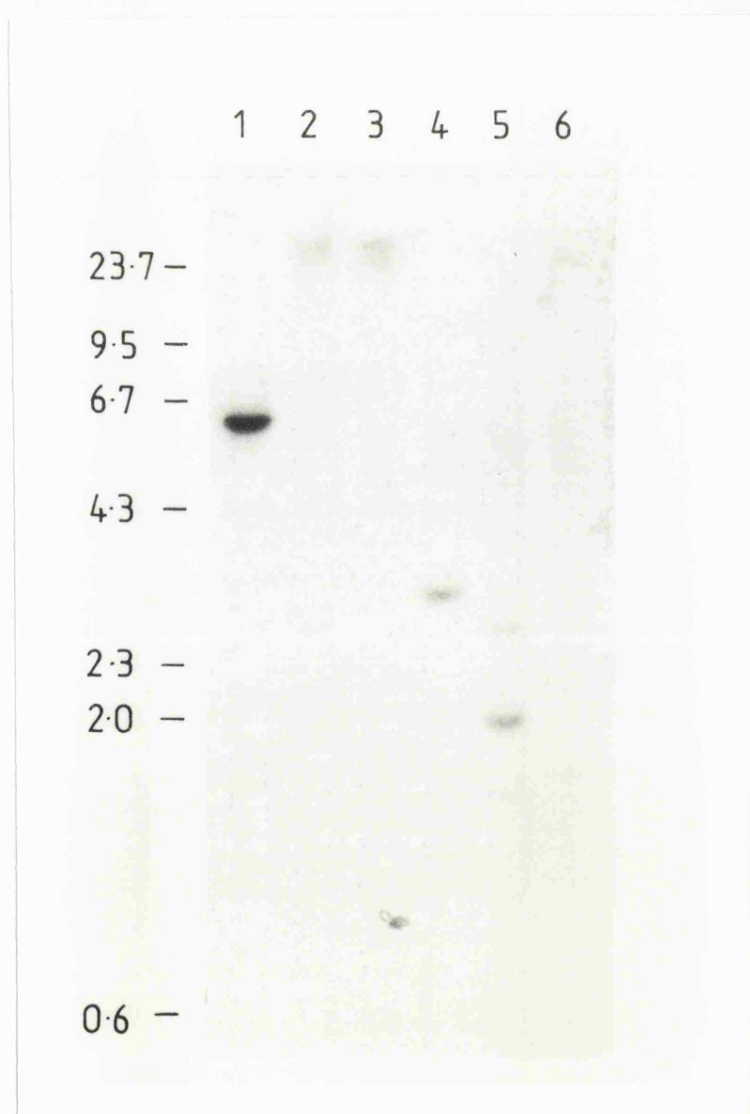
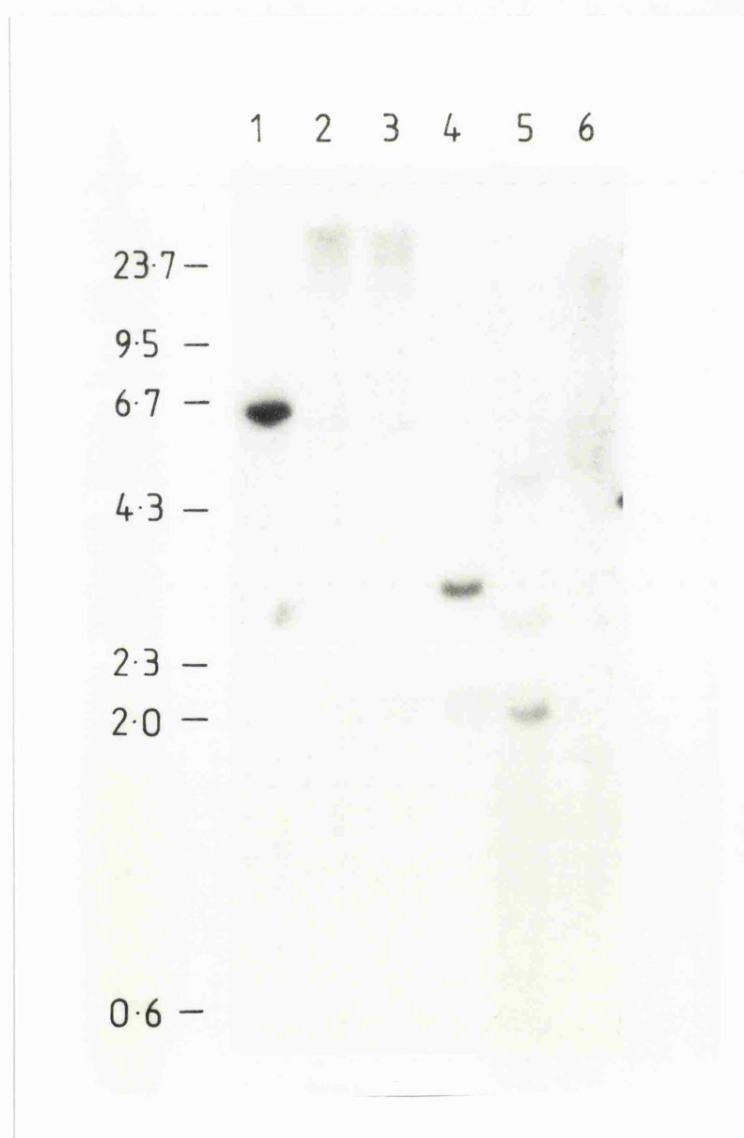


Figure 81: Blot hybridisation of genomic DNA from the strain loua sn⁶ with the probe CSSn9. The probe was nick translated, and hybridised to the restriction endonuclease digested genomic DNA using a nitrocellulose filter and protocol I. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI. Fragment lengths are in kb.



Apart from strain $\pi 2$ sn³ it was thus impossible to deduce the correct orientation and site of insertion of the P elements from the given data, there being two possibilities in each of the cases. EcoRI, XhoI double digests were thus run for each of the sn strains, and DNA Southern blot hybridisations carried out with the CSSn9 probe, in order to resolve the problem. There is a XhoI site present in the P factor between the two HindIII sites, and thus this will be present in the elements retaining both HindIII sites, and also perhaps in the elements with the +877 bp HindIII site deletion. If present, then this site can be used as a second restriction endonuclease marker against the HindIII sites for orientation of the elements. It can also be used as a check that the insertions are actually P elements, as if the deletions are each to the right of the XhoI site (as orientated in figure 74) then they should all contain the 0.7 kb HindIII-XhoI fragment. The autoradiograph produced from the EcoRI, XhoI double digests can be seen in figure 82. Restriction endonuclease maps were constructed for Canton-S and each of the sn strains analysed, using the data from both the original and from the EcoRI, XhoI autoradiographs. These are shown in figures 83-89.

The $\pi 2$ sn¹ and $\pi 2$ sn² mutations were found to be due to 1.15 and 1.1 kb insertions of P elements, possessing 1.75 and 1.8 kb deletions, respectively, removing the SalI and EcoRI sites of the P factor in both cases (figures 84 and 85). The elements were inserted in the 1.9 kb EcoRI-SalI fragment of the CSSn9 EcoRI sn region fragment, at a position approximately 0.65 kb from the EcoRI site. They were both orientated with their +39 bp HindIII sites towards the SalI

Figure 82: Blot hybridisation of genomic DNA digested with the restriction endonucleases EcoRI and XhoI. The probe was nick translated, and hybridised to the restriction endonuclease digested genomic DNA using a nitrocellulose filter and protocol I. The lanes were loaded with the genomic DNA from the following strains: Lane 1- π2 sn¹; 2- π2 sn³; 3- π2 sn²; 4- loua sn⁵; 5- loua sn⁴; 6- loua sn⁶. Fragment lengths are in kb.

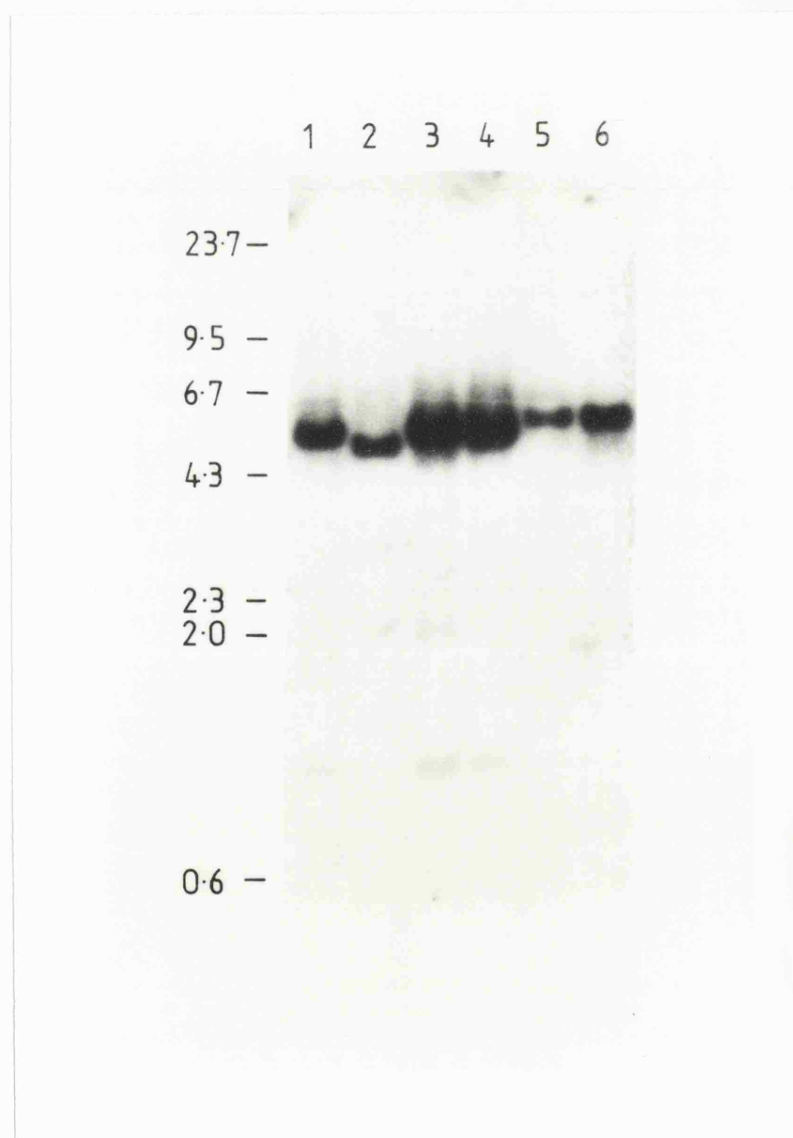


Figure 83 Restriction endonuclease map of the sn region of Canton-S

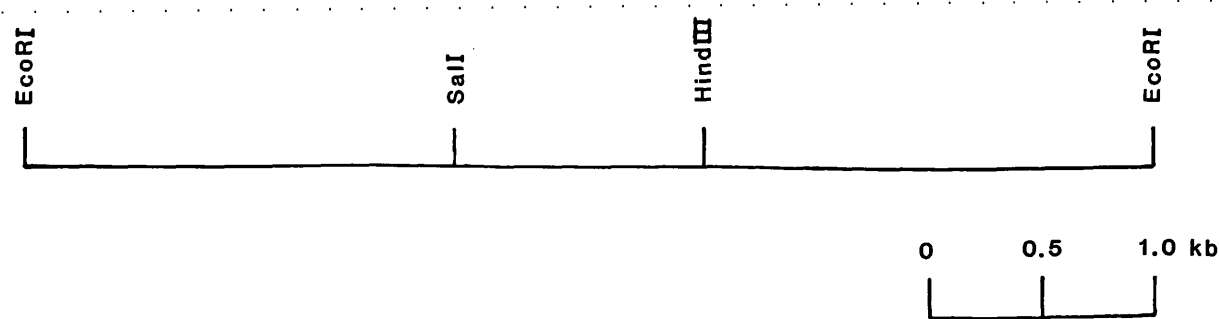


Figure 84 Restriction endonuclease map of the P element insert in the sn region of strain π2 sn¹

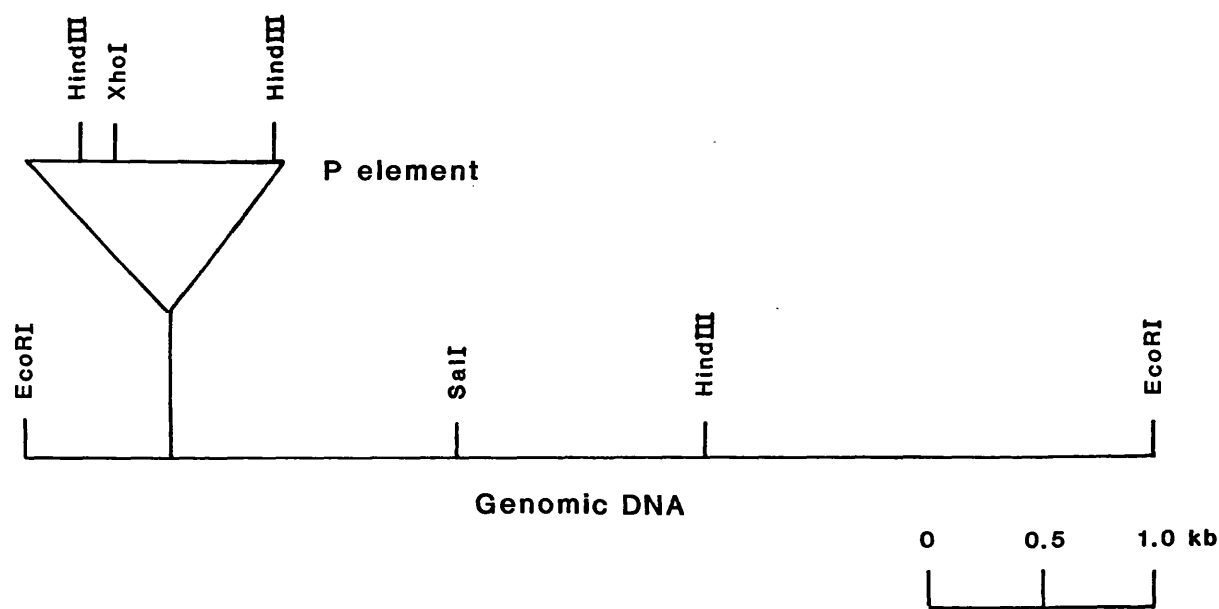
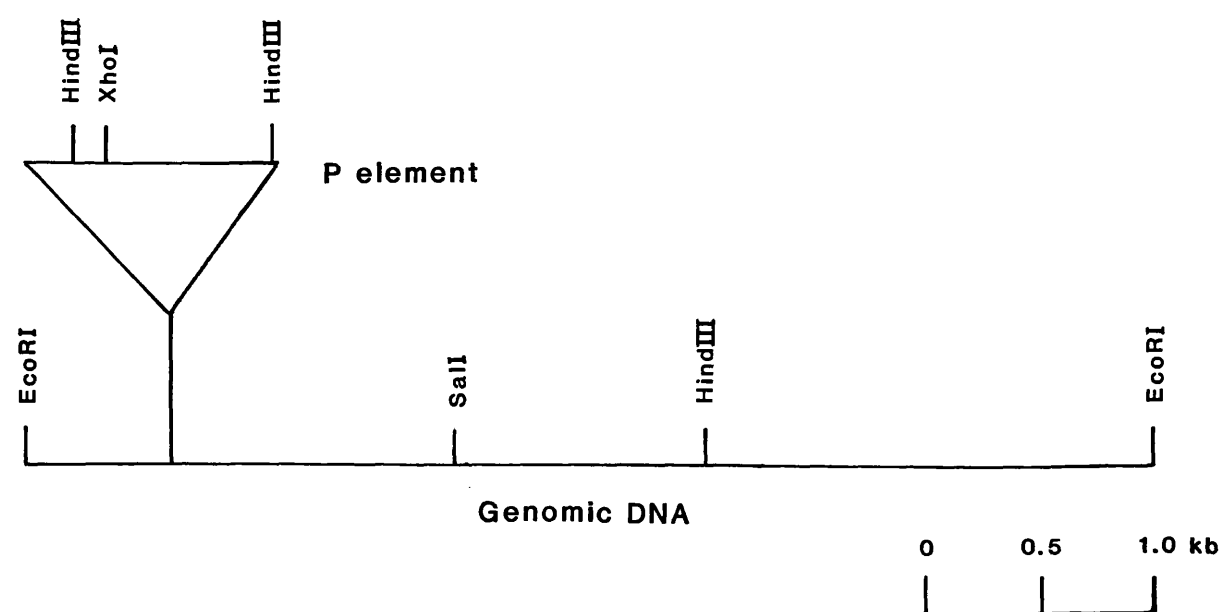


Figure 85 Restriction endonuclease map of the P element insert in the sn region of strain π2 sn²



site.

The loua sn⁵ mutation was due to the insertion of a 1.15 kb P element with a 1.75 kb deletion removing the SalI and EcoRI sites, and also the +877 bp HindIII site (figure 86). The element was inserted in a similar position and with the same orientation as the π2 sn¹ and π2 sn² insertions.

Strain π2 sn³ was found to possess a sn mutation due to a 1.2 kb P element with a 1.7 kb deletion spanning the SalI, EcoRI and +877 bp HindIII sites of the P factor (figure 87). It was again inserted in the genome at a similar position to the elements of strains π2 sn¹, π2 sn² and loua sn⁵, but the orientation was reversed such that the +39 bp HindIII site was towards the CSSn9 1.9 kb EcoRI-SalI fragment EcoRI site.

The loua sn⁴ and loua sn⁶ mutations both contained 1.25 kb P element insertions with 1.65 kb deletions removing the SalI, EcoRI and +877 bp HindIII sites (figures 88 and 89). The orientation was the same as for π2 sn¹, π2 sn² and loua sn⁵ but the position of the insertion, although still being in the CSSn9 1.9 kb EcoRI-SalI fragment, was only approximately 0.3 kb from the EcoRI site. These two strains, in fact, probably possess exactly the same mutations, as they were originally isolated from the same bottle in a hybrid dysgenesis X chromosomal mutation screen (Brookfield and Mitchell 1985). The two initial flies, from which the lines were constructed, were thus probably siblings, each containing the same insertion from a single premeiotic event in a parental germ cell.

Genomic DNA was isolated from adult flies of the sn strains, π2 sn⁸, π2 sn⁵, sn^{vw}, loua sn¹⁰ and loua sn⁹, and digested with the restriction endonucleases EcoRI, HindIII,

Figure 86 Restriction endonuclease map of the P element insert in the sn region of strain loua sn⁵

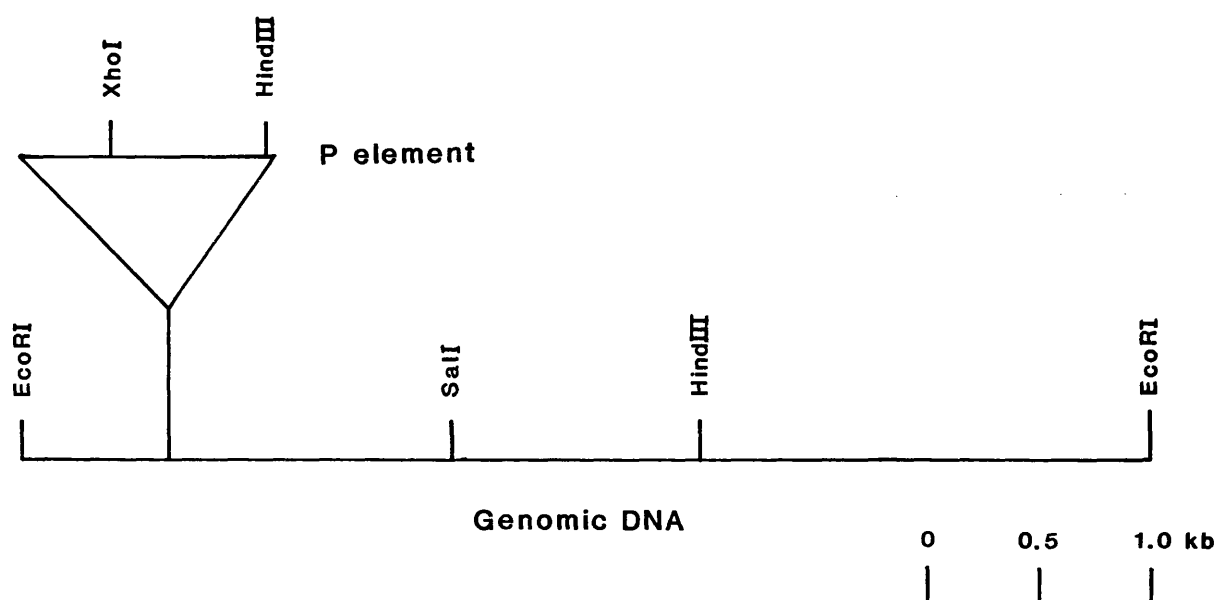


Figure 87 Restriction endonuclease map of the P element insert in the sn region of strain π2 sn³

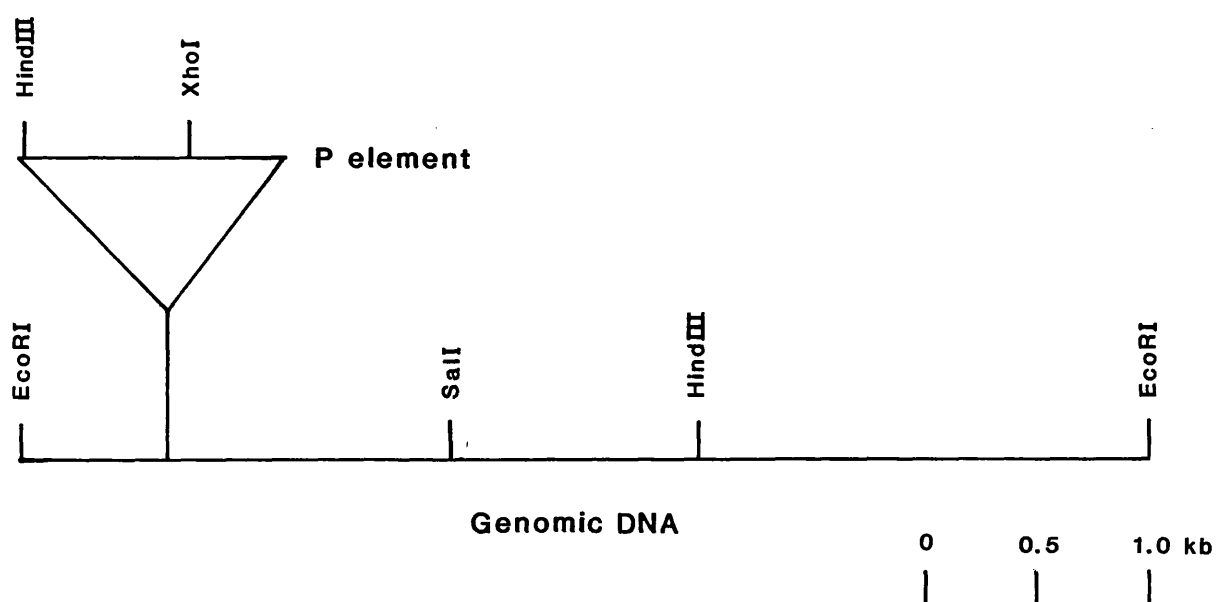


Figure 88 Restriction endonuclease map of the P element insert in the sn region of strain loua sn⁴

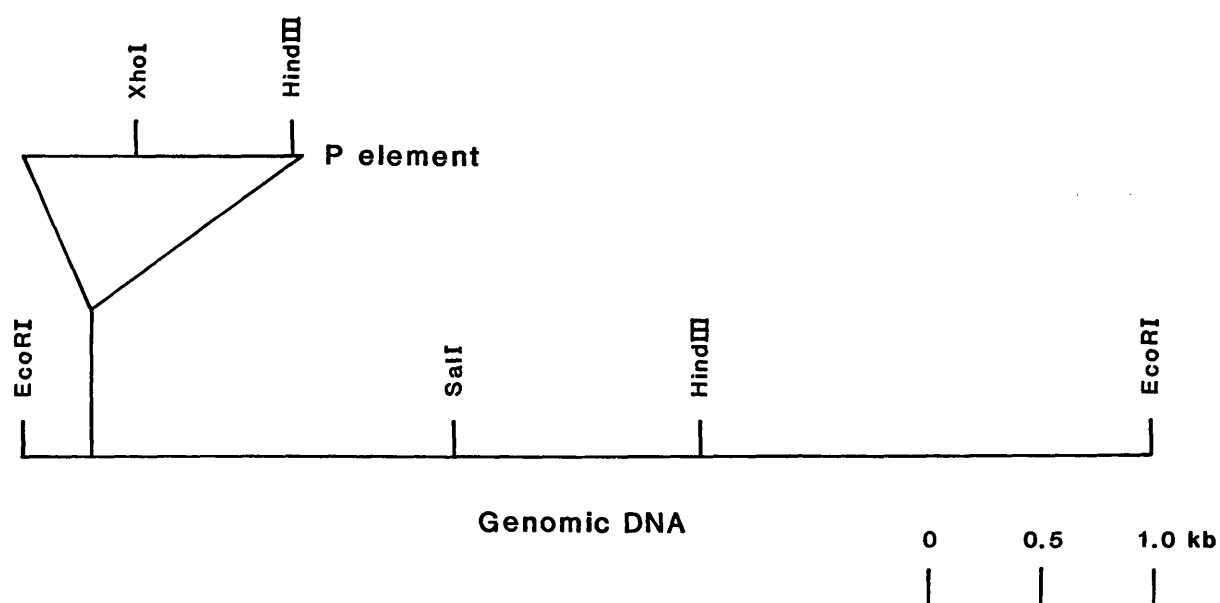
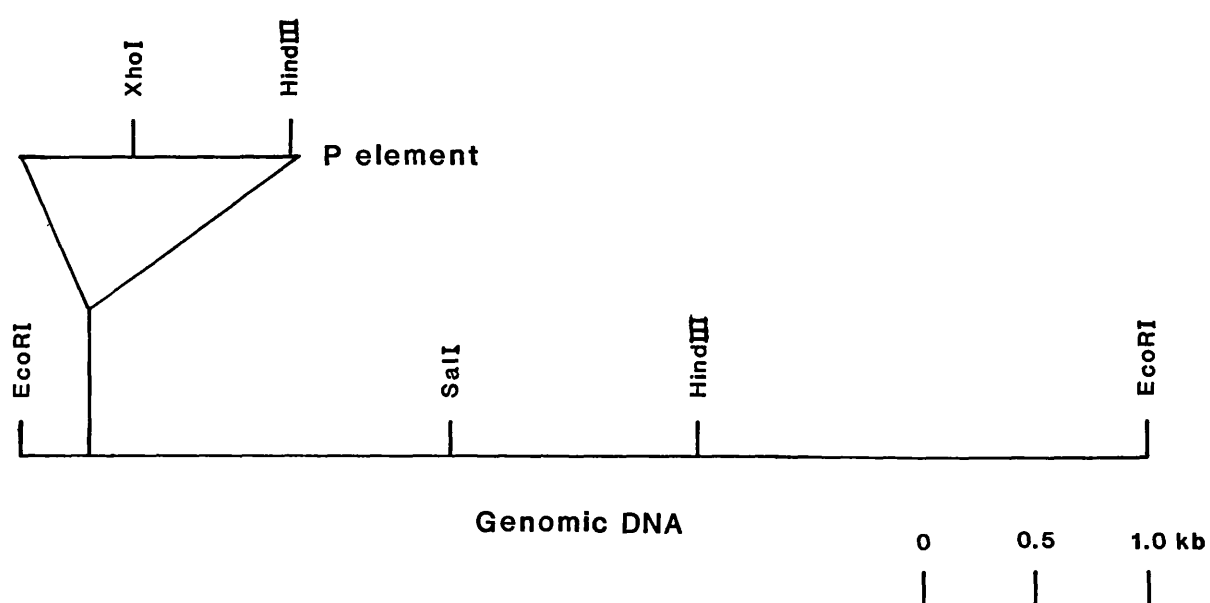


Figure 89 Restriction endonuclease map of the P element insert in the sn region of strain loua sn⁶



SalI and XhoI, both singly and in a variety of double combinations. The gels were Southern hybridised with the CSsn9 probe, and autoradiographs produced, which can be seen in figures 90-94. Restriction endonuclease maps were constructed from the data.

The $\pi 2$ sn⁸ mutation was due to the insertion of a 1.15 kb P element possessing a 1.75 kb deletion removing the SalI, EcoRI and +877 bp HindIII sites present in the P factor (figure 95). The element was inserted at a position similar to the elements of the strains loua sn⁴ and loua sn⁶, that is, at about 0.3 kb from the EcoRI site of the 1.9 kb CSsn9 genomic EcoRI-SalI fragment. The insert was orientated with its +39 bp HindIII site towards the genomic SalI site.

Strain $\pi 2$ sn⁵ was found to possess a P element of 1.0 kb in length with a 1.9 kb deletion again removing the P factor SalI, EcoRI and +877 bp HindIII sites (figure 96). The element was positioned at the insertion site found in strains $\pi 2$ sn¹, $\pi 2$ sn², loua sn⁵ and $\pi 2$ sn³, and was orientated with its +39 bp HindIII site towards the CSsn9 1.9 kb EcoRI-SalI fragment EcoRI site.

On analysis of strain sn^{vw} there appeared to be two elements present inserted in close proximity to each other (figure 97). The inserts were 1.25 and 0.65 kb long, the former being a P element possessing a deletion removing the EcoRI, SalI and +877 bp HindIII P factor sites. The smaller element was found to possess a single Hind III site only. As all the other elements characterised so far have been P elements, however, it was assumed that the 0.65 kb element was also a P, thus having a deletion of 2.25 kb spanning the EcoRI, SalI, +877 HindIII and XhoI sites. The elements were

Figure 90: Blot hybridisation of genomic DNA from the strain $\pi 2 \text{ sn}^8$ with the probe CSsn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol III. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- EcoRI, SalI; 4- EcoRI, HindIII; 5- HindIII, SalI; 6- EcoRI, XhoI. Fragment lengths are in kb.

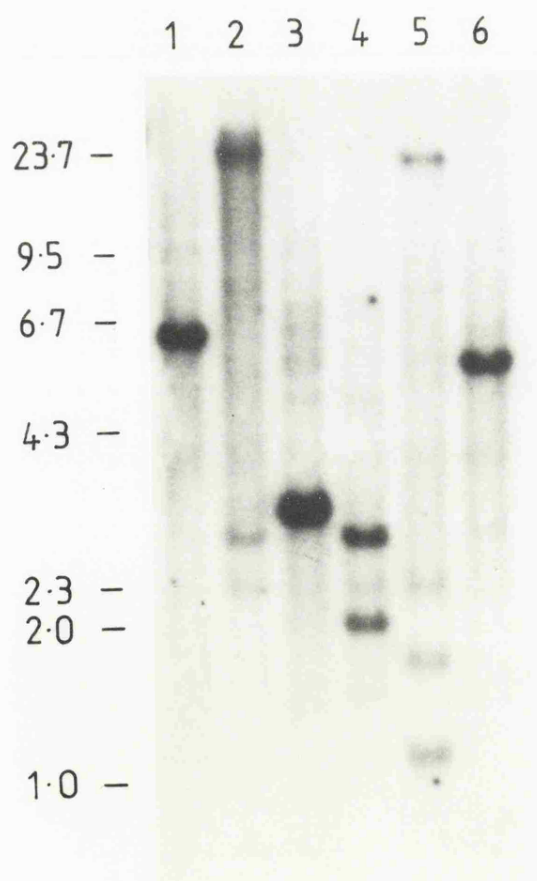


Figure 91: Blot hybridisation of genomic DNA from the strain $\pi 2 \text{ sn}^5$ with the probe CSSn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol II. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI; 7- EcoRI, XhoI. Fragment lengths are in kb.

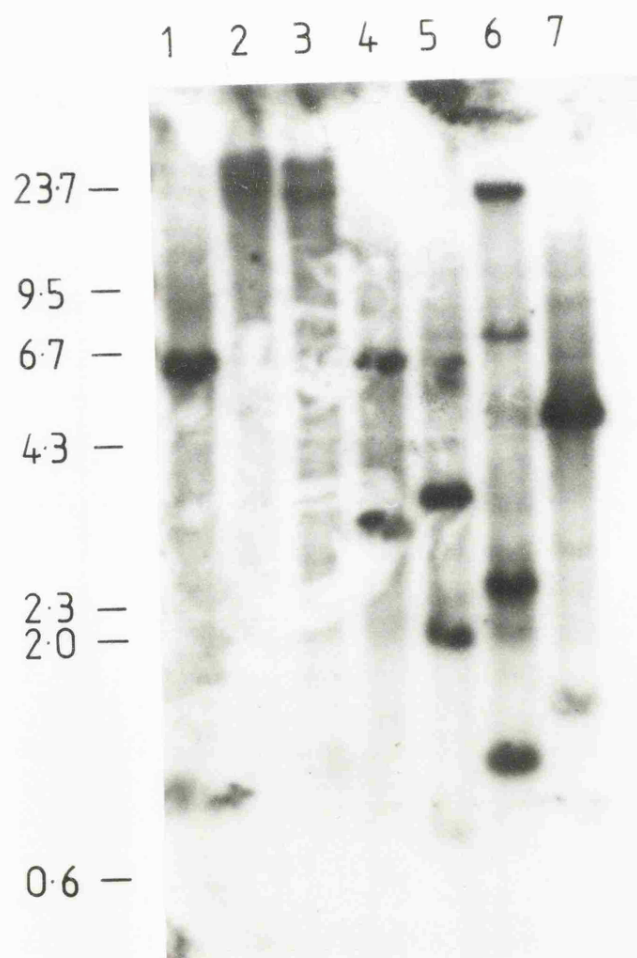


Figure 92: Blot hybridisation of genomic DNA from the strain sn^{vw} with the probe CSsn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol II. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI; 7- EcoRI, XhoI. Fragment lengths are in kb.

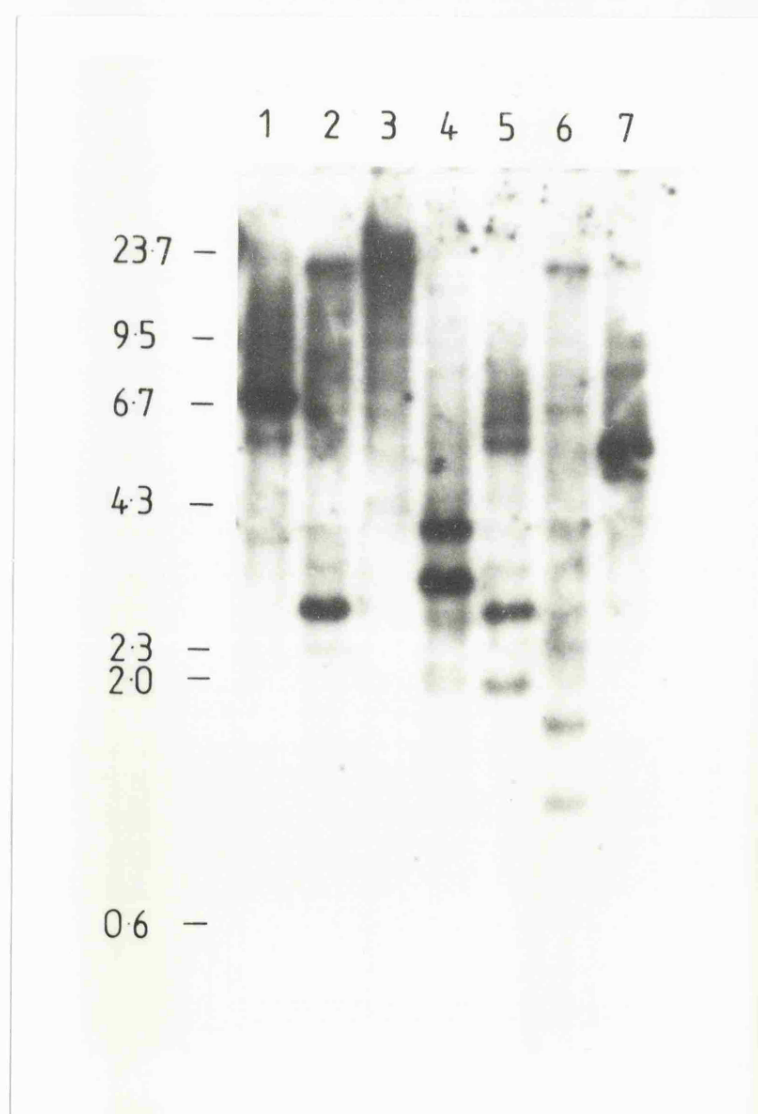


Figure 93: Blot hybridisation of genomic DNA from the strain loua sn¹⁰ with the probe CSsn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol I. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- SalI; 4- EcoRI, SalI; 5- EcoRI, HindIII; 6- HindIII, SalI; 7- EcoRI, XhoI. Fragment lengths are in kb.

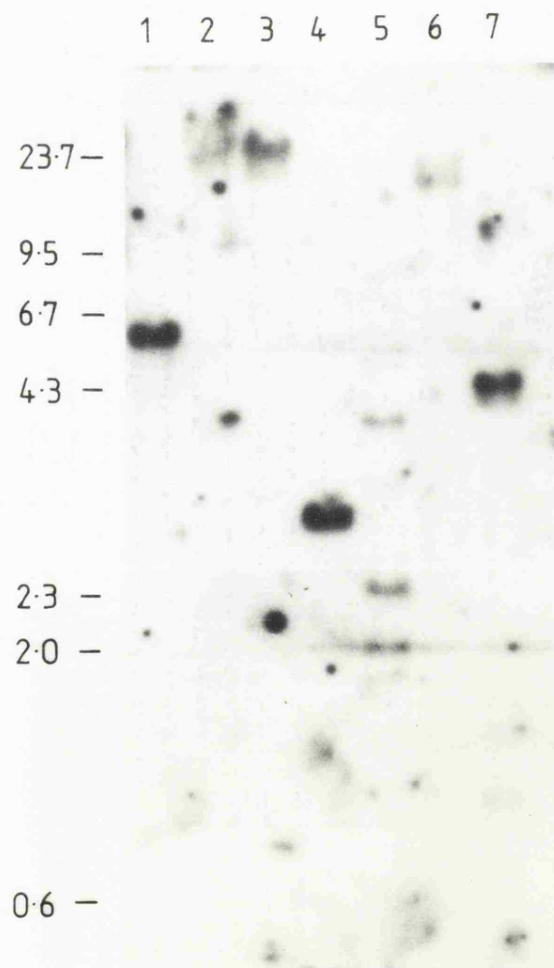


Figure 94: Blot hybridisation of genomic DNA from the strain loua sn⁹ with the probe CSSn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol II. The DNA was digested with the following restriction endonucleases: Lane 1- EcoRI; 2- HindIII; 3- EcoRI, SalI; 4- EcoRI, HindIII; 5- HindIII, SalI; 6- EcoRI, XhoI. Fragment lengths are in kb.

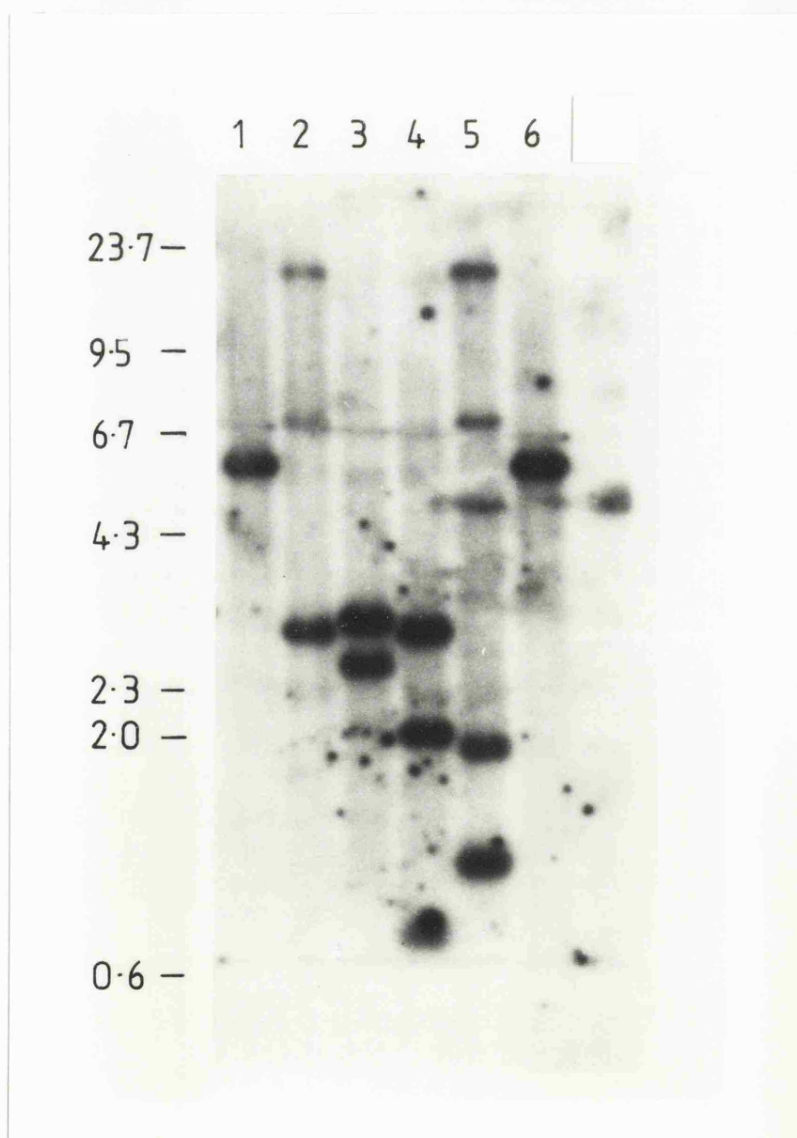


Figure 95 Restriction endonuclease map of the P element insert in the sn region of strain π 2 sn⁸

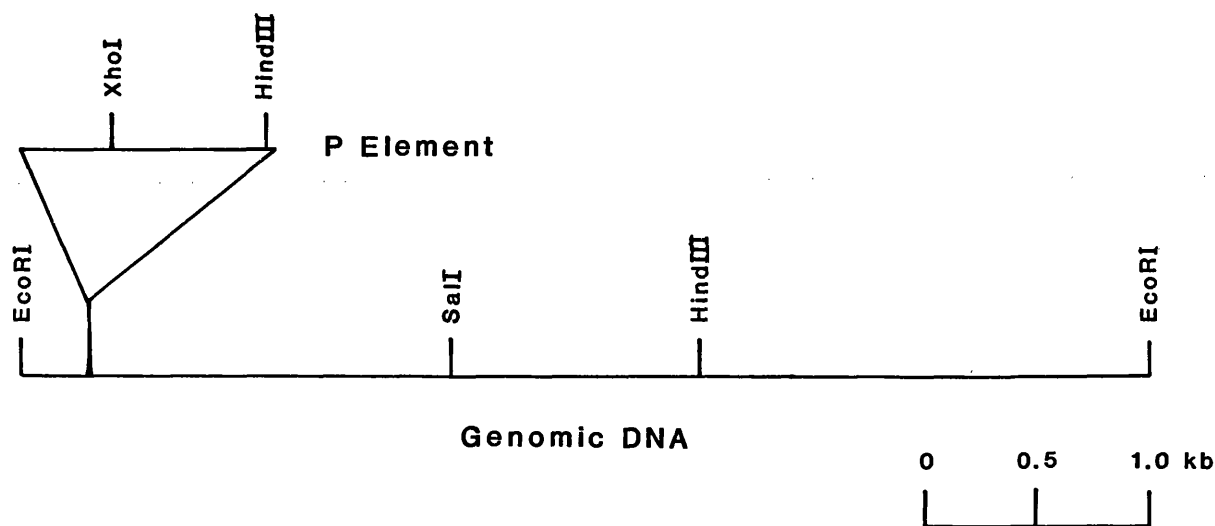


Figure 96 Restriction endonuclease map of the P element insert in the sn region of strain π 2 sn⁵

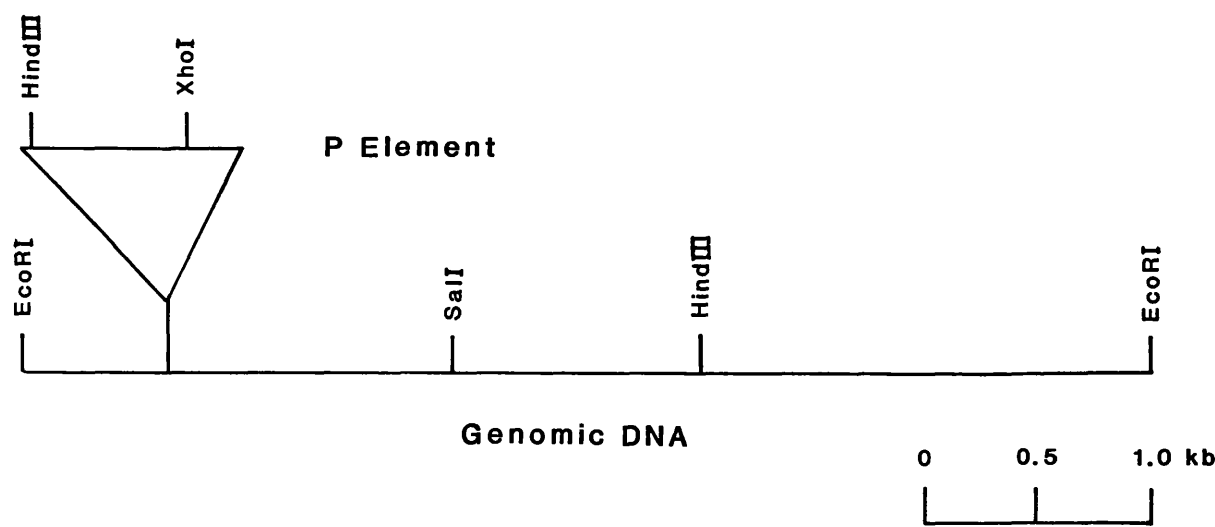
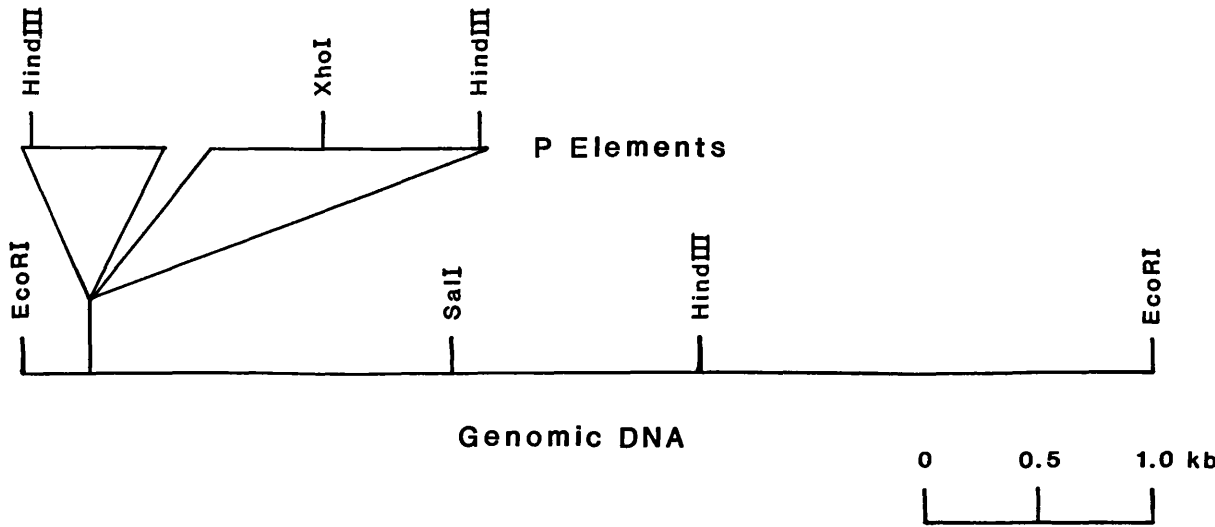


Figure 97 Restriction endonuclease map of the P element inserts in the sn region of strain sn^{VW}



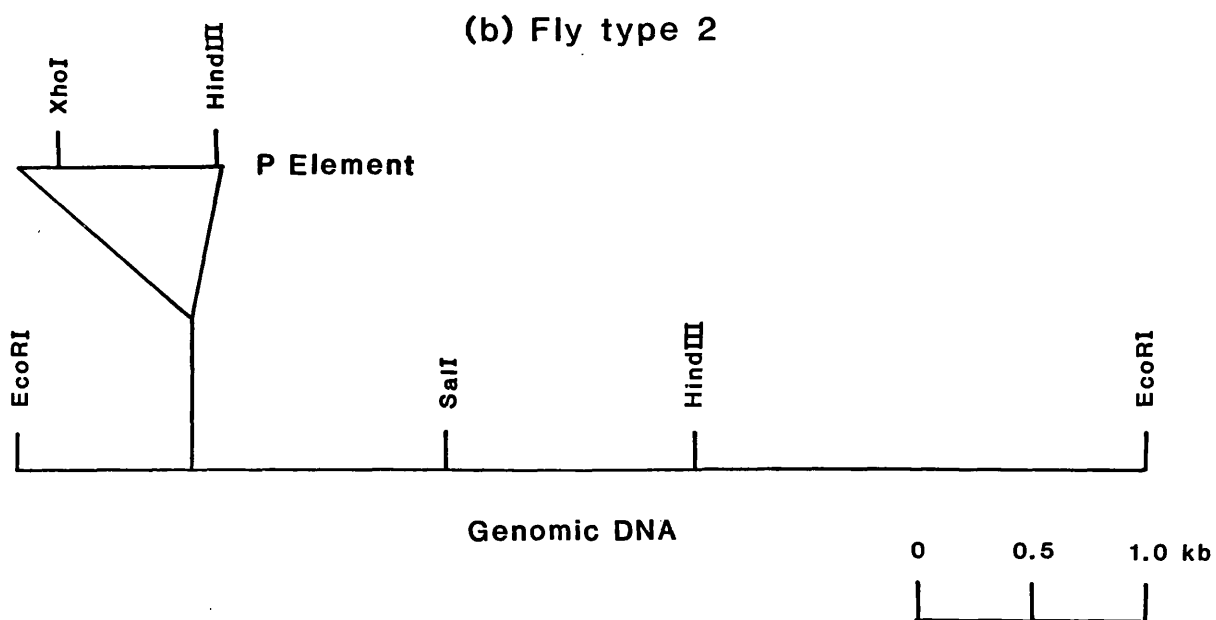
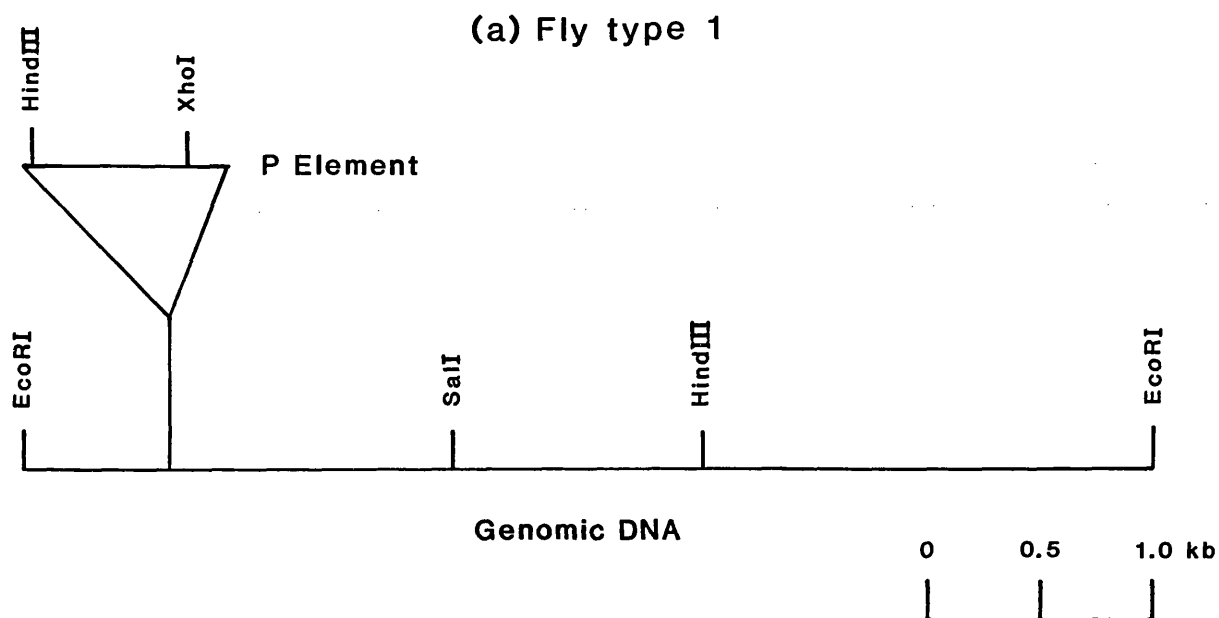
inserted in reverse orientation with their +39 bp HindIII sites outwards. They were positioned at the same site as the insertions of strains loua sn⁴, loua sn⁶ and π2 sn⁸. The strain was initially isolated as a partial germ cell revertant of loua sn⁶ (Brookfield and Lewis, submitted), and thus appears to have been produced by the insertion of a 0.65 kb element next to the original 1.25 kb insert of loua sn⁶. In addition to bands corresponding to both insertions in the autoradiograph, there were also some fainter bands which were consistent with the insertion of the 0.65 kb P element only. The sn^{vw} stock was maintained as males with C(1)DXyf females, and thus only adult males were collected for the analysis. In some of the males germ cell reversions had occurred producing, instead of the very weak sn phenotype of sn^{vw}, a strong sn phenotype. These flies had been discounted from the DNA isolation. It appears that these revertants were most probably due to the germline excision of the 0.65 kb P element producing a molecular organisation and sn phenotype similar to that of the loua sn⁶ strain from which originally derived. In the flies collected for analysis, however, there must also have been some revertants, but these flies were due to the germline excision of the 1.25 kb P element, and produced the weak bands in the autoradiograph. On reexamining the sn^{vw} stock it was found that there did appear to be some flies which were wild-type in phenotype for bristle morphology, although it was difficult to see without scrutinising the flies in detail, as the sn^{vw} phenotype was so weak. These flies may be the revertants generated by excision of the 1.25 kb P element.

Strain loua sn¹⁰ was found to be made up of two sets of

flies with different P element insertions (figure 98). Both inserts were 0.9 kb P elements with 2.0 kb deletions removing the EcoRI, SalI and +877 bp HindIII sites, but they were inserted in opposite orientations in two different sites. In the one case the element was positioned in the same site as in the strains $\pi 2$ sn¹, $\pi 2$ sn², loua sn⁵, $\pi 2$ sn³ and $\pi 2$ sn⁵ with its +39 HindIII site orientated towards the CSsn9 1.9 kb EcoRI-SalI fragment EcoRI site. The other flies, however, possessed elements in the opposite orientation positioned at a site within the 1.9 kb CSsn9 EcoRI-SalI genomic fragment, 0.8 kb away from the EcoRI site. Thus it appears that the original fly from which the line was derived, probably possessed the P element inserted at the common location, 0.65 kb from the EcoRI site. Due to an inversion, however, the element was flipped over into the opposite orientation. The inversion also extended into the genomic DNA 0.15-0.8 kb towards the SalI site, and 0.15 kb less than this distance in the opposite direction, such that the P element insertion site in the restriction endonuclease map was shifted 0.15 kb to the right. On examining the loua sn¹⁰ stock in more detail it was found that the two different types of fly could in fact be visually divided, one having a slightly more extreme sn phenotype than the other. They were thus separated into two stocks, loua sn^{w10} and loua sn^{s10}, although these have not been analysed molecularly to determine which have the different insertions.

Strain loua sn⁹ was found to possess a 0.65 kb insert with a 2.25 kb deletion removing the EcoRI, SalI, +877 bp HindIII and XhoI sites. The element only contained one site for the restriction endonucleases used in the digests, a HindIII site. For further analysis it was assumed that the

Figure 98 Restriction Endonuclease maps of the P element insert in the sn region of strain loua sn¹⁰



insert was a P element, and that the HindIII site of the element was the +39 bp site of the P factor (as all the other elements so far characterised have been P elements). It was impossible to orientate or site the element on the given data, there being two arrangements possible (figure 99). Suitable restriction endonucleases were therefore sought in order to solve the dilemma.

The P factor sequence (O'Hare and Rubin 1983) was fed into a computer and a programme run to search for restriction endonuclease sites. The enzyme PvuII was found to cut at two sites, +585 and +1481 bp. Assuming that the 0.65 kb loua sn⁹ P element only contained one deletion, it was possible for the element to possess the +585 bp PvuII site (the deletion being towards the +2907 bp end of the element), but not the +1481 bp site (the P element being too small). The restriction endonuclease SphI cut at only one site, +1883 bp, and the loua sn⁹ P element was not large enough to possess this site. The 1.9 kb EcoRI-SalI fragment of the CSSn9 EcoRI sn region contains one PvuII site, 1.15 kb from the EcoRI site (Roiha *et al.* 1987), and two SphI sites at distances of 0.6 and 1.5 kb from the EcoRI site (K. O'Hare, pers comm.).

In a double restriction endonuclease digest of EcoRI and PvuII, one can propose different results depending upon whether or not the loua sn⁹ P element contains the +585 bp PvuII site. If absent, one would expect the 1.15 kb CSSn9 EcoRI-PvuII fragment simply to be increased in length to 1.8 kb. If the site is present, however, one would expect one of two fragment banding patterns depending upon the position of the P element insertion. If inserted 0.1 kb from the EcoRI site (figure 99(a)) one would expect the 1.15 kb EcoRI-PvuII

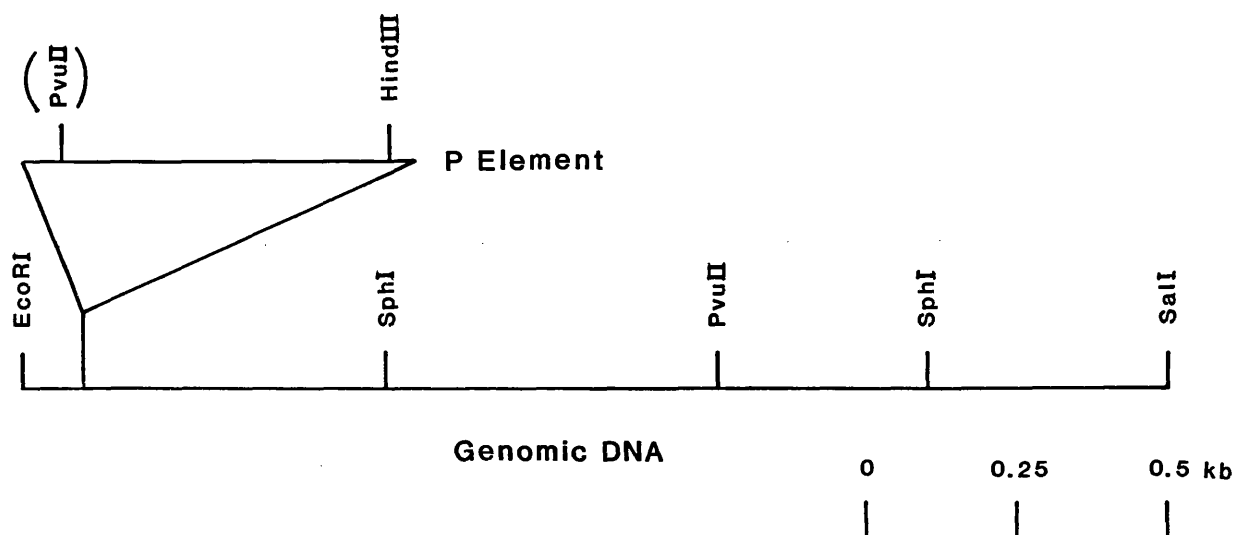
fragment to be split into a 0.15 and 1.65 kb fragments, but if inserted 0.65 kb from the EcoRI site (figure 99(b)), 0.55 and 1.25 kb fragments would result. Thus, if the P element does contain the PvuII site one will be able to deduce the insertion site of the element.

One can also use SphI to derive the P element insertion position. In an EcoRI, SphI double restriction endonuclease digest one would again expect different results in the two different cases. If inserted 0.1 kb from the EcoRI site (figure 99(a)) one would expect the 0.6 kb CSSn9 EcoRI-SphI fragment to be increased to a length of 1.25 kb. If inserted at the second site (figure 99(b)), however, the 0.9 kb SphI fragment would become 1.55 kb in length.

EcoRI, PvuII and EcoRI, SphI double restriction endonuclease digests of loua sn⁹ and Canton-S were carried out, and Southern hybridisations using the CSSn9 probe. The autoradiographs for the strains loua sn⁹ and Canton-S can be seen in figures 100(a) and 100(b) respectively. The 1.15 kb EcoRI-PvuII fragment (labelled A in figure 100) was increased in length to 1.8 kb, thus indicating that the loua sn⁹ P element insertion did not possess any PvuII restriction endonuclease sites. One could not therefore use this double digest to position the site of insertion. In the EcoRI, SphI digest, however, it was found that the 0.6 kb EcoRI-SphI fragment (labelled C in Figure 100) was not altered by the insert, but the 0.9 kb SphI fragment (labelled B in figure 100) was increased to 1.55 kb in length. Thus it was determined that the loua sn⁹ P element insertion was positioned approximately 0.65 kb from the EcoRI site with its +39 bp HindIII site orientated towards the same site. This can

Figure 99 Possible restriction endonuclease maps of the P element insert in the sn region of strain loua sn⁹

(a)



(b)

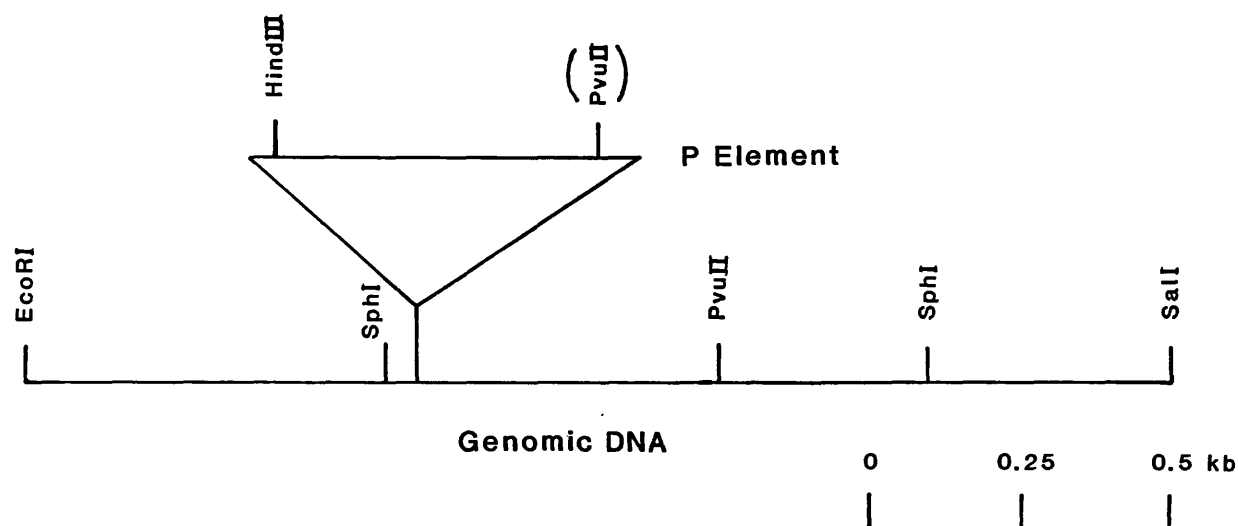


Figure 101 Restriction endonuclease map of the P element insert in the sn region of strain loua sn⁹ determined using EcoRI /PvuII and EcoRI/SphI digests

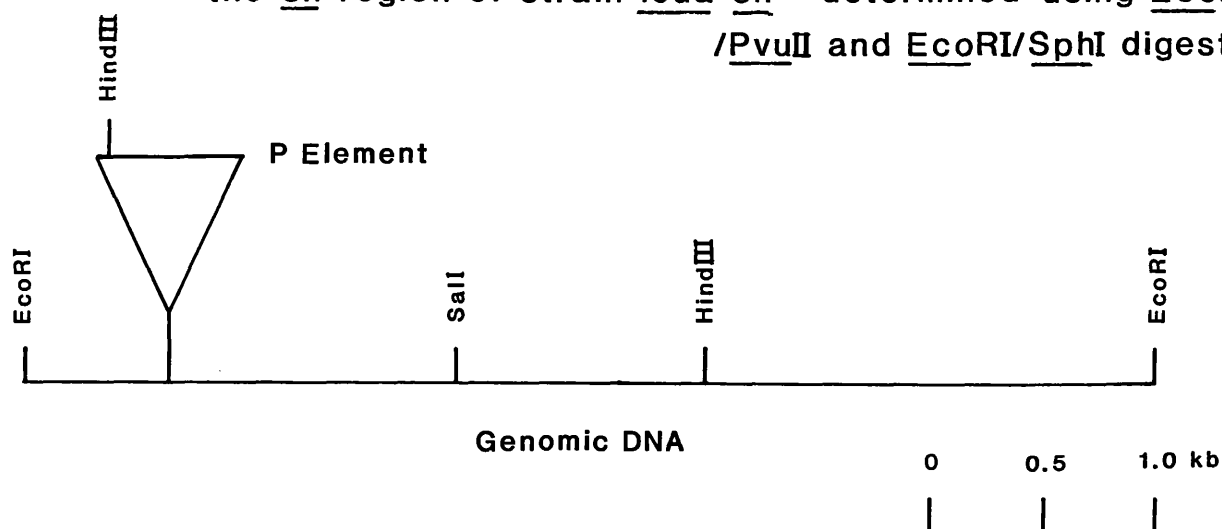


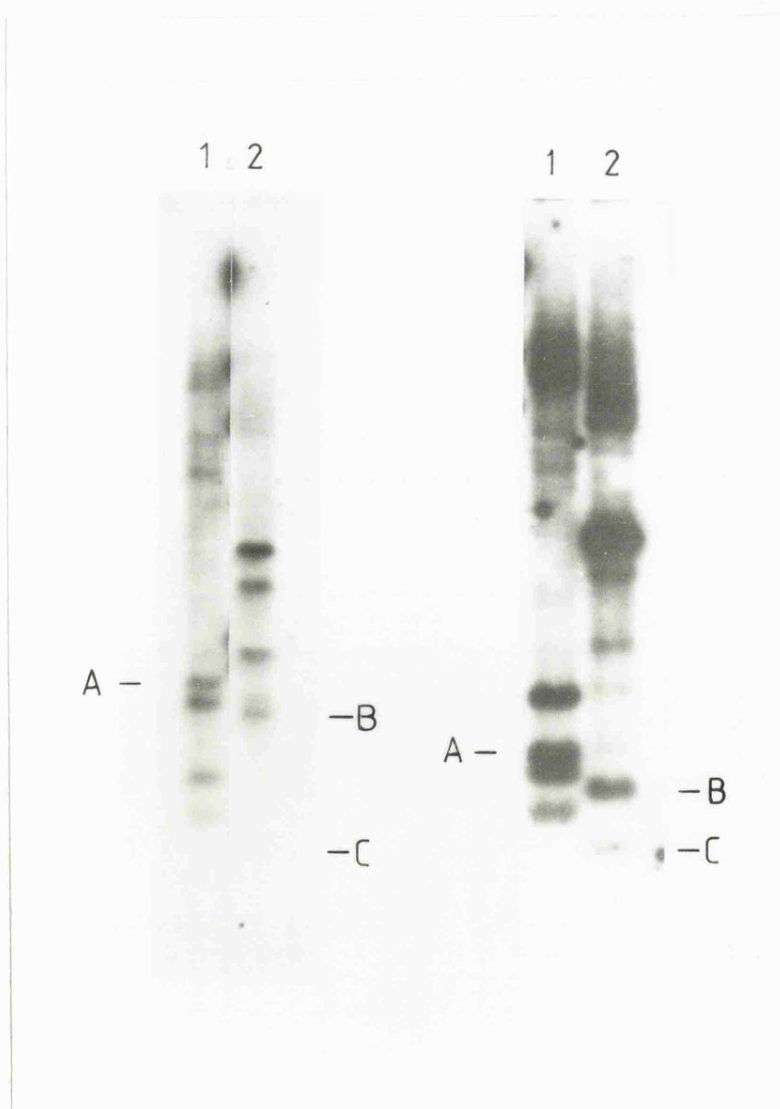
Figure 100: Blot hybridisation of loua sn⁹ and Canton-S genomic DNA treated with restriction endonucleases EcoRI, PvuII and SphI, and probed with CSsn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using nylon filters and protocol II. The DNA was digested with the following restriction endonucleases: Lanes 1- EcoRI, PvuII; 2- EcoRI, SphI.

(a) loua sn⁹ genomic DNA

A- 1.8 kb EcoRI-PvuII fragment
 B- 1.55 kb SphI fragment
 C- 0.6 kb EcoRI-SphI fragment

(b) Canton-S genomic DNA

A- 1.15 kb EcoRI-PvuII fragment
 B- 0.9 kb SphI fragment
 C- 0.6 kb EcoRI-SphI fragment



be seen in Figure 101.

4.3 Analysis Of singed mRNA From The singed Mutation Strains

Poly(A)⁺ RNA was isolated from a number of the sn strains and analysed, with respect to sn, to find what effects the P element insertions have on the sn gene mRNA species. It might be expected that in some cases the quantity of mRNA produced may be reduced, or in others, the size of the mRNA species altered. Such observations may help in determining why the different sn strains have different sn phenotypic strengths.

Pupal RNA was used in the analysis, as sn mRNA is more abundant at this stage of development (K. O'Hare, pers comm.). Unfortunately, however, this meant that in stocks in which the sn mutation was kept in males with C(1)DXy^f females, wild-type sn mRNA would be present in the pupal poly(A)⁺ RNA, produced from the C(1)DXy^f females. Most stocks chosen for analysis, therefore, (apart from sn^{vw}) were those in which both the males and females possessed the sn mutation.

Total RNA was isolated from the pupae of each of the strains Canton-S, sn^{vw}, loua sn^{w10}, loua sn⁶, π2 sn⁵, loua sn⁹, π2 sn³ and loua sn⁵, and poly(A)⁺ RNA subsequently prepared. The poly(A)⁺ RNA was run on a gel, approximately 0.2-2 μg per lane, and after electrophoresis, blotted onto a nylon filter. Length standards used consisted of an RNA ladder (BRL) of 6 RNA components derived from bacteriophage T7, yeast 2μ circle and bacteriophage λ DNA. The probe used was pBSP6 which contained a sn cDNA sequence cloned in a Bluescribe vector (a derivative of pUC19). This was oligo-labelled with

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 α -[^{32}P]-dCTP, and hybridised to the RNA on the filter using protocol III. The autoradiograph produced can be seen in figure 102(b).

Canton-S pupae were found to produce a sn mRNA transcript of approximately 3.4 kb in length, which agrees with results achieved by K. O'Hare (pers comm.). sn transcripts produced by the strains sn^{vw}, loua sn^{w10}, n2 sn⁵, loua sn⁹, n2 sn³ and loua sn⁵ were also of a similar size, but the 3.4 kb poly(A)⁺ RNA of loua sn⁶ was replaced by two larger transcripts of 3.7 and 4.1 kb.

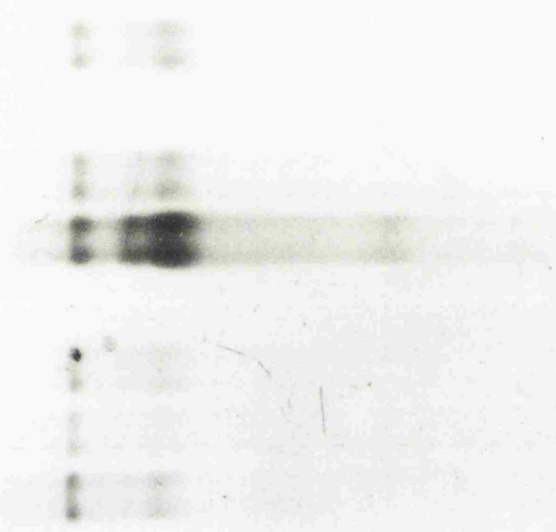
Figure 102(a) shows the poly(A)⁺ RNA's electrophoresed on the agarose gel, and thus can be used in a quantitative capacity to compare the relative amounts of mRNA run in each lane. The photograph is in a negative form, the RNA fluorescence showing up dark on a light background. The amount of fluorescence is proportional to the amount of ethidium bromide bound, which is in turn proportional to the quantity of RNA present. Thus, one can use the level of fluorescence in each lane as a rough guide to the amount of RNA loaded. The banding that can be seen in the lanes corresponds to ribosomal RNA not completely removed in the poly(A)⁺ isolation. The poly(A)⁺ RNA itself is present as a smear in the lanes.

Figures 102(a) and 102(b) were compared to determine the approximate levels of sn poly(A)⁺ RNA produced in each sn strain per fixed quantity of RNA originally loaded. The amount of Canton-S RNA loaded on the gel, when compared to the other strains, was very low, and yet this lane possessed the most abundant sn transcript. Thus it appears that the level of mRNA transcript from sn was reduced in each of the sn strains. A very rough quantitative assessment of the RNA concentrations

Figure 102: Northern blot hybridisation of singed mRNA from Canton-S and singed mutation strains with the probe PBSP6. The probe was oligo-labelled, and hybridised to the mRNA's using a nylon filter and protocol III. The lanes were loaded with the poly(A)⁺ RNA from the following strains: Lanes 1 - Canton-S; 2 - sn^{VW}; 3 - loua sn^{w10}; 4 - loua sn⁶; 5 - π2 sn⁵; 6 - loua sn⁹; 7 - π2 sn³; 8 - loua sn⁵.

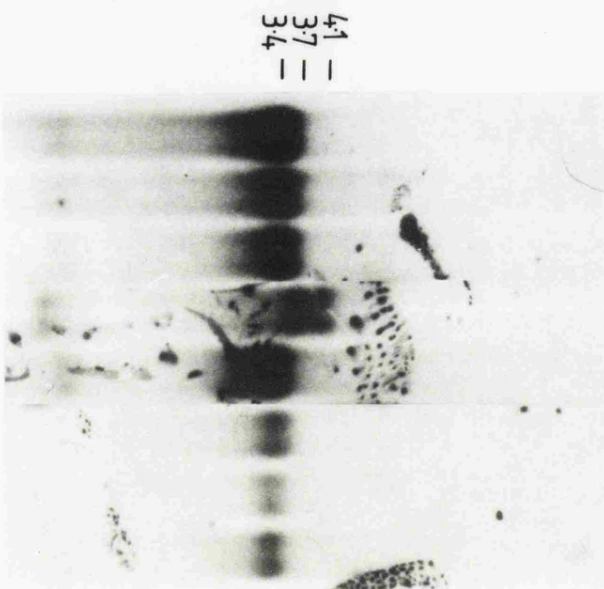
(a) mRNA visualised on the electrophoresed gel

1 2 3 4 5 6 7 8



(b) Autoradiograph

1 2 3 4 5 6 7 8



was carried out by estimating relative intensities of bands on the gel photograph and autoradiograph. The Canton-S lane was taken as a base level for the gel calculations, and $\pi 2 \text{ sn}^3$ as a base in the autoradiographs. The band intensities of the other lanes were estimated as multiples of these levels. From these figures the relative decrease in sn transcript, per fixed level of total poly(A)⁺ RNA, was calculated for each strain, compared to the wild-type level of Canton-S (figure 103).

The least reduction in the level of the 3.4 kb transcripts produced was seen in sn^{vw} where 5/16th of the level of Canton-S mRNA was observed. The abundance of sn poly(A)⁺ RNA is high in this strain as presumably half the pupae used were C(1)DXy^f females, wild-type at the sn locus. Loua sn⁵ was at the other end of the scale with a 32-fold reduction, and $\pi 2 \text{ sn}^3$, loua sn⁹, $\pi 2 \text{ sn}^5$ and loua sn^{w10} possessed intermediate levels of 1/24th, 1/20th, 1/13th and 1/8th of the amount of Canton-S, respectively. In loua sn⁶ the 3.7 kb poly(A)⁺ RNA was found to have a level 21 times lower than that of the wild-type Canton-S transcript (labelled lower in figure 103), whilst the 4.1 kb species was only 1/32nd as abundant (labelled upper in figure 103). It must be emphasised that these figures are only rough estimates due to the method used for determining the relative band intensities, that is, by eye. However, they do give a good indication as to the order of magnitude of the sn transcript reductions in the different strains.

Figure 103: Rough estimation of the reduce in level of sn poly(A)⁺ in the sn strains when compared to Canton-S.

RNA GEL: The RNA in the Canton-S lane was designated as having an intensity of 1 unit, and the intensities of the other lanes roughly estimated from this base.

Lane	1	2	3	4	5	6	7	8
Strain	Canton-S	<u>sn</u> ^{vw}	<u>loua</u> <u>sn</u> ^{w10}	<u>loua</u> <u>sn</u> ⁶	<u>π2</u> <u>sn</u> ⁵	<u>loua</u> <u>sn</u> ⁹	<u>π2</u> <u>sn</u> ³	<u>loua</u> <u>sn</u> ⁵
Intensity (Units)	1	$\frac{2}{5}$	$\frac{3}{6}$	$\frac{4}{8}$	$\frac{5}{10}$	$\frac{6}{5}$	$\frac{7}{3}$	$\frac{8}{7}$

AUTORADIOGRAPH: The sn mRNA band in the π2 sn³ lane was designated as having an intensity of 1 unit, and the intensities of the other lanes roughly estimated from this base.

Lane	1	2	3	4	5	6	7	8
Strain	Canton-S	<u>sn</u> ^{vw}	<u>loua</u> <u>sn</u> ^{w10}	<u>loua</u> <u>sn</u> ⁶	<u>π2</u> <u>sn</u> ⁵	<u>loua</u> <u>sn</u> ⁹	<u>π2</u> <u>sn</u> ³	<u>loua</u> <u>sn</u> ⁵
Intensity (Units)	8	$\frac{5}{5}$	$\frac{6}{6}$	$\frac{2}{3}$ (upper) 3 (lower)	$\frac{6}{6}$	$\frac{2}{2}$	$\frac{1}{1}$	$\frac{1.75}{1.75}$

$$\text{Fraction of wild-type level} = \frac{\text{Canton-S total poly(A)}^+}{\text{sn strain total poly(A)}} \times \frac{\text{RNA intensity}}{\text{sn strain sn mRNA intensity}}$$

Strain	<u>sn</u> ^{vw}	<u>loua</u> <u>sn</u> ^{w10}	<u>loua</u> <u>sn</u> ⁶	<u>π2</u> <u>sn</u> ⁵	<u>loua</u> <u>sn</u> ⁹	<u>π2</u> <u>sn</u> ³	<u>loua</u> <u>sn</u> ⁵
Fraction of wild-type level	$\frac{5}{16}$	$\frac{1}{8}$	$\frac{1}{32}$ (upper) $\frac{1}{21}$ (lower)	$\frac{1}{13}$	$\frac{1}{20}$	$\frac{1}{24}$	$\frac{1}{32}$

Chapter 5 : Attempts To Clone The P Element Insertions At The singd Locus In Strain singd-(very weak)

5.1 singd-(very weak) And singd-weak

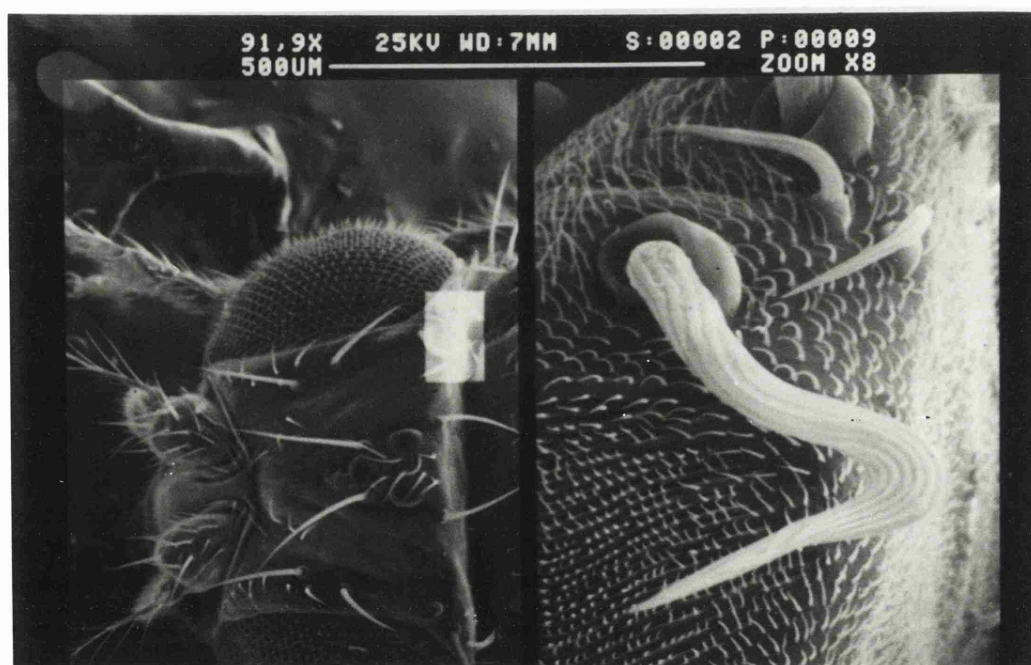
The strain singd-(very weak) (sn^{vw}) possessed a number of interesting features that prompted a more detailed investigation into its structure. It was initially isolated as a partial germ cell revertant of loua sn⁶ (Brookfield and Lewis, submitted), its origin being the insertion of a 0.65 kb presumably P element adjacent to the 1.25 kb element at sn of loua sn⁶ (see Chapter 4). The two elements were inserted in reverse orientation with their +39 bp HindIII restriction endonuclease sites facing away from each other. From restriction endonuclease maps, however, it was impossible to determine how long a stretch of genomic DNA, if any, separated their adjacent termini. The strain sn^w has been analysed at the molecular level (Roiha et al. 1987), and possesses a structure very similar to that of sn^{vw}. Two P elements of 0.95 and 1.15 kb were found to be inserted at the same site, within the 1.9 kb EcoRI-SalI fragment of the CSsn9 EcoRI sn region fragment, at a position 582 to 589 bp from the EcoRI site. They were again inserted in reverse orientation, but with their +39 HindIII sites towards each other, and were found to be separated by just the 8 bp duplicated target site (which would normally flank the elements if only 1 was present).

sn^{vw} was found to revert to a strong sn phenotype in germ cells, and also to a possibly wild-type phenotype, at quite a high frequency. This was thought to be due to, in the former case, the excision of the 0.65 kb P element, thus

producing the loua sn⁶ structure, and in the latter, the excision of the 1.25 kb element. The strain was also found to be able to be somatically destabilised, but at a frequency 4-fold greater than any other sn strain. The mosaic areas observed in such flies, however, consisted of strong sn phenotypic bristles within the very weak bristles of the sn^{vw} phenotype. An example of such an area can be seen in figure 104. These somatic reversions are again probably the result of 0.65 kb P element excisions at the sn locus, and thus one would also expect excision of the 1.25 kb element to occur. Such wild-type patches of bristles upon the very weak sn phenotypic background, however, would be impossible to observe. If such excision events were occurring, and with as high a frequency as that of the 0.65 kb P element, then the somatic destabilisation rate would be doubled, thus becoming perhaps 8-fold greater than any other mosaic strain. sn^w is again very similar to sn^{vw} in that, although its somatic destabilisation frequency is negligible, its germline reversion can be as great as 50% in a dysgenic cross (Engels 1979). It mutates to either a wild-type or to a more extreme sn phenotype, the former state being the result of precise excision of the 1.15 kb P element at sn, and the latter, the excision of the 0.95 kb element (Roiha et al. 1987). Other alleles of sn have been described (Golubovsky et al. 1977; Golubovsky 1978) which again show mutability to both wild-type, or near wild-type, and to more extreme phenotypes. The mutations are thought to be due to transposable element insertions, and such characteristics suggest that they may again be due to double insertions of P elements.

sn^{vw} and sn^w thus seem to share certain characteristics.

Figure 104: Scanning electron micrograph of a region of sn bristles affecting the orbital bristles of a sn^{vw} mosaic male.



They are both thought to be caused by double insertions of P elements in reverse orientation at the sn locus, but located at different sites in the two strains. Both are hypermutable, although sn^{vw} being especially so with somatic reversions, and sn^w with germline events, and in both cases the P elements may perhaps excise singly to produce a wild-type or extreme bristle phenotype.

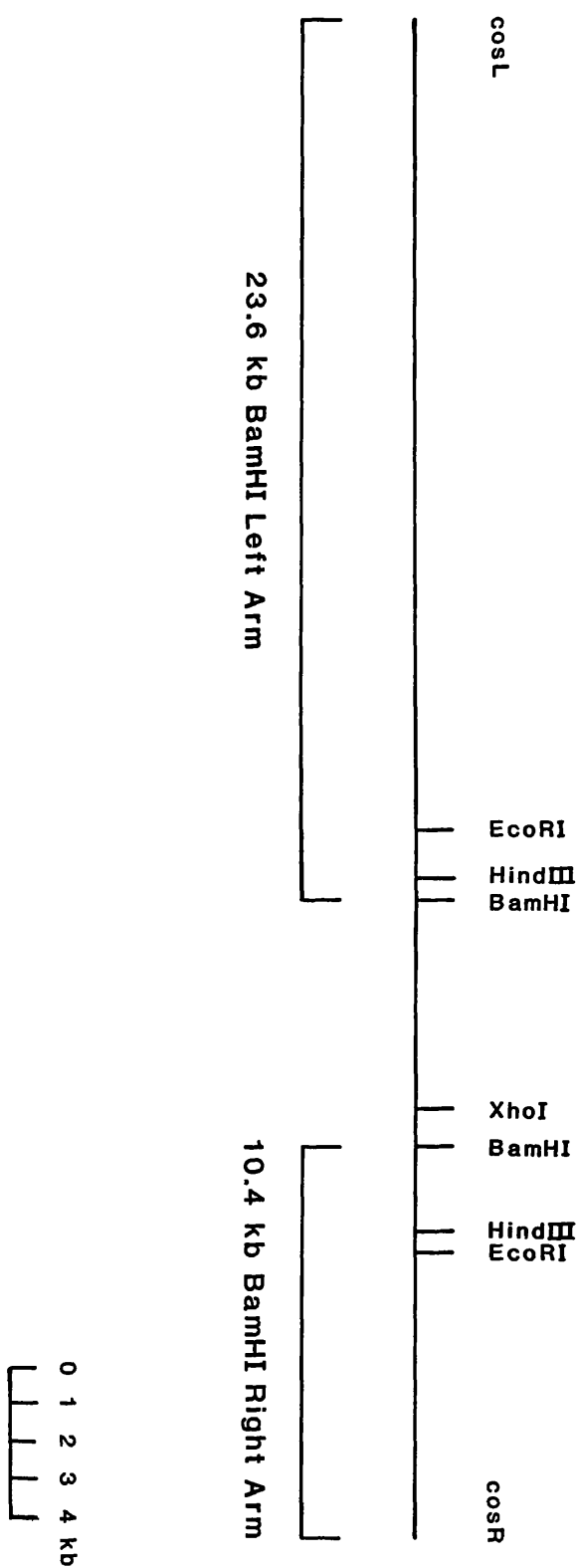
An attempt was made to clone the P elements at the sn locus of the sn^{vw} strain so that initially they could be mapped by restriction endonuclease digestion in more detail, using a P factor probe instead of the CSsn9 probe. Only a limited amount of information can be achieved by trying to map P elements indirectly with flanking DNA probes, and yet one can not use a P factor probe against genomic DNA for mapping purposes, due to the presence of other P elements, in addition to those at sn, in the genome. Once cloned, however, elements can be characterised with such a probe, the additional elements having been eradicated. Cloning was also necessary to investigate the junction between the two element inserts in sn^{vw}, to determine the length of the interposing DNA sequence, and to see if, like sn^w, it was the 8 bp target sequence only. A number of P element target sites have been sequenced by Roiha et al. (1987), all within 100 bp of each other in the 1.9 kb EcoRI-SalI fragment of the CSsn9 EcoRI sn region fragment, at about 0.65 kb from the EcoRI site. The P elements in sn^{vw}, however, are located at a position approximately 0.3 kb from the EcoRI site, and thus cloning of the elements would allow the sequencing of this new target site.

5.2 The Preparation And Initial Screening Of singed-(very weak) Genomic Libraries

The replacement vector, bacteriophage λ L47.1 (Loenen and Brammar 1980), was used for construction of the genomic libraries, a restriction endonuclease map of which is shown in figure 105. The middle portion of the λ genome is not essential for lytic growth, and can thus be replaced by foreign DNA. The viability of phages, however, is dramatically reduced when DNA longer than 105% or shorter than 78% of the wild-type λ genome is packaged, and thus the sizes of vector and foreign DNA used in the cloning process are crucial. λ L47.1 is 40.6 kb long, and possesses two BamHI sites which can be used to remove a 6.6 kb fragment from the central portion of the vector, leaving two arms of 23.6 and 10.4 kb. Wild-type bacteriophage λ is approximately 50 kb in length, and thus one can use the BamHI arms of λ L47.1 to package DNA fragments of between 5 and 18.5 kb. Sau3A produces similar termini to BamHI, and cuts DNA, on average, every 256 bp. It can thus be used to partially digest foreign genomic DNA, producing fragments of the correct sizes for ligation between the λ L47.1 arms, and then for packaging in vitro with bacteriophage λ packaging extracts.

λ L47.1 DNA was isolated from phage, and 100 μ g digested with BamHI. Samples were run on 0.5% agarose gel to ensure that the DNA was completely digested into the 3 fragments. A deep 0.5% agarose gel was prepared on a 1% agarose gel base, using 0.5 x TAE electrophoresis buffer, with a glass plate clamped vertically to produce a wide slot large enough to accomodate the volume of digested DNA. Ethidium bromide was

Figure 105 Restriction Endonuclease Map Of Bacteriophage λ Replacement Vector λ L47.1



added to the DNA, to a concentration of approximately 250 $\mu\text{g/ml}$, which was run on the gel in the dark using 0.5 x TAE buffer. The 23.6 and 10.4 kb BamHI fragments were recovered from the gel by electroelution onto dialysis membrane, and after ethanol precipitation redissolved in 20 μl 10 mM Tris-HCl, pH 7.5. The cohesive termini of the $\lambda\text{L47.1}$ arms were annealed by adding 2 μl 1 M Tris-HCl, pH 7.5 and 1 μl 0.2 M MgCl_2 , and incubating for 1 hour at 42°C. A sample was run on a 0.5% gel at low voltage (high voltage tends to melt the cohesive ends) and was found to have annealed.

The sn^{vw} mutation was maintained as males with C(1)DXyf females, and thus genomic DNA was isolated from adult males only. 20 μg was partially digested with Sau3A to produce a smear of DNA fragments, the greatest mass of which was in the 10-20 kb range. This was tested by initially digesting 1 μg with 0.2 units of Sau3A, and removing and testing samples every couple of minutes, until the correct period of digestion was determined. The reaction was then scaled up to digest the remaining DNA. A similar gel to that used for recovering the $\lambda\text{L47.1}$ arms was constructed, except that two small slots were also prepared, one either side of the wide slot. The partially digested DNA was run in a similar manner to the $\lambda\text{L47.1}$, together with HindIII digested bacteriophage λ markers in the flanking slots. DNA fragments of between 10 and 20 kb were recovered from the gel by electroelution onto dialysis membrane, and finally redissolved in 20 μl 10 mM Tris-HCl, pH 7.5.

The $\lambda\text{L47.1}$ annealed arms were ligated to the genomic fragments using the μl volume ratios 1:1, 1:0.5 and 1:0.2 (arms:partials) in a total reaction volume of 20 μl . A control

was also carried out, with λ L47.1 arms only in the ligation, in order to check that no intact, undigested λ L47.1 had been recovered in the arm isolation procedure. FTL and SE packaging extracts were prepared, and used to in vitro package half of each ligation reaction. 0.1 μ g of intact λ L47.1 DNA was also packaged to test the efficiency of the packaging extracts. Each packaging reaction of ligated DNA was added to 100 μ l LB and plated out with 150 μ l of E. coli WL95. 1 μ l of the λ L47.1 packaging reaction was diluted 10^2 , 10^3 , 10^4 and 10^5 -fold in LB, and each dilution plated out with 150 μ l of E. coli WL87. 1 μ l of the packaged λ L47.1 was also plated out with 100 μ l LB on 150 μ l of E. coli WL95 to check the WL95 phenotype (small plaques should be produced). E. coli WL87 and WL95, and LB only, were also plated out as controls, to test for any contamination.

The plates were observed after overnight incubation at 37°C. The plate possessing the packaged λ L47.1 arms contained approximately 50 plaques, thus suggesting that the isolated arms were reasonably pure, with little intact λ L47.1 DNA present. The number of plaques on the plates containing the packaged intact λ L47.1 dilutions were counted, and an efficiency of approximately 5×10^6 plaque forming units per μ g of DNA calculated. No plaques, however, were visible on any of the genomic library plates, apart from a number of plaques corresponding to the intact λ L47.1 DNA in the arms solution, even though all of the other controls were as expected. The results thus suggested that the genomic DNA/ λ arms ligation reactions had not worked.

Three ligations were set up, one containing sn^{vw} genomic partials and λ L47.1 arms, and two others with just the

partials or arms separately. After incubating overnight, samples were run on a 0.5% gel to determine whether the DNA had been ligated to form large catenates. There was found to have been no ligation of the genomic partials, and only a minor amount of ligation of the λ L47.1 arms, thus suggesting that the DNA isolation procedures were not producing DNA of a pure nature, but instead perhaps DNA possessing destroyed restriction endonuclease termini.

20 μ g of sn^{vw} genomic DNA was partially digested and electrophoresed as above, and 10-20 kb fragments recovered from the gel by electroelution in a Schleicher and Schnell biotrap. The DNA fragments were ethanol precipitated, and redissolved in 20 μ l 10 mM Tris-HCl, pH 7.5. λ L47.1 arms were kindly provided by A. J. Jeffreys, and were tested for the presence of intact λ L47.1 by ligation, packaging, and plating out with E. coli WL95. 100-150 plaques were observed per plate, thus implying a reasonably pure λ L47.1 arms preparation.

Ligation reactions were carried out of the new genomic partials to both sets of λ L47.1 arms, using ratios as above. Half of each reaction was in vitro packaged and plated out on E. coli WL95. Approximately 500 plaques were observed on the overnight plates produced from the 1:1 ligations achieved using the arms made by myself, and approximately 10^4 per plate from the 1:1 ligations with the arms obtained from A. J. Jeffreys. It thus appeared that the new sn^{vw} partials, recovered using the biotrap, were of a reasonable purity for the preparation of genomic libraries. The second half of the ligation reaction, produced using the λ arms from A. J. Jeffreys, was packaged and plated out with E. coli WL95, and a

second plate thus obtained possessing approximately 10^4 plaques. The λ arms prepared by myself were discarded.

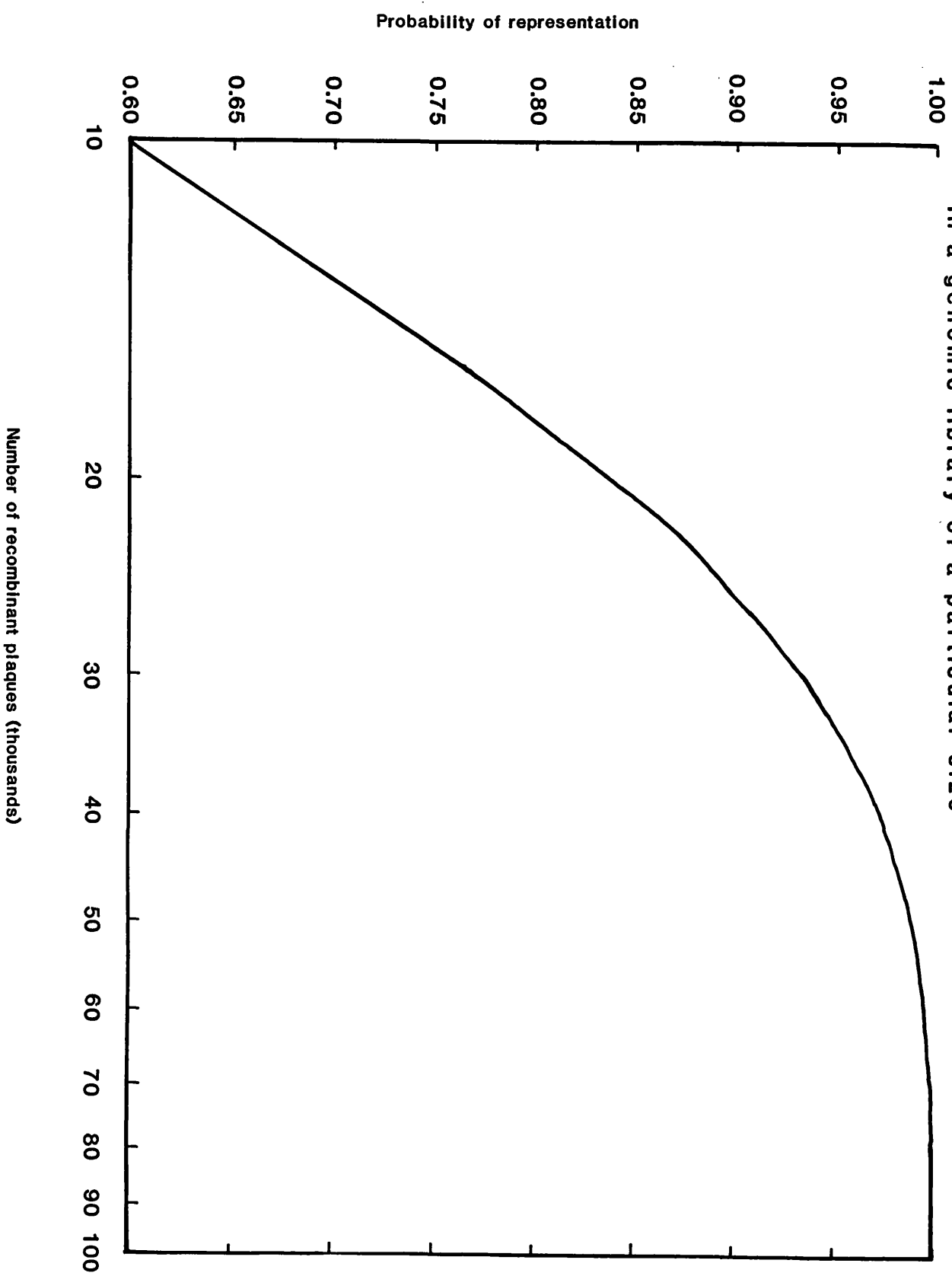
One can calculate the probability of having a DNA sequence represented in a genomic library using the formula:

$$N = \frac{\ln(1 - P)}{\ln(1 - f)}$$

where N is the necessary number of recombinants, P the desired probability, and f the fractional proportion of the genome in a single recombinant (Clarke and Carbon 1976). The D. melanogaster genome is 165000 kb in length, and one expects an average length of genome in a single recombinant of approximately 15 kb. Using a value of 15/165000 for f, one can produce a graph of probability of representation against necessary number of recombinants (figure 106). This can be used to calculate the probability of possessing the desired DNA sequence in a genomic library of 20000 recombinants. A value of 0.84 was determined.

The plaques on the two library plates were lifted onto Hybond-N membrane filters, which were hybridised with α -[32 P]-dCTP oligo-labelled CSSn9 probe using hybridisation protocol III, to screen for the sn region in sn^{vw} containing the P elements. The alignment marks on the membrane filter were labelled by spotting with radioactive ink. On observation of the autoradiograph produced, however, no positive plaques were found. The filters were therefore stripped of CSSn9 probe, by incubating at 45°C for 30 minutes in 0.4 M NaOH followed by 30 minutes in 0.1 x SSC, 0.1% (w/v) SDS, 0.2 M Tris-HCl, pH 7.5 at 45°C, and reprobbed with p π 25.1 to screen for P element sequences. Presuming that strain sn^{vw} possesses

Figure 106: A graph to show the probability of a desired DNA sequence being represented in a genomic library of a particular size



30-50 P elements, one would definitely expect to pick up a number of positive plaques if the libraries actually contained sn^{vw} genomic DNA. 12 positives were observed, thus confirming the presence of P elements, and thus genomic DNA, in the library.

Three further ligations were set up using a 1:1 μ l volume ratio of the λ L47.1 arms and the sn^{vw} genomic Sau3A partials recovered using the biotrap, in order to create a larger library. However, after packaging and plating each half of the ligation reactions (thus producing 6 plates), and incubating overnight, it was found that only a few hundred plaques were produced on the E. coli WL95 plates. The λ L47.1 arms and sn^{vw} partials were thus again ligated to each other, and themselves, and samples run on a 0.5% gel to check whether the DNA had ligated. The λ arms were discovered to have ligated, but not the genomic partials, thus suggesting that the partials solution was again impure, and that perhaps the restriction endonuclease termini had been degraded, but after a period of time.

A number of further solutions of sn^{vw} genomic partials were prepared, using the Schleicher and Schnell biotrap for their recovery and changing various solutions, and genomic libraries were constructed using the λ L47.1 arms. In some cases small libraries were made at first, but on reuse the partials were unable to ligate, and in other circumstances no plaques were produced from the DNA, even from the initial ligation. Approximately 10^5 plaques had been produced in total from these library plates, and these were lifted and screened for the presence of sn sequences using hybridisation protocol III and oligo-labelled CSsn9. There was calculated to be a

probability of 0.9999 of the correct sequence being represented in the library, but no positive plaques were found.

It was discovered that the phenol/chloroform stock solution, that had been used in the above DNA preparations, was defective, and had been contaminating the DNA instead of purifying it. A new stock solution was therefore prepared, and genomic DNA again isolated from sn^{vw} male adult flies.

The probability of a sequence being represented in 10^5 recombinants of a D. melanogaster genomic library is 0.9999, and yet no positive plaques were found in the screen above. The reason for such a result could be due to the number of Sau3A sites present within the CSsn9 genomic DNA and flanking regions. Genomic regions containing very few Sau3A sites will require more complete digestion than regions with many Sau3A sites to be represented in a given fragment size. If the CSsn9 genomic DNA, for example, possessed many Sau3A sites compared to the average number of sites per stretch of D. melanogaster DNA, then one might expect the Sau3A fragments from this region to be represented in the Sau3A partial digest smear beneath the 10 kb fragment band, and thus would not be recovered from the gel. The sn^{vw} genomic DNA was thus partially digested with Sau3A in 6 different reactions. A test digest was run initially to determine the time taken to produce a smear with the greatest mass of DNA fragments in the 10-20 kb range, and then 5 similar reactions, but with incubation periods greater and less than the determined time. Regions of DNA with many Sau3A sites would thus be digested to fragments of the required length in the digests of short incubation, whilst those with few sites would produce

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fragments of a similar length in the longer digests. The different digests were pooled, electrophoresed, and 10-20 kb fragments recovered using the biotrap protocol. A sample was ligated to itself, and run on a 0.5% gel. Large DNA catenates were observed, produced by the ligation of the genomic partials.

The λ L47.1 arms obtained from A. J. Jeffreys had been used up in the previous ligation reactions, and thus λ L47.1 DNA was isolated from phage and new arms prepared, again using the biotrap in the DNA recovery procedure. The cohesive termini of the arms were annealed, and the arms then tested for their ability to ligate. Large DNA fragments were produced.

The λ L47.1 arms were ligated to the sn^{VW} genomic Sau3A partials at a ratio of 1:1 μ l volumes, and the λ arms also ligated to themselves as a control. Half of each ligation reaction was packaged, and plated out on E. coli WL95 overnight. The control plates only possessed about 50 plaques each, thus indicating that the λ L47.1 arms were reasonably pure with little intact, undigested λ L47.1 DNA present. The library plates each contained in excess of 10^4 plaques, and thus further ligations and packagings were carried out, using a similar arms to partials ratio, to produce a library of approximately 300000 recombinants. The library was lifted, and screened for sn genomic CSsn9 DNA using hybridisation protocol III and oligo-labelled CSsn9 probe. The probability of the correct sequence being represented in the library was 1.000, and on investigation 4 positive plaques were found which were named λ SVW1, λ SVW2, λ SVW3 and λ SVW4.

5.3 Analysis Of Genomic Library Plaques Positive For CSsn9 Genomic Sequences

Positive recombinants were picked for 2nd and 3rd cycle screening to produce stocks containing each positive phage only, and no other recombinants. The petri dishes and autoradiographs were aligned using the reference spots, and a pin driven through the positive plaque positions on the autoradiographs to mark the undersides of the plates. The plaques were picked using the narrow end of a pasteur pipette, and each put into 500 μ l LB. Large enough areas were picked to ensure inclusion of the positives. One drop of chloroform was added to each suspension, which was gently mixed. 10 μ l was diluted 10-fold and 100-fold, and 100 μ l of each phage solution plated out with 50 μ l of ED8910 and 50 μ l LUB plus 10 mM $MgSO_4$.

A dense, but not confluent, plate from each positive recombinant was lifted, and screened with α -[^{32}P]-dCTP oligo-labelled CSsn9 probe using hybridisation protocol III. There was found to be between 5 and 20 positive plaques per plate, and because the plates were not too dense, on alignment one could restrict the plaque positions on the autoradiographs to individual plaques on the plates. 3 positive plaques were picked per plate, and each put into 500 μ l LB with a drop of chloroform. The suspensions were diluted, and plated out on ED8910 as above. Plates possessing clear separate plaques were lifted (one plate from each positive), and screened as above. In each case all plaques were found to be positive, and thus stocks were produced, one for each of the 4 initial positive recombinants. LB was pipetted onto the plates possessing all

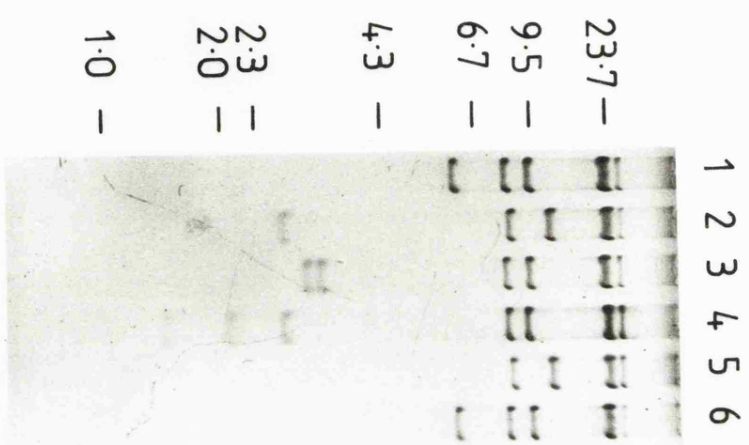
positive plaques, 1.5 ml per plate, and left for 1 hour with occasional shaking. The suspensions were decanted, and spun for 2 minutes at 13 krpm in an MSE Micro Centaur to pellet the cells. The supernatants were each stored at 4°C above 2 drops of chloroform.

DNA was prepared from each of the positive recombinant λ phage, λ SVW1, λ SVW2, λ SVW3 and λ SVW4, and was digested with the restriction endonucleases, EcoRI, HindIII, SalI and XhoI, both singly, and in a variety of double combinations. The digested DNA was run on gels, and Southern blotted onto Hybond-N nylon filters which were hybridised with α -[³²P]-dCTP oligo-labelled CSsn9 probe (using protocol III). HindIII digested bacteriophage λ length standards (BRL) were used for sizing the DNA. The autoradiographs produced can be seen in figures 107-110, together with photographs of the gels from which derived. Restriction endonuclease maps were constructed from the data (figures 111-114).

Each of the recombinant λ clones was found to contain DNA sequences from the 2 kb HindIII-EcoRI fragment end of the CSsn9 genomic DNA, but none possessed the P elements, the genomic DNA stopping short of the insertion site in each case. λ SVW1 possessed 10.9 kb of sn^{vw} genomic DNA cloned in the BamHI site of the λ L47.1 arms, which included 4.1 kb of the CSsn9 sequence, the cloned DNA stopping 0.6 kb short of the P elements' insertion site. λ SVW2 recombinants contained 10.35 kb of the sn^{vw} genome, of which only 1.35 kb was CSsn9 genomic DNA. The clones λ SVW3 and λ SVW4 possessed 16.3 and 13.7 kb of the sn^{vw} genomic sn region, of which in both cases, 4.7 kb consisted of DNA present in CSsn9. One end of the cloned DNA in these recombinants was thus terminated at a site which, by

Figure 107: Blot hybridisation of genomic DNA from the recombinant λ clone λ SVW1 with the probe CSn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol III. The DNA was digested with the following restriction endonucleases: Lanes 1 - EcoRI; 2 - HindIII; 3 - EcoRI, SalI; 4 - EcoRI, HindIII; 5 - HindIII, SalI; 6 - EcoRI, XhoI.

(a) DNA visualised on the electrophoresed gel



(b) Autoradiograph

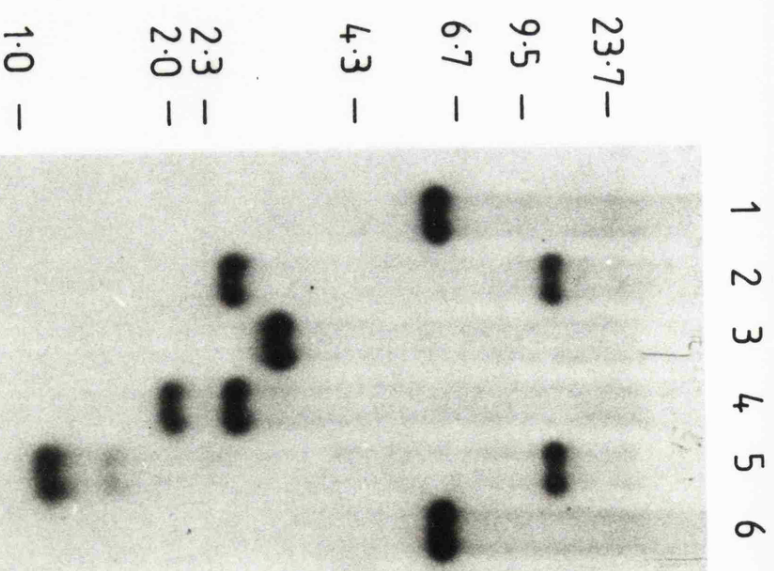
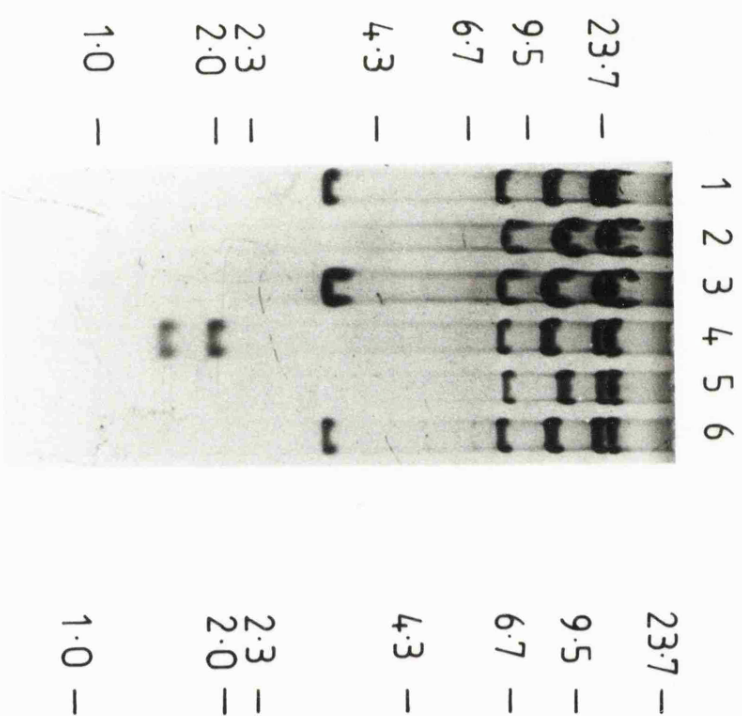


Figure 108: Blot hybridisation of genomic DNA from the recombinant λ clone λ SVW2 with the probe CSSn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol III. The DNA was digested with the following restriction endonucleases: Lanes 1 - EcoRI; 2 - HindIII; 3 - EcoRI, SalI; 4 - EcoRI, HindIII, SalI; 5 - HindIII, SalI; 6 - EcoRI, XhoI.

(a) DNA visualised on the electrophoresed gel



(b) Autoradiograph

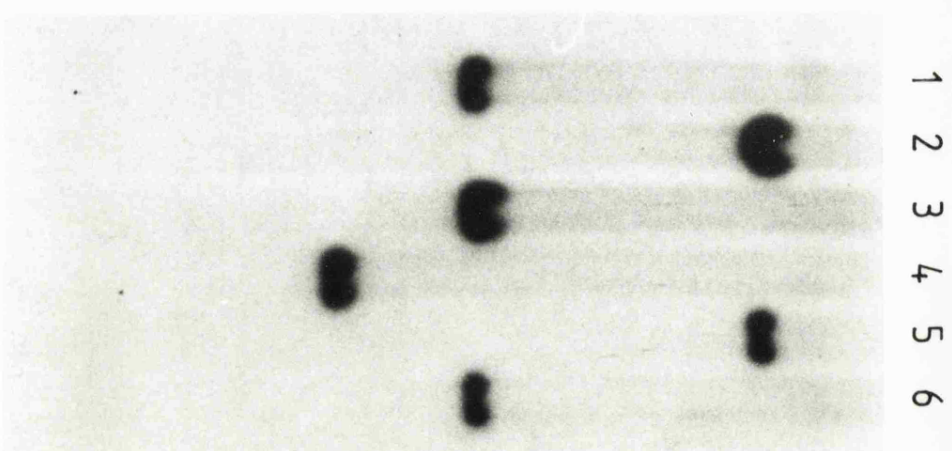


Figure 109: Blot hybridisation of genomic DNA from the recombinant λ clone λ SVW3 with the probe CSSn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol III. The DNA was digested with the following restriction endonucleases: Lanes 1 - EcoRI; 2 - HindIII; 3 - EcoRI, SalI; 4 - EcoRI, HindIII; 5 - HindIII, SalI; 6 - EcoRI, XhoI.

(a) DNA visualised on the electrophoresed gel

(b) Autoradiograph

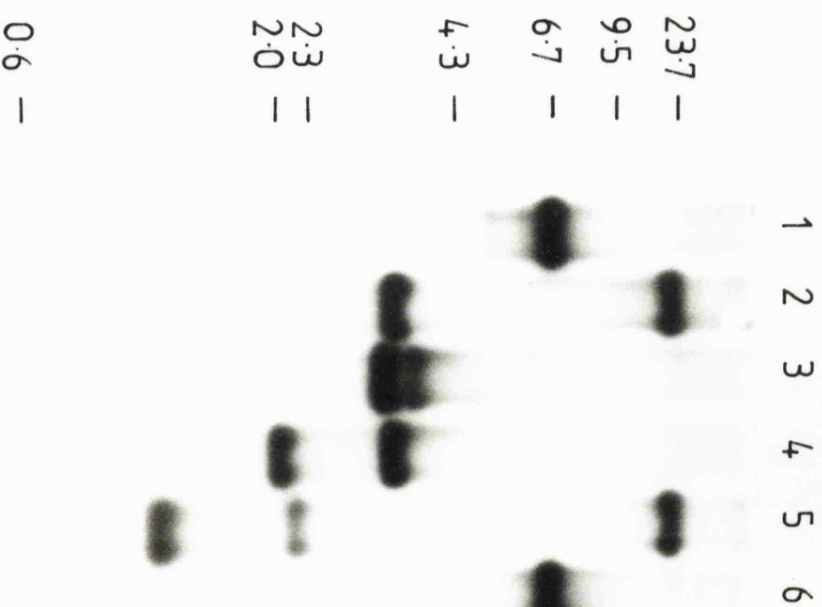
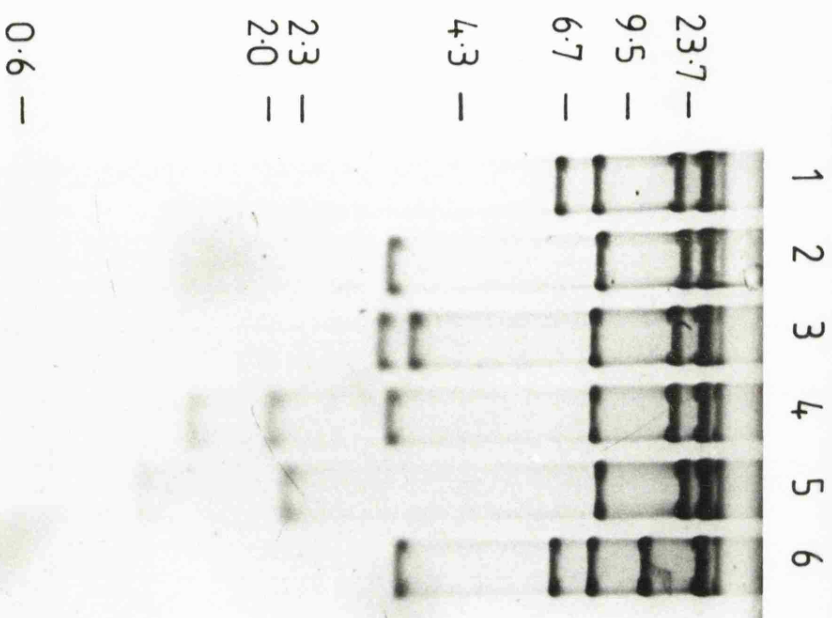


Figure 110: Blot hybridisation of genomic DNA from the recombinant λ clone λ SVW4 with the probe CSSn9. The probe was oligo-labelled, and hybridised to the restriction endonuclease digested genomic DNA using a nylon filter and protocol III. The DNA was digested with the following restriction endonucleases: Lanes 1 - EcoRI; 2 - HindIII; 3 - EcoRI, SalI; 4 - EcoRI, HindIII; 5 - HindIII, SalI; 6 - EcoRI, XhoI.

(a) DNA visualised on the electrophoresed gel

(b) Autoradiograph

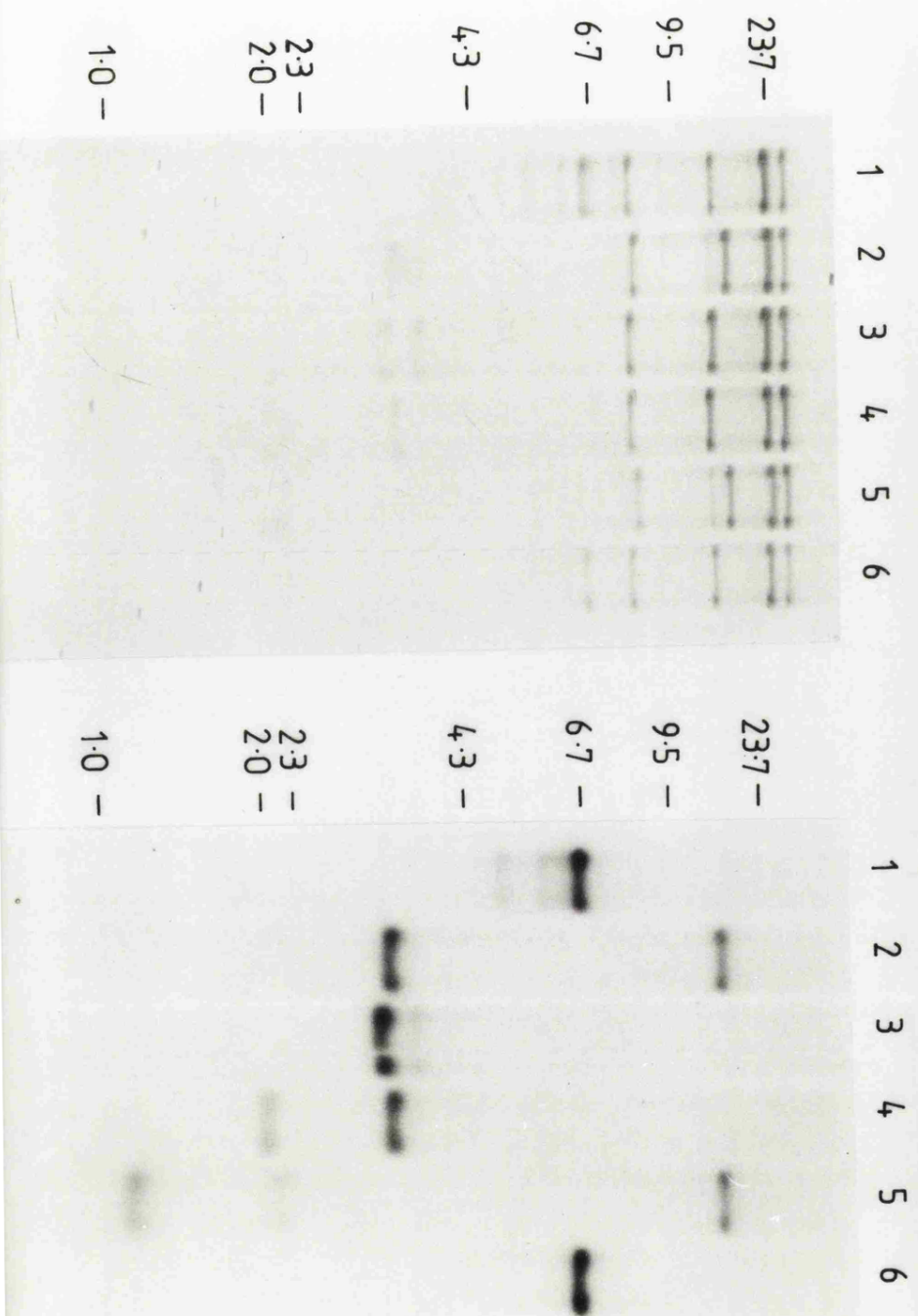


Figure 111 Restriction endonuclease map of bacteriophage λ recombinant λ SVW1

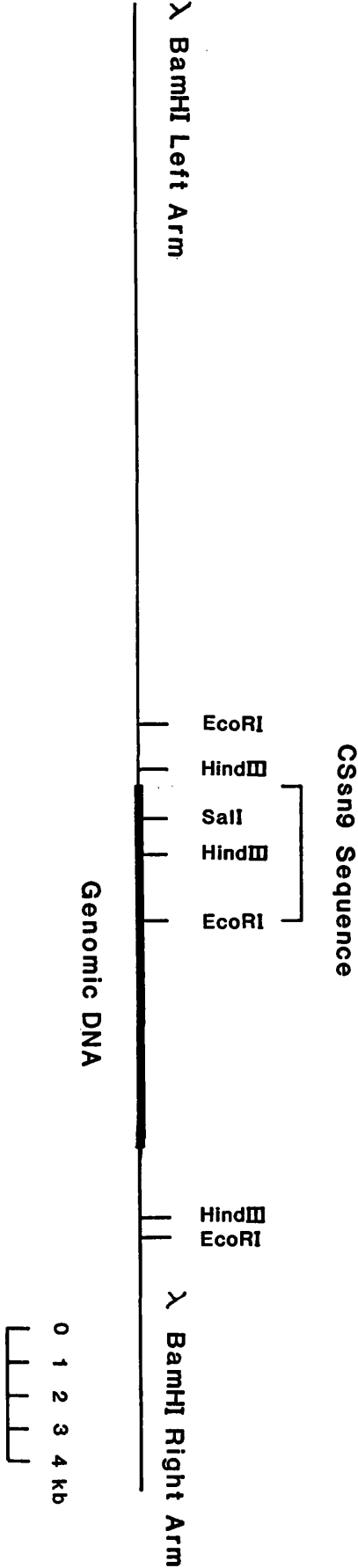


Figure 112 Restriction endonuclease map of bacteriophage λ recombinant λ SVW2

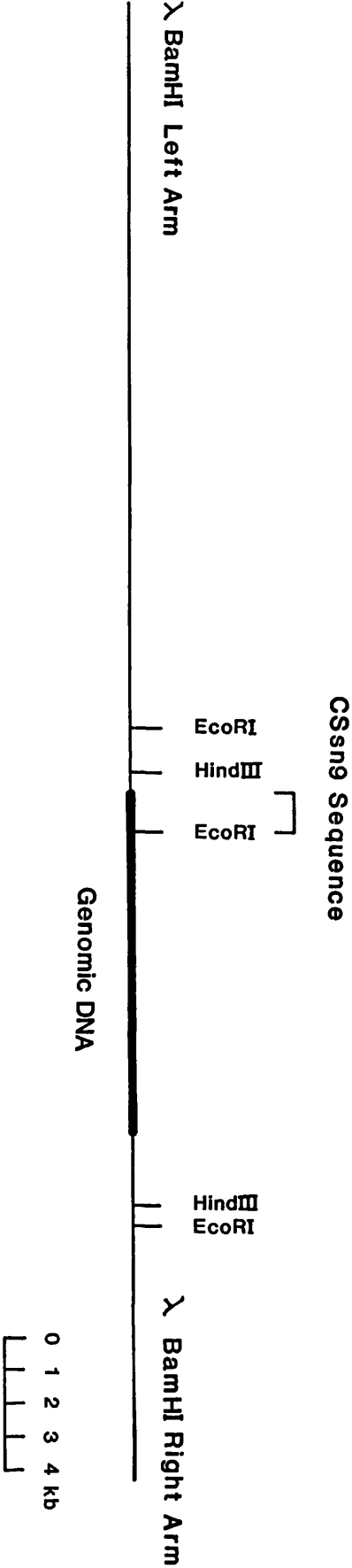


Figure 113 Restriction endonuclease map of bacteriophage λ recombinant λ SVW3

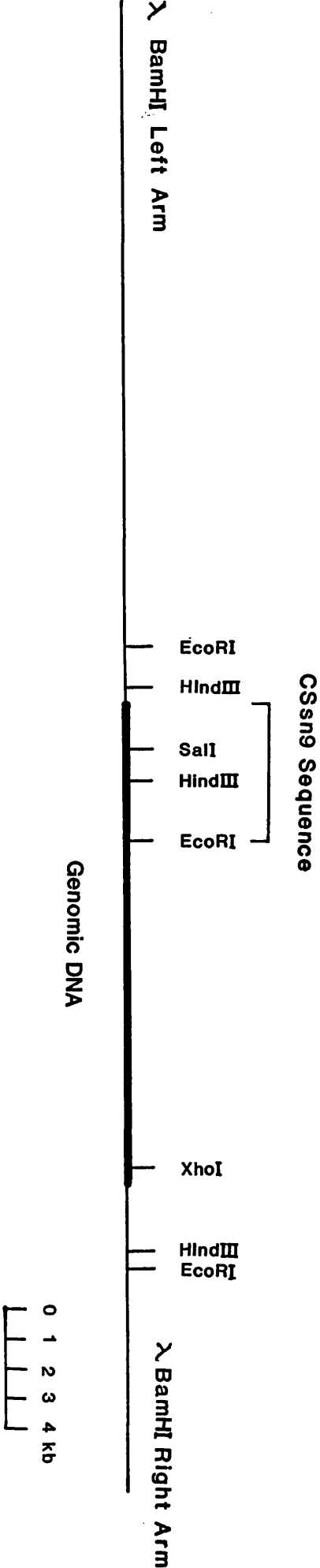
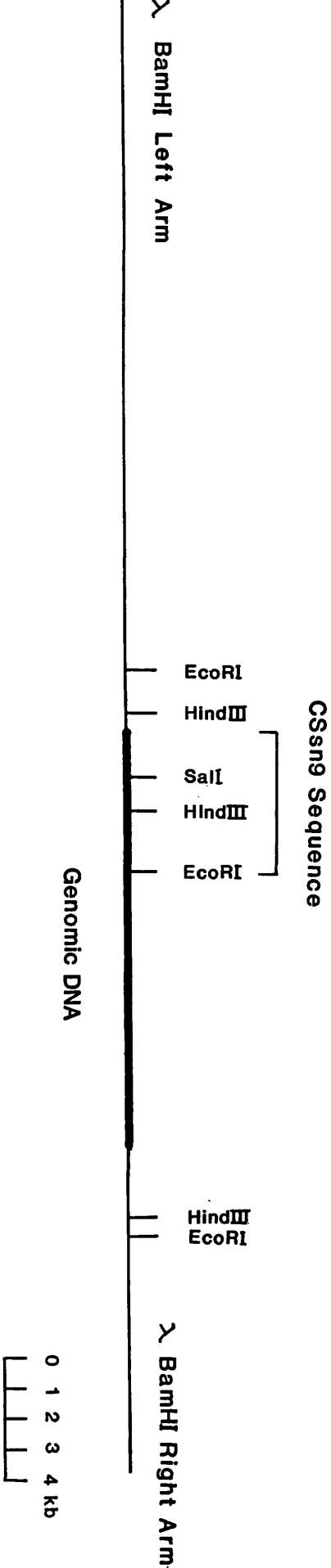


Figure 114 Restriction endonuclease map of bacteriophage λ recombinant λ SVW4



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Southern blotting analysis, was very close to the site at which the sn^{vw} P elements were inserted. In all 4 recombinant λ clones the inserted genomic DNA was orientated with its CSsn9 homologous DNA towards the 23.6 kb left arm of λ L47.1.

Chapter 6 : Discussion

6.1 Movement Of 412, copia And F Transposable Elements In P-M Hybrid Dysgenesis

The transposition rate of the elements 412, copia and F was seen to be increased under the conditions of P-M hybrid dysgenesis in the cross between Harwich and Canton-S. It is possible that some of the new sites observed in the experiment represent polymorphic, but rare, transposable element sites present in the original stocks which, by chance, were seen only in single experimental dysgenic lines. The significant excess of these new sites in the dysgenic lines relative to the controls, however, argues that this is not the case for the majority of new sites observed.

There is a suggestion of some clustering of new sites in certain of the dysgenic sublines. Out of the 9 different lines produced in the experimental cross which were separated at the F3 generation, 5 of the lines possessed new insertion sites for the elements (discounting P elements). Line Aa was found to have 4 new sites of insertion, Ab2(b) to have 5 new sites, Ac1 to possess 2 sites, Ac2 to have again 2, and Ac4 to possess a single new site. Of the 4 sites observed in line Aa, all were seen in 1 subline only, and thus one could not determine the generation at which the transposition events had occurred. The new sites in line Ac1, however, into which the elements copia and F had transposed, were both found to have been mobilised in the germ cells of an F1 generation fly. Of the 5 new transposable element sites observed in the line Ab2(b), one was able to determine that the F element at region

31A had transposed at the F3 generation or earlier, and that the copia element observed in region 35D had undergone transposition in the germ cells of an F5 fly or earlier. There is the possibility that all 5 of these new sites may have arisen at the same time, the transposition of the 2 F elements, the 2 412 elements and the single copia element all occurring in the same germ cell. If this is the case, one may be observing the transposition bursts described by Gerasimova et al. (1984a). Clearly, even if these multiple transpositions did not stem from the same germ cell, certain lines do seem to be more susceptible to transpositions of these elements. Analysis of the lines with other transposable element probes may confirm or refute this observation, if such elements are also mobilised, and may even perhaps determine whether or not transposition bursts have indeed occurred.

D. melanogaster cells contain at least 50 different transposable element families dispersed throughout the genome. If copia, 412 and F are typical of such elements, moving at approximately 1/30th the rate of P elements during hybrid dysgenesis, and one assumes an average of 30 representatives of each family in the genome, one might expect in total as many (if not more) transposition events of these elements to occur as those of P elements. One would thus expect such elements to be found inserted into genes mutated in P-M hybrid dysgenesis with as great a frequency as that with which P elements are found. This, however, does not seem to be the case. Perhaps the reason for this observation is that the literature has become biased, in that most hybrid dysgenic mutation screens are done in an attempt to clone genes by "transposon tagging". If mutations generated in such screens

were in fact due to the insertion of elements other than the P element, no P element homology would be seen by in situ hybridisation at the correct chromosomal site for the mutated gene, and thus one would not be able to use such an observation for the cloning of the gene. Such a study would not therefore be published, and only in experiments in which the gene was in fact mutated by a P element insertion, thereby leading to the cloning of the locus, would one see such published data.

The experimental results suggest that some component is inducing copia, 412 and F element transposition when under the P-M hybrid dysgenic conditions of a Harwich, Canton-S cross. The component may in fact be a constituent of the P-M hybrid dysgenic process. P factor transposase seems a likely candidate for such a role, but it is not clear how this could mechanistically produce an increase in the transposition rates of transposable element families which transpose using different mechanisms. Copia-like elements are thought to transpose via RNA intermediates, produced by transcription, which are used to generate new DNA copies by the action of reverse transcriptase-like enzymes encoded by the elements themselves (Flavell and Ish-Horowicz 1981; Flavell and Ish-Horowicz 1983; Shiba and Saigo 1983; Flavell 1984; Mount and Rubin 1985; Yuki et al. 1986). DNA sequence analysis has suggested that there are at least 2 major groups of copia-like elements in D. melanogaster, with respect to their putative reverse transcriptases, of which copia and 412 are in separate groups (Yuki et al. 1986). It is thought that the F elements may also transpose by a mechanism that involves transcription and reverse transcription, followed by reinsertion into the

genome, as they possess an oligo(A) stretch, preceded by a polyadenylation signal, at one end which is homologous between different members of the F family (DiNocera et al. 1983). Sequence analysis of an F element has also revealed a large open reading frame encoding a polypeptide which exhibits extensive homology to the reverse transcriptase-like domains of the potential products of D. melanogaster I factors and mammalian LINE-1 or L1 sequences (DiNocera and Casari 1987). The P element data, however, seems to suggest that this element transposes in a different manner to the above, using a "cut and paste" mechanism (O'Hare 1985). There is a possibility that the P factor transposase may also be able to mobilise the copia-like and F elements by the "cut and paste" method. This seems unlikely, however, as these elements do not possess the 31 bp P element terminal repeats on which the transposase is thought to act, and also analysis of a copia element inserted in white during P-M hybrid dysgenesis has revealed 5 bp target site duplications, as normally observed with copia, and not the 8 bp duplications of P elements (K. O'Hare, pers comm.). Perhaps it is repression of transposition that is the key to the problem and not in fact induction. The actual means of repression of different transposable elements is not very well understood, and there is a possibility that P transposase (or some other component of the P element transposition mechanism) may interfere detrimentally with the repression of transposition in the other transposable element families such that their repression is partially overcome, and mobilisation of these elements occurs to a certain extent.

Although the copia, 412 and F element transposition only occurred in the dysgenic, and not in the reciprocal cross,

there still remains the possibility that this movement was not connected with an aspect of P-M hybrid dysgenesis, but instead due to some other difference between the Harwich and Canton-S stocks. Two other hybrid dysgenic systems are known to exist in D. melanogaster, I-R hybrid dysgenesis involving transposable elements termed I elements (Bucheton et al. 1984), and an H-E hybrid dysgenic system mediated by hobo elements (Blackman et al. 1987; Yannopoulos et al. 1987).

In the I-R hybrid dysgenic system the dysgenic effect is seen in the progeny of crosses between R (reactive)-strain females and I (inducer)-strain males (Bucheton et al. 1976). Both I and R strains possess I elements, but only I strains contain the complete 5.4 kb I factors (Bucheton et al. 1984). Harwich and Canton-S are both I strains, and thus I-R hybrid dysgenesis can not have played a part in the transposable element mobilisation observed in this experiment.

The H-E hybrid dysgenic system involves H and E strains, the former containing complete 3.0 kb and defective hobo elements, and the latter being lacking in such elements (Streck et al. 1986). Blackman et al. (1987) have analysed various strains by genomic Southern blotting and classed them as H or E strains depending on the presence or absence of hobo elements. By such characterisation Canton-S was classified as an E strain, but Harwich as an H. Yannopoulos et al. (1987), however, have carried out a number of crosses involving an H strain, 23.5 MRF (Stamatis et al. 1981), with a number of other strains. They found that both Harwich and Canton-S acted as if E strains, suggesting that the hobo elements present in Harwich are non-functional in causing H-E hybrid dysgenesis, and thus eliminating hobo elements as candidates for the

mobilisation of copia, 412 and F in the P-M hybrid dysgenic lines. H-E hybrid dysgenesis, like the P-M system, has GD sterility as one of its dysgenic traits. In the H-E system, however, no temperature dependence for the sterility is observed, the trait being observed with as high a frequency at 18°C as at 25°C. This may suggest that the Harwich stock, used in the Harwich, Canton-S crosses in this thesis, had somehow acquired the ability to cause H-E hybrid dysgenesis, as a high sterility effect was observed in the F1 generation of the P-M dysgenic cross at 22°C. Blackman et al. (1987), however, have found that levels of mutability are similarly high in both H strain male with E strain female, and the reciprocal E male with H female crosses, thus suggesting that H-E hybrid dysgenesis occurs in both cases. If copia, 412 and F elements were being mobilised by H-E hybrid dysgenesis one would therefore not expect to see any differences in the frequency of their transposition in the reciprocal crosses.

I-R and H-E hybrid dysgenesis do not therefore appear to have played a role in the transposable element movements observed in the P-M hybrid dysgenic lines. However, there still may be other transposable elements, differing between the two stocks, which might be responsible for the transpositions. To exclude these other factors one would have to use stocks that were identical apart from the presence and absence of P elements. This could perhaps be accomplished by using germline transformation of an M strain with a P factor.

Woodruff et al. (1987) have analysed a number of mutant alleles caused by the insertion of transposable elements such as copia, gypsy, B104, F, foldback and hobo. They found that the excision of these elements was not elevated under P-M

hybrid dysgenesis, which seems to disagree with the results obtained in this thesis. The experiment of Woodruff et al. (1987), however, investigated the excision of transposable elements, whilst my work concentrated on new insertions. Such events may occur by different mechanisms, as appears to be the case in prokaryotes (Kleckner 1981), and thus P-M hybrid dysgenesis may be capable of elevating the rate of insertion of these other elements, but not the frequency of excision.

It has been suggested that transposition bursts or explosions may have a significant role in evolution, by inducing multiple insertions and thus creating an organism that differs from its parents in a number of features (Gerasimova et al. 1984a; Gerasimova et al. 1985). Such a system could explain the appearance of new complex properties requiring a number of independent genetic changes, each of which has no selective advantage, as multiple mutagenesis might perhaps occasionally create such features. If transposition bursts are under the control of P elements, however, and such elements only appeared in D. melanogaster 30-60 years ago, one can not attribute such changes in this species to P-mediated bursts. It would thus be interesting to see if other hybrid dysgenic systems can produce multiple transposable element mobilisations.

It is possible that other elements, such as copia-like elements, which do not show any dysgenic properties, were once also able to mediate hybrid dysgenesis and perhaps transposition bursts. These elements appear to be repressed in D. melanogaster, as their movement only occurs at a very low rate when compared to the frequency of P element transposition in P-M hybrid dysgenesis. In the hybrid dysgenic systems

described, increased transposition occurs in crosses between strains in which one possesses intact elements able to produce an active transposase. The mobilisation is not increased in crosses between strains in which both contain the intact elements, presumably due to the presence of some form of repressor produced by the complete elements in the cellular environment of the germline in these strains, and nor in crosses between strains both containing defective elements, due to a lack of active transposase production. Such a situation might therefore exist in the elements such as copia-like elements, as all D. melanogaster strains analysed appear to possess these elements. It is possible that when these elements were first introduced into Drosophila populations, they too acted as hybrid dysgenic systems in crosses between two flies in which the elements were absent and present respectively. Eventually, however, the whole population may have come to possess active elements and consequently repressor, such that repression of transposition occurred. Alternatively, in some of the transposable element families, active elements may have been lost from the population leaving defective elements only, unable to produce active enzymes for their transposition. This is not the case for copia, 412 and 297 where the frequency of sites investigated in natural D. melanogaster populations suggests that these elements do transpose (Montgomery and Langley 1983). The appearance of spontaneous mutations due to transposable element insertion also indicates that these elements are not inactive in transposition. If present-day non-dysgenic transposable elements were in fact at one time able to cause hybrid dysgenesis, these dysgenic crosses might

also have produced transposition bursts of other resident transposable elements, thus perhaps playing an active role in the evolution of Drosophila. One might test whether such elements are able to cause hybrid dysgenesis by transforming flies of a Drosophila species, not containing a specific transposable element, with that element, and investigating its effect in crosses with non-transformed flies.

6.2 Analysis Of The P Element-Induced singed Mutations

6.2.1 Bristle Phenotypes

The restriction endonuclease maps of the sn mutation strains were analysed to look for any correlations between the P element insertions in the sn locus at the molecular level, and the bristle phenotypic strengths of the mutations generated. No such recognisable correlations were observed, however, when concerning the sizes of the P inserts, their orientations, or their positions of insertion. This can be demonstrated, for example, when considering strains π2 sn² and loua sn⁵. Both strains possess P elements of similar sizes, inserted in the same orientation at a similar site, and yet π2 sn² possesses bristles of a weak phenotypic strength whilst loua sn⁵ has a strong sn phenotype. One can also consider the pairs of strains, π2 sn³ with loua sn⁹, π2 sn¹ with π2 sn³, and loua sn⁵ with loua sn⁴. The P element insertions are very similar apart from their size, orientation, and insertion position respectively for each pair, and yet the strains within each pair have similar phenotypic strengths.

The results from the Northern blot analysis of the strains were investigated with reference to their sn

phenotypic strengths. It was found that in each case the abundance of poly(A)⁺ RNA corresponding to sn wild-type Canton-S transcript was reduced, but that the mRNA lengths, apart from in strain loua sn⁶ where 2 larger transcripts of 3.7 and 4.1 kb were produced, were of a similar 3.4 kb size to the wild-type. It thus appears that with the exception of loua sn⁶, the P element insertions in the strains analysed cause the amount of sn poly(A)⁺ RNA to be reduced, thus leading to the bristle phenotypes observed. In the case of loua sn⁶ the P insertion causes both the loss of the wild-type length transcript, 2 larger mRNA species resulting, and also a decrease in the abundance of these transcripts when compared to the wild-type level.

The reduction in sn poly(A)⁺ RNA in the sn mutation strains suggests that the P elements might be inserted within the 5' promoter region of the sn locus, thus having an adverse affect on transcription of the gene. The presence of 2 larger transcripts in loua sn⁶, on the other hand, suggests that the element in this strain, and thus also in sn^{vw}, is perhaps inserted within an intron of the sn gene, thus affecting the splicing of this intron. K. O'Hare (pers comm.), however, has found that transcription at the sn locus initiates at a position within the 1.9 kb CSSn9 EcoRI-SalI genomic fragment, approximately 0.7 kb from the EcoRI site, and proceeds in a direction towards the EcoRI site. The P elements analysed by Roiha et al. (1987) have been found to be inserted within the 5' end of the 1st sn exon, which extends 0.6 kb to approximately 0.1 kb from the same EcoRI site. It thus appears that the P elements at the sn locus in each of the strains that I have analysed, are also inserted within the 1st exon.

One would expect the P elements to be transcribed within the sn mRNA producing poly(A)⁺ RNA species longer than the wild-type length. With the exception of loua sn⁶, however, this is not the case, and in strain loua sn⁶ neither of the 2 larger transcripts produced are increased by a length equal to the 1.25 kb P element DNA inserted.

The strains in which the transcripts synthesized are of a similar length to the wild-type sn poly(A)⁺ RNA shall be initially considered. One can imagine a number of different ways in which these mRNA species could be produced. The transcription could be initiating at a different position to that in the wild-type situation. This seems unlikely, however, as the position of initiation, in each case, would have to be within the P element insertions. Promoter sequences (TATA box and upstream promoter elements) are required for accurate initiation of transcription, and need to be positioned immediately upstream from the transcription start site, the TATA box being typically 30 bp from this site (Maniatis et al. 1987). As the P elements in the different sn mutation strains are of various sizes and are inserted in different locations with different orientations, the likelihood of such promoter sequences being present at the correct position in each case, to cause transcription initiation at a site producing a 3.4 kb poly(A)⁺ RNA, seems very remote. The original transcription initiation would also have to have been abolished, as otherwise sn poly(A)⁺ RNA's larger than 3.4 kb would still be produced.

An alternative mechanism to a change in transcription initiation, such that 3.4 kb mRNA species could be produced, involves the processing of the sn HnRNA's synthesized in the

transcription. RNA sequences of the correct lengths corresponding to the size of the P element insertions could have been spliced out. The P element inserts, however, were of different lengths, and thus it is difficult to imagine how 3.4 kb mRNA's could be produced in each case, as this would mean different lengths of RNA being spliced out in the different strains. The most plausible method seems to be that the actual RNA corresponding to the P element DNA sequences is spliced. RNA splicing is dependent on a number of factors of which one concerns the actual nucleotide sequences of the splice sites (Aebi and Weissmann 1987). The 5' consensus sequence is (C/A)AG'GU(G/A)AGU of which the GU following the 5' cleavage site is highly conserved. The 3' consensus sequence is (C/U)₁₁NCAG, consisting of a pyrimidine-rich stretch of variable sequence followed by the strongly conserved AG nucleotides which precede the 3' cleavage site. Such splice sites may be present near the ends of the RNA produced from the P elements, such that the P element RNA is spliced out of the HnRNA. The nucleotide sequences most likely to be involved in such a process are the 31 bp P element terminal inverted repeats, as such sequences would be present in the HnRNA in a similar manner whichever way around the P element was orientated. The few nucleotides from the P sequences remaining in the mRNA would not be traceable by Northern blotting analysis. One can search for sequences in the P terminal repeats homologous to the splice site consensus sequence. A sequence of AAGGTGGTC, of which the 1st 6 nucleotides are identical to the 5' consensus sequence, is present in the 5' terminal repeat of the 5'-3' DNA strand, and although no AG nucleotide pairs are present in the 3' P element terminal

repeat, a stretch of 22 nucleotides, of which 16 are pyrimidines, does exist. Whether such sequences would operate as splice sequences, however, is difficult to predict.

One further method by which 3.4 kb poly(A)⁺ RNA's could be produced in the sn mutation strains, is if the P element sequences in the HnRNA are actually excised by a form of P transposase. The mechanism by which transposase acts is not known, and thus it might be possible that the enzyme can recognise and act upon the 31 bp terminal repeats when present in RNA form. P factor transposase is in some way repressed in somatic cells, unable to act upon P element DNA, but some form of transposase might be present which is active upon RNA. The element RNA sequences might be precisely excised from the HnRNA by this process, leaving mRNA exactly the same as the wild-type form.

The 3.7 and 4.1 kb mRNA species produced in the strain loua sn⁶ are most probably due to altered splicing of the HnRNA. In addition to the splice sequences, RNA splicing is also dependent upon the spatial relationship between the two different splice sites (Kühne et al. 1983; Aebi and Weissmann 1987). If two sites are unfavourably located in relation to each other, then a more favourable cryptic splice site may be used. The presence of the P element in loua sn⁶ could be affecting the tertiary structure of the HnRNA produced, such that processing is altered, and the two poly(A)⁺ RNA's result.

The mRNA species produced by the sn mutation strains analysed were reduced in abundance. One mechanism by which this may have been achieved is polyadenylation terminus formation in the P element inserts. Zachar et al. (1985) have analysed the effects of a copia element inserted in the 2nd

intron of the white locus producing a w^a mutation. They found that polyadenylation terminus formation occurred in the copia 3' LTR which was dependent upon sequences present in the LTR and also sequences internal to the nonrepeated portion of the transposable element. The P element mRNA is polyadenylated at +2710 bp (Laski et al. 1986), and thus if DNA sequences around this site are present, and also presumably other necessary P sequences more internal to the element, then polyadenylation of sn transcripts at this site may occur. The P element polyadenylation site is leaky, leading to readthrough past this site (Laski et al. 1986), and thus this could account for the presence of the large sn poly(A)⁺ transcripts at a reduced level. For termination to occur in this manner a P element would have to be inserted in the correct orientation with its +2907 bp facing downstream with respect to sn transcription. Thus, if such a mechanism is occurring, it could only occur in strains in which the P elements are correctly orientated and possess the correct sequences.

sn transcription might also be reduced by affects of the P element insertions upon the sn promoter, causing reduced efficiency. The TATA box of the promoter is required primarily for accurate initiation of transcription, whilst the upstream promoter elements (UPE's) increase the rate of transcription (Maniatis et al. 1987). It is thought that one or more proteins bind to the UPE's and interact with a protein (or proteins) bound to the TATA box, controlling the transcription process. This interaction is believed to require a stereospecific alignment of the proteins on the DNA helix (Takahashi et al. 1986). Enhancer sequences are also necessary for transcription, possessing DNA sequence elements that

specifically interact with proteins to increase the rate of transcription from promoters (Serfling et al. 1985). It is thus possible that the insertion of P elements quite close to the promoter and enhancer sequences of the sn locus might affect the tertiary structure of the DNA in that region, thus perhaps having a detrimental effect on the protein-protein and protein-DNA interactions of the transcription machinery, reducing its efficiency.

On studying the approximate levels of reduced transcription from the Northern blotting analysis, it can be seen that they do not directly correlate with the bristle phenotypic strengths of the sn mutation strains. This can, for example, be seen when comparing loua sn^{w10} and π2 sn³. From the analysis, the abundance of poly(A)⁺ RNA in loua sn^{w10} was estimated to be 3-fold of that in π2 sn³, and yet the former strain has a strong sn phenotype and the latter a weak one. Such a discrepancy might perhaps be due to P element sequences, if still present to some degree in the poly(A)⁺ RNA. Presuming that the sn mRNA is translated to produce a functional polypeptide, such sequences, if untranslated, might affect the binding of ribosomes to the mRNA in some way, thus reducing the efficiency of translation. If translated, however, they may affect the function of the polypeptide produced.

The strain loua sn^{s10}, derived from loua sn¹⁰, possessing a very strong sn phenotype, can be presumed to be the stock in which the P element and some flanking genomic DNA is inverted. The inversion most probably extends into the promoter region and 1st exon of the sn gene, thus drastically affecting transcription and resulting in the very strong

bristle phenotype.

Analysis of the poly(A)⁺ RNA's in more detail, perhaps by cDNA cloning and sequencing, might reveal further insight into their structure, and thus into the exact reasons for the different sn bristle phenotypes.

6.2.2 Germline Reversion Frequency

The restriction endonuclease maps of the sn mutation strains were studied to look for any correlations between the P insertions in sn and the reversion frequencies of the elements. As for bristle phenotypes, however, no such recognisable correlations were observed. This can be seen, for example, by considering strains π2 sn¹ and π2 sn². Both stocks possess similar sized P elements inserted in the same orientation at a similar site of insertion, and yet the former demonstrates quite a high reversion rate as opposed to a rate of almost zero for π2 sn².

Roiha et al. (1987) have sequenced the target sites of 11 strains possessing P elements inserted within the 1.9 kb CSSn9 EcoRI-SalI genomic fragment approximately 0.65 kb from the EcoRI site. It was found that instead of the elements being present in a single target site, 4 such sequences were used, of which 7 elements utilised 1 of the sites, 2 elements another, and the 2 other target sites were found independently in 2 of the strains. It is thus possible that the reversion frequency of a P element is dependent upon the target site at which the element is inserted. Elements inserted at a particular target site may be more readily excised from the genome than elements at other sites. The P transposase might possibly interact with the target sequences in some way, on

excision of the P elements, and may recognise certain targets with greater efficiency than others. Alternatively, the tertiary structure of the elements inserted at particular target sites might make them more amenable to interaction with the transposase. Sequencing analysis of the sn strains' P element insertion target sites, and comparison with the reversion rates, may determine whether the target sequence is responsible for the observed frequencies.

6.2.3 Somatic Destabilisation

The excision of P elements at the sn locus in somatic cells, again appears to be concerned with the particular target sites at which the elements are inserted. The somatic reversions were not well correlated with the germline instabilities, however, as can be seen in an extreme case with sn^W, which possessed a 50% germline reversion rate but a somatic destabilisation frequency of almost zero. It thus appears that somatic destabilisation of P elements occurs more readily with a different subset of target sites than those at which the elements revert in the germline. All of the P elements situated within the 1.9 kb CSsn9 EcoRI-SalI genomic fragment approximately 0.3 kb from the EcoRI site were found to have high somatic destabilisation frequencies, together with a few of the elements 0.65 kb from the EcoRI site. In the analysis performed by Roiha et al. (1987) all of the sn mutations were found to be due to P elements inserted around the 0.65 kb position, thus suggesting that the 0.3 kb site is in fact only rarely used as a target site for P element insertions. Out of 11 strains that I analysed, however, 4 possessed P elements situated at the 0.3 kb site. On the other

hand, of these, loua sn⁴ and loua sn⁶ were most probably originally derived from brothers, thus possessing the same P element insertion, and sn^{vw} was derived from the loua sn⁶ strain. I was also, in fact, indirectly selecting for this site as, out of the sn mutation strains available (Brookfield and Lewis, submitted), I was preferentially analysing strains that showed a high somatic instability. The P elements located at the 0.65 kb site exhibiting high somatic reversion were thus possibly present at one of the rare target sites revealed by Roiha et al. (1987).

One question raised by the somatic instability seen in the strains is what form the mutation causing this phenomenon takes. One hypothesis could be that the germline specific splicing of the ORF2-ORF3 intron in the P factor transcript, has been altered by a mutation in the process which controls this tissue specificity. Splicing of the transcript would thus occur in somatic cells such that active P transposase would be produced which could act on the P element insertions. One problem with this theory, however, is that the somatic instability observed should be correlated with the germline reversion frequencies of the strains. An alternative hypothesis is that the mutation is in fact a mutated P factor present in the genome of these strains. The altered P factor would produce an active transposase with its spectrum for P element mobilisations changed. Such a P factor might produce a transcript which can now be spliced in somatic cells in addition to germ cells, thus allowing synthesis of the transposase in all cells, or alternatively might produce a transcript which encodes the active transposase without the requirement for a tissue specific splice to occur. The

maternal effect exhibited by the mosaicism mutation can be explained by the transposase being expressed in the mother and then being passed into the eggs, and thus zygotes, where it can act on the P elements. It is possible that there is no somatic expression of transposase genes occurring at this stage, and that all the somatic destabilisation results from protein stored in the egg.

The mosaicism mutation has been tentatively mapped to the 2nd chromosome. By using multiply marked 2nd chromosomes the mutation could be more accurately mapped, and by in situ hybridisation with P factor probes one could determine whether this position corresponds to the location of a P element. The P element could thus be cloned and analysed to determine the structure of the transcript and polypeptide produced.

6.3 singed-(very weak)

An attempt was made to clone the elements (assumed both to be P) causing the sn^{VW} mutation in λ L47.1. This, however, proved unsuccessful, the 4 positives achieved by screening with CSsn9 all containing genomic sequences from the DNA to the right of the insertions as orientated in figure 97. Similar attempts by Roiha et al. (1987) to clone the two P elements of sn^W in λ libraries have also proved unsuccessful. The element in the allele sn⁽⁺⁾ derived from sn^W, however, has been cloned without any difficulty (Roiha et al. 1987). It thus appears that the double P element structure of the sn^{VW} and sn^W alleles has an adverse affect on the cloning of the elements in λ vectors. This may be concerned with the elements being inserted in reverse orientation, and possessing quite

large stretches of homology to each other in close proximity. The two elements at the sn locus of sn^{vw} can be estimated to possess at least 0.5 kb of homology to each other, whilst those of sn^w contain at least 0.55 kb. On cloning, stem-loop type structures might possibly be produced due to this homology, and recombination events may perhaps occur destroying the phage.

The high reversion rates observed in the sn^{vw} and sn^w strains, respectively, are of great interest. Presumably the structure of the double P element inserts is important in this phenomenon. W. Eggleston and W. R. Engels have carried out a preliminary investigation into a number of other hypermutable sn alleles, the results of which suggest that some of these may also possess double P element insertions (Roiha et al. 1987). Roiha et al. (1987) have suggested that P transposase might bind independently to each end of a P element insert, and that an interaction occurs between the proteins at each terminus for excision to occur. This interaction may occur more frequently in double insertions due to the close proximity of two P element termini.

An alternative mechanism might be that P transposition is initiated by binding of transposase to one end of a P element, and that the two termini close to each other in the double insertion causes an increase in the frequency with which transposase binding occurs to these sites. The homology between the two P elements might cause the production of stem-loop structures if in the reverse orientation, which might also create a more favourable construction for the binding reaction, the adjacent termini being positioned at the end of the stem-loop, held outwards from the main body of the

DNA. Such a structure would not be produced in double P element inserts in direct orientation. It would thus be interesting to see if elements arranged in such a fashion would possess a high reversion frequency.

6.4 Mutagenesis By Transposable Element Insertion

Many spontaneous mutations in D. melanogaster are caused by the insertion of transposable elements, and such mutations, together with insertion mutations induced by P-M hybrid dysgenesis, have been used as an aid in the mapping of the molecular organisation of a number of genetic loci (Bender et al. 1983; Scott et al. 1983; Côté et al. 1986). Mutations can be produced by the insertion of the elements into two major areas of a locus, the structural transcribed region of the gene, and the upstream control sequences. There can be differing mutational effects on the gene phenotype depending on the structure of the transposable element and where within the locus it is actually inserted. Such effects can be seen in the mapping of the bithorax complex (BX-C) in D. melanogaster (Bender et al. 1983). The complex consists of a number of genetic loci such as anterobithorax, bithorax, bithoraxoid and hyperabdominal, which transform entire, or parts of, thoracic and abdominal segments into the form of other segments when mutated. In a number of spontaneous mutations 4 transposable elements were found to be inserted in different regions of the bithorax locus (transformation of anterior 3rd thoracic segment into anterior 2nd thoracic segment). The 4 alleles had varying phenotypes of strong, intermediate, weak and apparently wild-type, due to the insertion of gypsy, gypsy,

412 and Doc elements respectively in different regions of the gene.

The transcribed sequences of a gene can be situated in 1 of 3 regions which possess differing fates in the RNA transcript. The sequences may be present in an intron and spliced out of the HnRNA produced by transcription, they may be in regions of the transcript which are present in the final mRNA, but are untranslated, or they may be in transcribed and translated regions of the gene. Insertions into different areas of the transcribed DNA may have a number of effects upon transcription, processing and translation of the locus, thus producing a variety of observed phenotypes. RNA corresponding to inserted DNA may perhaps be inserted in an intron and spliced out of the transcript, thus having no effect on the locus, or alternatively may affect the splicing machinery producing an abnormal mRNA and protein. The transposable element, on the other hand, may be inserted in exon sequences which, if untranslated, may again have no effect on the phenotype, or may perhaps alter the translation mechanism. If present in translated regions, however, a mutated protein would be synthesized, but again differing phenotypes may be produced depending upon the alterations of the protein.

An example of phenotypic variation caused by differing insertions in transcribed regions can be seen in the mapping of the Antennapedia (Antp) complementation group of the Antennapedia complex (ANT-C; Scott et al. 1983). Mutations in this homoeotic gene result in a variety of dominant and recessive phenotypes. 2 mRNA transcripts of 5 and 3.5 kb are produced from a transcription unit of over 100 kb in the wild-type situation. The insertion of an unknown transposable

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element into a 5' region of the transcribed DNA was found to produce a dominant Antp mutation with an Extra Sex Combs phenotype resulting in the partial transformation of 2nd and 3rd legs into 1st legs. F elements inserted into sequences 5 kb apart, just 5' to the middle of the transcribed region, however, were found to have no phenotypic effect. These elements were observed individually in the TM3 balancer chromosome and in the strain Canton-S. Moving 3' to the middle of the transcription unit, however, again produces a different phenotype if mutated by transposable element insertions. An F element inserted in such a region produced a recessive lethal mutation with no dominant effect, and yet a B104 element inserted 6 kb 3' distant to this insert caused the production of a dominant gain-of-function allele, with the antennae of the mutated flies transformed to legs. A similar phenotype to the latter was also observed in a mutation possessing an unknown transposable element inserted into the 3' region of the transcribed DNA, 36 kb away from the B104 site of insertion. It can thus be seen that not only may genes be altered by different insertions causing a gradation of phenotypic strengths, but in loci such as Antennapedia, which controls the development of D. melanogaster, one can actually observe completely different phenotypes by a variety of insertion mutations in the same gene.

The insertion of transposable elements into the 5' non-translated region of a mRNA transcript can have differing effects upon a phenotype. In addition to examples perhaps in the experimental work of this thesis, such a situation was also observed in an investigation of the Notch locus by P-M hybrid dysgenesis-induced mutations (Kelley et al. 1987). 13 P

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element insertion mutations were produced which were lethal when hemizygous or homozygous, but possessed either no mutant phenotype or various degrees of wing nicking when heterozygous with the wild-type gene. Although the alleles showed a variety of heterozygous phenotypes, all 13 P element insertions were determined to be located in the 5' non-translated region of the transcript, one at about 400 bp from the transcription start site, but all 12 others within 100 bp of each other and the transcription start.

In some situations the apparent complete loss-of-function of a gene can be caused by the insertion of an element into the transcribed DNA. Chia et al. (1986) analysed the yellow (y) locus of Drosophila using P-M hybrid dysgenesis. 2/3rds of the mutations induced were classed as type 1 alleles possessing a total loss of pigmentation from all parts of the cuticle. 3 of these mutants were found to possess P elements inserted into amino acid coding regions of the locus, but 8 others had elements located in the 5' non-translated sequences. An example of how the different structure of the transposable element insert can affect the phenotype was observed in one of the mutations when a deletion of internal P element sequences occurred in one of the alleles (5' non-translated insertion). This transformed the type 1 mutant to a type 2, in which the pigmentation was only lost from particular areas of the cuticle. Similar P element deletions were found to alter the phenotype of a mutation RpII215^{D50} (Voelker et al. 1984; Searles et al. 1986). This allele was a lethal mutation caused by a 1.3 kb P element inserted into the 5' noncoding region of the 215 kilodalton subunit RNA polymerase II transcript. The protein product was

found to be qualitatively normal, but its abundance was less than normal and below the threshold necessary for survival. Imprecise excisions of the element, however, were found to restore non-lethality, presumably due to increases in the amount of protein produced.

There are a number of regulatory sequences upstream of genes which play a part in the control of transcription (Maniatis et al. 1987). Transposable element insertions in the different control sequences can produce different phenotypic effects.

The promoter is located immediately upstream of the transcription start site of a gene, and consists of the TATA box, which ensures accurate initiation of transcription, and the upstream promoter elements (UPE's) which control the rate of transcription. Snyder et al. (1982) analysed a mutation in the cuticle protein gene 3 (CP3) in which no CP3 protein was detectable. They found that a transposable element, H. M. S. Beagle, was inserted immediately adjacent to the TATA box, producing a 4 bp target site duplication of the TATA box sequence, thus affecting the transcription and inactivating the CP3 gene. McGinnis et al. (1983) have characterised a hobo element insertion located between the TATA box and UPE's of the D. melanogaster salivary gland glue protein Sgs-4 gene, which duplicates the TAT nucleotides of the TATA box sequences as part of the 8 bp target site. The expression of the locus was reduced 50 to 100-fold, and it was found that 4 transcripts were produced instead of the normal 1. It was determined that the position of the 5' end of the normal transcript had been altered by the hobo insertion, such that 2 transcripts were produced possessing transcription start sites

within the transposable element, 1 was present with the normal start site used, and 1 with the site situated within the 2nd hundred bps of the transcribed DNA sequences. The element thus altered both the efficiency and accuracy of transcription of the gene. The tissue and temporal specificity of the locus, however, was unaffected, the transcripts being expressed only in late larval salivary glands as in the wild-type situation.

The tissue specificity of a gene is controlled by certain upstream regulatory sequences called tissue-specific enhancers (Maniatis et al. 1987). An example of such 5' control regions that regulate the same promoter in different tissues is seen in the yolk protein genes, yp1 and yp2, which are expressed in the fat body and ovaries of D. melanogaster adult females (Garabedian et al. 1986). The expression of both genes in the fat body and ovaries is controlled by 2 distinct cis-acting sequences, each of which acts on the promoters of both genes. An example of transposable elements inserting into such tissue-specific enhancers may have been observed by Chia et al. (1986), in their analysis of the y locus. A number of type 2 mutants were produced with allele-specific mosaic pigmentation patterns, due to the insertion of P elements (and in one case gypsy) upstream within 700 bp of the transcription start site. The elements may be inserted into different tissue-specific enhancers, thus causing loss of pigmentation in the tissues controlled by these enhancers.

A second type of enhancer present in the control regions of certain genes, is the inducible enhancer (Maniatis et al. 1987). Examples of such enhancers in D. melanogaster are those that respond to heat shock. Eissenberg and Elgin (1987) have described a P element inserted in the heat shock gene Hsp28^{stl}

between the TATA box and the transcription initiation site. In normal adult males, on heat shock at 37°C for 45 minutes, an Hsp28 RNA transcript is produced. On analysis of the mutant allele, however, heat shocked adult flies produced no detectable normal transcript, but instead formed a heat inducible larger mRNA due to initiation of transcription within the P element by the action of the normal TATA and regulatory sequences upstream of the element. The normal Hsp28 gene is subject to developmental regulation, high levels of expression being observed in white prepupae and in the ovaries of fertile adult females. On analysis of fertile adult females from the mutant stock, it was found that low levels of the normal RNA were present in non-heat shock flies. On heat shock, however, the larger novel RNA species appeared, but the level of normal size RNA was not increased. It was thought that the low level of normal RNA present in non-heat shock females was perhaps due to some regulatory sequences present downstream of the insert which were not activated by the heat shock enhancer due to the presence of the insertion. On analysis of white prepupae, there was found to be no RNA produced from the Hsp28 locus of either size, whether heat shocked or not. In this stage of development the transcription thus appeared to be inactivated, and was not stimulated by the heat shock enhancer. Transposable element insertions can thus cause different effects on the expression of genes at different developmental stages.

It has been seen that transposable elements can insert into a variety of regions in a genetic locus, and may have a number of different effects upon the phenotype of the gene.

The insertions may produce mutations of different phenotypic strengths, or may perhaps cause a complete loss of any genetic function of the locus. They might alter the tissue specificity or inducibility of a gene, or may alter the developmental regulation of its expression. Such changes may be potentially advantageous to an evolving species, and thus spontaneous insertion mutations, combined with the elevated rates of transposition seen in hybrid dysgenic systems and transposition bursts, may implicate transposable elements as one of the major factors in the evolution of D. melanogaster, and perhaps also in the evolution of other transposable element-bearing species.

Appendix

It was found that in the P-M hybrid dysgenic cross between Harwich and Canton-S, many new sites were shared between different sublines, thus suggesting that particular chromosomes receiving transposed elements in the germ cells of F1 flies have come to be found in more than one line tested. One would expect this to occur frequently as a result of the amplification of the lines at various generations. The 27 lines tested therefore represent a smaller number of gametes examined from the F1 flies. The expected number of different F1 genomes examined can be calculated from the phylogeny of the cross (figure 4). To do this a number of assumptions are made:

(i) Transpositions occur at meiosis of the F1 generation of the dysgenic cross. The number of different F1 gametes are calculated for the final lines scored. If the F1 transpositions occur well before meiosis it would be expected that the F1 gametes would share transposable element sites. If transpositions occurred after sublines were all taken, such events would be independent in the various sublines. The number of events in this latter example would be calculated using the total number of sublines scored, that is, 27. The sharing of new sites between sublines, however, indicates that at least some transpositions occurred early. One can in fact estimate the number of lines effectively scored, not assuming that all transpositions occur in the F1 generation. There are 14 new sites, but these sites have a total of 20 occurrences across all of the sublines. Each new site is thus represented on average in $20/14$ sublines, and the effective number of

haploid genomes scored (defined in terms of the degree of separation of the genomes at the times of transposition) can be estimated as $(27 \times 14/20 =) 18.9$. Using this figure as the number of genomes scored still yields a χ^2 result which is significant at the 1% level.

(ii) When 2 females are removed from the same vial to form sublines, it is assumed that they have been fertilized by different males. Were they fertilized by the same mate, their offspring would be more similar, and the effective number of gametes scored would be less.

(iii) It is assumed that all of the genotypes are selectively equivalent.

Using these assumptions the expected number of F1 gametes represented in the final lines can be calculated.

One can consider the initial female used to establish any line. Such a fertilized fly has 4 haploid genomes which can be termed A, B, C and D, where A and B are her own genomes, and C and D the genomes in the sperm stored from the male which fertilized her. In subsequent generations each new generation will be initiated by a female with 4 genomes descended from A, B, C and D. The calculations are based upon the probabilities that 1, 2, 3 or 4 of these 4 genomes are still represented in the generations. We can classify these fertilized females depending upon the representation of A, B, C and D in such flies. There are 13 possible classes:

Class I: All genomes are descended from one parental genome - AAAA, BBBB, CCCC and DDDD.

Class II: 2 genomes are represented, both from the same parental generation parent (A and B, or C and D), with one copy of the one genome and 3 of the other - ABBB (and BABB), AAAB, BBBA, BAAA, CDDD, CCCD, DDDC and DCCC.

Class III: 2 genomes are represented, each from different parental generation parents, with 1 copy of one genome and 3 of the other - AAAC, AAAD, ACCC, AD DD, CAAA, DAAA, CCCA, DDDA, BBBC, BBBD, BCCC, BDDD, CBBB, DBBB, CCCB and DDDB.

Class IV: 2 genomes are represented, both from the same parental generation parent, 2 copies of one genome being in the female genomes and 2 of the other in the male - AABB, BBAA, CCDD and DDCC.

Class V: 2 genomes are represented, each from different parental generation parents, 2 copies of one genome being in the female genomes and 2 of the other in the male - AACC, AADD, CCAA, DDAA, BBCC, BBDD, CCBB and DDBB.

Class VI: 2 genomes are represented, each from the same parental generation parent, with each genome being found once in the female genomes and once in the male - ABAB and CDCD.

Class VII: 2 genomes are represented, each from different parental generation parents, with each genome being found once in the female genomes and once in the male - ACAC, ADAD, BCBC and BDBD.

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Class VIII: 3 genomes are represented, one of which is present in both the female and male genomes, and 2 others, of which one is from the same parental generation parent as the doubly represented genome - ABAC, ABAD, ACAB, ADAB, BABC, BABD, BCBA, BDBA, CDCA, CDCB, CACD, CBCD, DCDA, DCDB, DADC and DBDC.

Class IX: 3 genomes are represented, one of which is present twice in either the female or male genomes, and 2 others, of which one is from the same parental generation parent as the doubly represented genome - AABC, AABD, BBAC, BBAD, CCDA, CCDB, DDCA, DDCB, BCAA, BDAA, ACBB, ADBB, DACC, DBCC, CADD and CBDD.

Class X: 3 genomes are represented, one of which is present in both the female and male genomes, and 2 others, both of which are from the same parental generation parent, and thus from a different one to the doubly represented genome - ACAD, ADAC, BCBD, BDBC, CACB, CBCA, DADB and DBDA.

Class XI: 3 genomes are represented, one of which is present twice in either the female or male genomes, and 2 others, both of which are from the same parental generation parent - AACD, CDAA, BBBD, CDBB, CCAB, ABCC, DDAB and ABDD.

Class XII: 4 genomes are represented, 2 from one parental generation parent being in either the female or male genomes - ABCD and CDAB.

Class XIII: 4 genomes are represented, 2 from one parental generation parent being split with one in the male genomes and the other in the female - ACBD, ADBC, BDAC and BCAD.

The probability of a certain line falling into a given class in a generation is dependent upon the probability of the line, from which derived in the previous generation, being in the different classes, and not upon its own genotype. Such probabilities can be determined by first calculating the probabilities of classes in generation 1 producing the different classes in generation 2. These can be seen in table 3. The method of calculation can be seen by taking, for example, class VI in generation 1. A fertilized female ABAB produces progeny of AB, AA and BB in the ratio 2:1:1 respectively. The following generation fertilized females may thus be of the classes ABAA (class II), AB BB (class II), ABAB (class VI), AAAA (class I), BBBB (class I) or AABB (class IV) in the ratio 4:4:4:1:1:2 respectively, thus producing probabilities of 0.5, 0.25, 0.125 and 0.125 for the classes II, VI, I and IV respectively.

From table 3 one can derive recursion equations to determine the probability of generation 2 having a certain class of fertilized female. These are seen in figure 115. The original parental class is XII (ABCD), thus with a probability of 1. The recursion equations of figure 115 can be used to determine the probabilities of the different classes occurring in subsequent generations. These are shown in table 4 for up to 14 generations.

Transpositions occurring in the gametes of F1 flies will produce F2 flies with different genomes, some possessing one

Table 3: Probability of a class of female in generation 1 producing the different classes in generation 2

Class in generation 1	Class in generation 2	Probability
I	I	1.0000
II	I	0.2500
	II	0.5000
	VI	0.2500
III	I	0.2500
	III	0.5000
	VII	0.2500
IV	VI	1.0000
V	VII	1.0000
VI	I	0.1250
	II	0.5000
	IV	0.1250
	VI	0.2500
VII	I	0.1250
	III	0.5000
	V	0.1250
	VII	0.2500
VIII	I	0.0625
	II	0.1250
	III	0.1250
	VI	0.0625
	VII	0.1250
	VIII	0.2500
	IX	0.1250
	X	0.1250
IX	VI	0.2500
	VII	0.2500
	VIII	0.5000
X	I	0.0625
	III	0.2500
	VI	0.0625
	VII	0.1250
	VIII	0.2500
	X	0.1250
	XI	0.1250
XI	VII	0.5000
	X	0.5000
XII	VII	0.2500
	X	0.5000
	XIII	0.2500
XIII	VI	0.1250
	VII	0.1250
	VIII	0.5000
	XII	0.1250
	XIII	0.1250

Figure 115: Recursion equations to determine the probability of a generation possessing a fertilized female of a particular class. I_1, II_1, III_1 etc. represent the probability of the female being of that class in generation 1. I_2, II_2, III_2 etc. represent the probability of the female being of that class in generation 2.

$$I_2 = I_1 + \frac{II_1}{4} + \frac{III_1}{4} + \frac{VI_1}{8} + \frac{VII_1}{8} + \frac{VIII_1}{16} + \frac{X_1}{16}$$

$$II_2 = \frac{II_1}{2} + \frac{VI_1}{2} + \frac{VIII_1}{8}$$

$$III_2 = \frac{III_1}{2} + \frac{VII_1}{2} + \frac{VIII_1}{8} + \frac{X_1}{4}$$

$$IV_2 = \frac{VI_1}{8}$$

$$V_2 = \frac{VII_1}{8}$$

$$VI_2 = \frac{II_1}{4} + IV_1 + \frac{VI_1}{4} + \frac{VIII_1}{16} + \frac{IX_1}{4} + \frac{X_1}{16} + \frac{XIII_1}{8}$$

$$VII_2 = \frac{III_1}{4} + V_1 + \frac{VII_1}{4} + \frac{VIII_1}{8} + \frac{IX_1}{4} + \frac{X_1}{8} + \frac{XI_1}{2} + \frac{XII_1}{4} + \frac{XIII_1}{8}$$

$$VIII_2 = \frac{VIII_1}{4} + \frac{IX_1}{2} + \frac{X_1}{4} + \frac{XIII_1}{2}$$

$$IX_2 = \frac{VIII_1}{8}$$

$$X_2 = \frac{VIII_1}{8} + \frac{X_1}{8} + \frac{XI_1}{2} + \frac{XII_1}{2}$$

$$XI_2 = \frac{X_1}{8}$$

$$XII_2 = \frac{XIII_1}{8}$$

$$XIII_2 = \frac{XII_1}{4} + \frac{XIII_1}{8}$$

Table 4: The probabilities of possessing females of a particular class in the 14 generations produced from a class XII female. They were determined from the recursion equations of figure 115.

Generation	Class												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Parental	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000
F1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2500	0.0000	0.0000	0.5000	0.0000	0.0000	0.2500
F2	0.0625	0.0000	0.2500	0.0000	0.0313	0.0625	0.1563	0.2500	0.0000	0.0625	0.0625	0.0313	0.0313
F3	0.1719	0.0625	0.2500	0.0078	0.0195	0.0391	0.2148	0.0938	0.0313	0.0859	0.0078	0.0039	0.0117
F4	0.2930	0.0625	0.2656	0.0049	0.0269	0.0537	0.1724	0.0664	0.0117	0.0283	0.0107	0.0015	0.0025
F5	0.4092	0.0664	0.2344	0.0067	0.0216	0.0431	0.1572	0.0308	0.0083	0.0180	0.0035	0.0003	0.0007
F6	0.5124	0.0586	0.2041	0.0054	0.0196	0.0393	0.1295	0.0167	0.0039	0.0080	0.0022	0.0001	0.0002
F7	0.6007	0.0510	0.1709	0.0049	0.0162	0.0324	0.1082	0.0082	0.0021	0.0043	0.0010	0.0000	0.0000
F8	0.6745	0.0428	0.1417	0.0041	0.0135	0.0271	0.0886	0.0042	0.0010	0.0021	0.0005	0.0000	0.0000
F9	0.7355	0.0354	0.1162	0.0034	0.0111	0.0222	0.0724	0.0021	0.0005	0.0011	0.0003	0.0000	0.0000
F10	0.7854	0.0291	0.0948	0.0028	0.0091	0.0181	0.0589	0.0010	0.0003	0.0005	0.0001	0.0000	0.0000
F11	0.8260	0.0237	0.0771	0.0023	0.0074	0.0147	0.0478	0.0005	0.0001	0.0003	0.0001	0.0000	0.0000
F12	0.8591	0.0193	0.0626	0.0018	0.0060	0.0120	0.0387	0.0003	0.0001	0.0001	0.0000	0.0000	0.0000
F13	0.8859	0.0157	0.0507	0.0015	0.0048	0.0097	0.0314	0.0002	0.0000	0.0001	0.0000	0.0000	0.0000
F14	0.9076	0.0127	0.0411	0.0012	0.0039	0.0079	0.0254	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000

type of transposition, others with a different type, and some lacking any new transposition sites. F3 vials set up from these F2 flies will therefore have different transpositions. Various lines separated at the F3 generation or earlier can be analysed using table 4 and figure 4 to determine the expected number of F1 genomes examined in each of these lines. Each F3 line is taken separately for analysis:

Line Aa

The fertilized F2 female used to set up the F3 vial possesses 4 genomes. The 2 F4 vials produced will be subsequently inbred to homozygosity, and thus will be essentially equivalent to sampling 2 haploid genomes of offspring in the F3 vial. These offspring will have, between them, 4 genomes (those in their F2 mother and father). There is a 1 in 4 chance that for any genomic region the same genome has been sampled in Aa2 as in Aa1. The expected number of different genomes examined in Aa1 and Aa2 is therefore 1.75.

Line Ab1

The F3 vial was established with a single fertilized F2 female with 4 genomes which can be called A, B, C and D, and 2 F3 females taken to set up 2 vials, Ab1(a) and Ab1(b). In each of these vials single F4 and F5 females were taken to form the F5 and F6 generations respectively, and then 3 F6 Ab1(a) females taken to set up sublines, and 2 F6 Ab1(b) females. The F2 female originally used to produce the F3 offspring, from which the Ab1(a) and Ab1(b) lines were derived, can be

considered as being the parental generation, and thus the F3 females taken as being the F1 generation, the F4's as the F2, and the F5's as the F3 generation.

The Abl(a) and Abl(b) sublines can be initially taken separately in the analysis. The 3 females from the F6 vial in figure 4, used to form the F7 vials, are equivalent to 3 gametes taken from the F5 male and female used to produce the F6 vial. The probabilities of representation of the various classes of fertilized female can thus be calculated for the 3 Abl(a) vials by using the F3 generation probabilities of table 4 (as the F5 generation is equivalent to the F3 generation produced from the F2). However, if samples are taken from the gametes of an individual with 4 genomes (which may be of different types), the number of different types represented in the sample needs to be calculated. This will vary for the different sample sizes as can be seen in table 5.

The probabilities that 1, 2 or 3 of the 4 genomes A, B, C and D (of the F2 generation Abl(a) female) are represented in the 3 Abl(a) vials can thus be calculated using tables 4 and 5. The probability of 1 genome being represented is calculated by multiplying the probabilities of the different classes of fertilized female occurring in the F3 generation (as explained above) by the probabilities of receiving 1 genome type for these different classes with a sample size of 3 (as 3 Abl(a) sublines were sampled).

Table 5: The probability of receiving 1, 2 or 3 different types of haploid genome A, B, C and D for various sample sizes taken from different fertilized female classes. Characters in brackets show examples of the classes, and also the genomes received by each sample from such examples.

Sample Size 1: 1 type of genome is received

Sample Size 2:

Class of female from which received	Number of types of genomes received	Probability
I (e.g.AAAA)	1 (AA)	1.0000
II or III (e.g.AAAB)	1 (AA or BB) 2 (AB)	0.6250 0.3750
IV, V, VI or VII (e.g.ABAB)	1 (AA or BB) 2 (AB)	0.5000 0.5000
VIII, IX, X, or XI (e.g.AABC)	1 (AA, BB or CC) 2 (AB, BC or AC)	0.3750 0.6250
XII or XIII (e.g.ABCD)	1 (AA, BB, CC or DD) 2 (AB, AC, AD, BC, BD or CD)	0.2500 0.7500

Sample Size 3:

Class of female from which received	Number of types of genomes received	Probability
I (e.g.AAAA)	1 (AAA)	1.0000
II or III (e.g.AAAB)	1 (AAA or BBB) 2 (ABB or AAB)	0.4375 0.5625
IV, V, VI or VII (e.g.ABAB)	1 (AAA or BBB) 2 (ABB or AAB)	0.2500 0.7500
VIII, IX, X or XI (e.g.AABC)	1 (AAA, BBB or CCC) 2 (AAB, AAC, ABB, ACC, BCC or CBB) 3 (ABC)	0.1563 0.6563 0.1875
XII or XIII (e.g.ABCD)	1 (AAA, BBB, CCC or DDD) 2 (AAB, AAC, AAD, BBA, BBC, BBD, CCA, CCB, CCD, DDA, DDB or DDC) 3 (ABC, ABD, ACD or BCD)	0.0625 0.5625 0.3750

Probability of 1 genome

$$\begin{aligned} &= [0.1719 \text{ (class I, F3; table 4) } \times 1.0000 \text{ (class I,} \\ &\quad \text{type 1, sample size 3; table 5)}] \\ &+ [0.3125 \text{ (class II + class III, F3) } \times 0.4375 \text{ (class II} \\ &\quad \text{or III, type 1, sample size 3)}] \\ &+ [0.2812 \times 0.2500 \text{ (classes IV-VII)}] \\ &+ [0.2188 \times 0.1563 \text{ (classes VIII-XI)}] \\ &+ [0.0156 \times 0.0625 \text{ (classes XII and XIII)}] \\ &= 0.4141 \end{aligned}$$

One can carry out a similar calculation for the probabilities of 2 and 3 genomes being represented by using the values from table 5 for 2 and 3 types of genome respectively.

Probability of 2 genomes

$$\begin{aligned} &= [0.3125 \times 0.5625 \text{ (classes II and III)}] \\ &+ [0.2812 \times 0.7500 \text{ (classes IV-VII)}] \\ &+ [0.2188 \times 0.6563 \text{ (classes VIII-XI)}] \\ &+ [0.0156 \times 0.5625 \text{ (classes XII and XIII)}] \\ &= 0.5391 \end{aligned}$$

Probability of 3 genomes

$$\begin{aligned} &= [0.2188 \times 0.1875 \text{ (classes VIII-XI)}] \\ &+ [0.0156 \times 0.3750 \text{ (classes XII and XIII)}] \\ &= 0.0469 \end{aligned}$$

A similar calculation can be carried out for the probability of representation of A, B, C and D in the 2 Abl(b) vials, but using a sample size of 2 from table 5.

$$\begin{aligned}
 \text{Probability of 1 genome} &= [0.1719 \times 1.0000] \\
 &+ [0.3125 \times 0.6250] \\
 &+ [0.2812 \times 0.5000] \\
 &+ [0.2188 \times 0.3750] \\
 &+ [0.0156 \times 0.2500] \\
 &= 0.5938
 \end{aligned}$$

$$\begin{aligned}
 \text{Probability of 2 genomes} &= [0.3125 \times 0.3750] \\
 &+ [0.2812 \times 0.5000] \\
 &+ [0.2188 \times 0.6250] \\
 &+ [0.0156 \times 0.7500] \\
 &= 0.4062
 \end{aligned}$$

From these probabilities one can calculate the probabilities that 1, 2, 3 or 4 of the genomes A, B, C and D are present in all 5 of the Abl(a) and Abl(b) vials together.

If 1 genome is represented in the Abl(a) vials (at a probability of 0.4141):

$$\begin{aligned}
 \text{Probability of 1 in all 5 vials} \\
 &= 0.5938/4 \times 0.4141 \\
 &= 0.0615
 \end{aligned}$$

$$\begin{aligned}
 \text{Probability of 2 in all 5 vials} \\
 &= [(0.5938 \times 0.75) + 0.4062/2] \times 0.4141 \\
 &= 0.2685
 \end{aligned}$$

.....

Probability of 3 in all 5 vials

$$= 0.4062/2 \times 0.4141$$

$$= 0.0841$$

If 2 genomes are represented in the Abl(a) vials (at a probability of 0.5391):

Probability of 2 in all 5 vials

$$= [0.5938/2 + 0.4062/6] \times 0.5391$$

$$= 0.1966$$

Probability of 3 in all 5 vials

$$= [0.5938/2 + (0.4062 \times 0.6667)] \times 0.5391$$

$$= 0.3060$$

Probability of 4 in all 5 vials

$$= 0.4062/6 \times 0.5391$$

$$= 0.0365$$

If 3 genomes are represented in the Abl(a) vials (at a probability of 0.0469):

Probability of 3 in all 5 vials

$$= [(0.5938 \times 0.75) + 0.4062/2] \times 0.0469$$

$$= 0.0304$$

Probability of 4 in all 5 vials

$$= [(0.5938 \times 0.25) + 0.4062/2] \times 0.0469$$

$$= 0.0165$$

The total probabilities of 1, 2, 3 or 4 genomes being represented in the 5 Ab1 vials are thus 0.0615, (0.2685 + 0.1966 =) 0.4651, (0.0841 + 0.3060 + 0.0304 =) 0.4205, and (0.0365 + 0.0165 =) 0.0530 respectively. The expected number of genomes examined in the 5 vials can thus be calculated from these probabilities.

Expected number of genomes in line Ab1

$$\begin{aligned}
 &= 0.0615 + (2 \times 0.4651) + (3 \times 0.4205) + (4 \times 0.0530) \\
 &= \underline{2.465}
 \end{aligned}$$

Line Ab2(a)

The F3 vial was established with a single fertilized F2 female possessing 4 genomes A, B, C and D, and then single females taken to form each subsequent generation. 2 females were taken from the F10 generation to produce 2 sublines, Ab2(a)I and Ab2(a)II. The F11 generation vials are equivalent to sampling 2 gametes from the F9 fertilized female which gave rise to the F10 offspring. The F2 female can be considered as the parental generation, and thus the F9 female as the F7 generation.

One can calculate the probability of 1 or 2 genomes being represented in the 2 Ab2(a) vials using the probabilities of the different fertilized female classes occurring in the F7 generation, and the probabilities of receiving 1 or 2 genome types in a sample size of 2.

$$\begin{aligned}
\text{Probability of 1 genome} &= [0.6007 \times 1.0000] \\
&+ [0.2219 \times 0.6250] \\
&+ [0.1617 \times 0.5000] \\
&+ [0.0156 \times 0.3750] \\
&= 0.8261
\end{aligned}$$

$$\begin{aligned}
\text{Probability of 2 genomes} &= [0.2219 \times 0.3750] \\
&+ [0.1617 \times 0.5000] \\
&+ [0.0156 \times 0.6250] \\
&= 0.1739
\end{aligned}$$

The expected number of genomes examined in the 2 Ab2(a) vials can thus be calculated to be:

$$0.8261 + (2 \times 0.1739) = \underline{1.174}$$

Line Ab2(b) (for the transposable elements 412, copia and F where all 3 of the Ab2(b)III lines (Ab2(b)IIIX, Y and Z) were examined)

The F3 vial was established with a single fertilized F2 female possessing a genome which can be called A, B, C and D, and then single females taken to form each subsequent generation. Ab2(b) was split into 3 vials, Ab2(b)I, II and III, by taking 3 single fertilized females of the F5 generation. The F4 fertilized female which produced these flies can be said to have genomes a, b, c and d. These Ab2(b) vials were again divided, the Ab2(b)I and III vials to form 3 sublines each at generation F7, and the Ab2(b)II vial to form 2 sublines at generation F8. If the F4 female is initially

regarded as being the parental generation, the 3 Ab2(b)I and III lines are each equivalent to 3 gametes sampled from her F2 generation, and the 2 Ab2(b)II lines the equivalent of sampling 2 F3 gametes.

One can calculate the probabilities of the genomes a, b, c and d being represented in the final Ab2(b) sublines. The sublines Ab2(b)I, II and III are initially considered separately.

Lines Ab2(b)I and III (using F2 probabilities from table 4 and a sample size of 3 from table 5):

$$\begin{aligned}
 \text{Probability of 1 genome} &= [0.0625 \times 1.0000] \\
 &+ [0.2500 \times 0.4375] \\
 &+ [0.2500 \times 0.2500] \\
 &+ [0.3750 \times 0.1563] \\
 &+ [0.0625 \times 0.0625] \\
 &= 0.2969
 \end{aligned}$$

$$\begin{aligned}
 \text{Probability of 2 genomes} &= [0.2500 \times 0.5625] \\
 &+ [0.2500 \times 0.7500] \\
 &+ [0.3750 \times 0.6563] \\
 &+ [0.0625 \times 0.5625] \\
 &= 0.6094
 \end{aligned}$$

$$\begin{aligned}
 \text{Probability of 3 genomes} &= [0.3750 \times 0.1875] \\
 &+ [0.0625 \times 0.3750] \\
 &= 0.0937
 \end{aligned}$$

Line Ab2(b)II (using F3 probabilities from table 4 and a sample size of 2 from table 5):

$$\begin{aligned}
 \text{Probability of 1 genome} &= [0.1719 \times 1.0000] \\
 &+ [0.3125 \times 0.6250] \\
 &+ [0.2812 \times 0.5000] \\
 &+ [0.2188 \times 0.2500] \\
 &+ [0.0156 \times 0.2500] \\
 &= 0.5938
 \end{aligned}$$

$$\begin{aligned}
 \text{Probability of 2 genomes} &= [0.3125 \times 0.3750] \\
 &+ [0.2812 \times 0.5000] \\
 &+ [0.2188 \times 0.6250] \\
 &+ [0.0156 \times 0.7500] \\
 &= 0.4062
 \end{aligned}$$

From these probabilities one can calculate the probabilities that 1, 2, 3 or 4 of the genomes a, b, c and d are present in all 8 of the Ab2(b) vials examined.

Ab2(b)I and III can be initially considered:

If 1 genome is represented in the Ab2(b)I vials (at a probability of 0.2969):

$$\begin{aligned}
 \text{Probability of 1 in all Ab2(b)I and III vials} \\
 &= 0.2969/4 \times 0.2969 \\
 &= 0.0220
 \end{aligned}$$

Probability of 2 in all Ab2(b)I and III vials

$$\begin{aligned} &= [(0.2969 \times 0.75) + 0.6094/2] \times 0.2969 \\ &= 0.1565 \end{aligned}$$

Probability of 3 in all Ab2(b)I and III vials

$$\begin{aligned} &= [0.6094/2 + (0.0937 \times 0.75)] \times 0.2969 \\ &= 0.1113 \end{aligned}$$

Probability of 4 in all Ab2(b)I and III vials

$$\begin{aligned} &= 0.0937/4 \times 0.2969 \\ &= 0.0070 \end{aligned}$$

If 2 genomes are represented in the Ab2(b)I vials (at a probability of 0.6094):

Probability of 2 in all Ab2(b)I and III vials

$$\begin{aligned} &= [0.2969/2 + 0.6094/6] \times 0.6094 \\ &= 0.1523 \end{aligned}$$

Probability of 3 in all Ab2(b)I and III vials

$$\begin{aligned} &= [0.2969/2 + (0.6094 \times 0.6667) + 0.0937/2] \times 0.6094 \\ &= 0.3667 \end{aligned}$$

Probability of 4 in all Ab2(b)I and III vials

$$\begin{aligned} &= [0.6094/6 + 0.0937/2] \times 0.6094 \\ &= 0.0904 \end{aligned}$$

If 3 genomes are represented in the Ab2(b)I vials (at a probability of 0.0937):

Probability of 3 in all Ab2(b)I and III vials

$$\begin{aligned} &= [(0.2969 \times 0.75) + 0.6094/2 + 0.0937/4] \times 0.0937 \\ &= 0.0516 \end{aligned}$$

Probability of 4 in all Ab2(b)I and III vials

$$\begin{aligned} &= [0.2969/4 + 0.6094/2 + (0.0937 \times 0.75)] \times 0.0937 \\ &= 0.0421 \end{aligned}$$

The total probabilities of 1, 2, 3 or 4 of the a, b, c and d genomes being represented in the Ab2(b)I and III vials are thus 0.0220, (0.1566 + 0.1523 =) 0.3089, (0.1113 + 0.3667 + 0.0516 =) 0.5296, and (0.007 + 0.0904 + 0.0421 =) 0.1395 respectively.

One can calculate the probabilities that 1, 2, 3 or 4 of the genomes are present in all of the Ab2(b) vials by combining these Ab2(b)I plus III probabilities with those determined for Ab2(b)II alone.

If 1 genome is represented in the Ab2(b)I and III vials (at a probability of 0.0220):

Probability of 1 in all Ab2(b) vials

$$\begin{aligned} &= 0.538/4 \times 0.0220 \\ &= 0.0033 \end{aligned}$$

Probability of 2 in all Ab2(b) vials

$$\begin{aligned} &= [(0.5938 \times 0.75) + 0.4062/2] \times 0.0220 \\ &= 0.0143 \end{aligned}$$

Probability of 3 in all Ab2(b) vials

$$\begin{aligned} &= 0.4062/2 \times 0.0220 \\ &= 0.0045 \end{aligned}$$

.....
If 2 genomes are represented in the Ab2(b)I and III vials (at a probability of 0.3089):

$$\begin{aligned} &\text{Probability of 2 in all Ab2(b) vials} \\ &= [0.5938/2 + 0.4062/6] \times 0.3089 \\ &= 0.1126 \end{aligned}$$

$$\begin{aligned} &\text{Probability of 3 in all Ab2(b) vials} \\ &= [0.5938/2 + (0.4062 \times 0.6667)] \times 0.3089 \\ &= 0.1754 \end{aligned}$$

$$\begin{aligned} &\text{Probability of 4 in all Ab2(b) vials} \\ &= 0.4062/6 \times 0.3089 \\ &= 0.0209 \end{aligned}$$

If 3 genomes are represented in the Ab2(b)I and III vials (at a probability of 0.5296):

$$\begin{aligned} &\text{Probability of 3 in all Ab2(b) vials} \\ &= [(0.5938 \times 0.75) + 0.4062/2] \times 0.5296 \\ &= 0.3434 \end{aligned}$$

$$\begin{aligned} &\text{Probability of 4 in all Ab2(b) vials} \\ &= [0.5938/4 + 0.4062/2] \times 0.5296 \\ &= 0.1862 \end{aligned}$$

If 4 genomes are represented in the Ab2(b)I and III vials (at a probability of 0.1395):

$$\begin{aligned} &\text{Probability of 4 in all Ab2(b) vials} \\ &= 0.1395 \end{aligned}$$

The total probabilities of 1, 2, 3 or 4 of the a, b, c and d genomes being represented in all 8 of the Ab2(b) vials are thus 0.0033, $(0.0143 + 0.1126 =) 0.1269$, $(0.0045 + 0.1754 + 0.3434 =) 0.5233$ and $(0.0209 + 0.1862 + 0.1395 =) 0.3466$ respectively.

The original abcd fertilized female, however, represents the F2 generation of the ABCD genome Ab2(b) fertilized female. The probability of 1, 2, 3 or 4 of the A, B, C and D genomes being represented in the 8 vials therefore has to be calculated.

The probabilities that the F2 a, b, c and d genomes represent 4 copies of the same genome (class I) from ABCD is 0.0625 (table 4). If this were the case, only 1 genome would be represented from ABCD.

The probability that abcd represents a class II or III fertilized female is 0.2500. If this were the case, and also only 1 copy of a, b, c or d was represented in all 8 of the Ab2(b) vials (probability of 0.0033), then only 1 genome also would be represented from ABCD. If, however, 2 copies of a, b, c and d were represented in the 8 Ab2(b) vials (probability of 0.1269), then there is a probability of 0.5 of 2 genomes of ABCD being represented, and 0.5 of 1. If 3 copies of a, b, c and d were present in the Ab2(b) vials examined (probability of 0.5233), then there is a probability of 0.25 that 1 genome of ABCD was represented, and 0.75 of 2 being present. If 4 copies of a, b, c and d were present (probability of 0.3466), however, then 2 genomes from ABCD would be represented.

The probability that abcd represents a fertilized female of class IV, V, VI or VII is 0.2500. If this were the case, and only 1 copy of a, b, c or d was represented in the final

Ab2(b) vials (probability of 0.0033), then only 1 genome would be represented from ABCD. If 2 copies of a, b, c and d were present in the 8 Ab2(b) vials (probability of 0.1269), then there is a probability of 0.3333 that 1 genome from ABCD was represented, and 0.6667 that 2 were present. If 3 or 4 copies were represented (probability of 0.5233 and 0.3466 respectively), then 2 genomes from ABCD would be present in the Ab2(b) vials.

The probability that abcd represents a class VIII, IX, X or XI fertilized female is 0.3750. If this were the case, and only 1 copy of a, b, c or d was represented in the 8 Ab2(b) vials (probability of 0.0033), then only 1 genome would be present from ABCD. If 2 copies of a, b, c and d were represented (probability of 0.1269), then the probability of 1 genome of ABCD being represented is 0.1667, and that of 2 genomes is 0.8333. If 3 copies of a, b, c and d were present in the 8 Ab2(b) vials (probability of 0.5233), then the probability that 2 or 3 genomes of ABCD were represented is 0.5 in each case. If 4 copies of a, b, c or d were represented (probability of 0.3466), then 3 genomes from ABCD were also present.

The probability that abcd represents a class XII or XIII fertilized female is 0.0625. If this were the case, and 1, 2, 3 or 4 copies of a, b, c and d were represented in the 8 Ab2(b) vials examined (probabilities of 0.0033, 0.1269, 0.5233 and 0.3466 respectively), then also 1, 2, 3 or 4 genomes, respectively, from ABCD were present in the 8 vials at a probability of 1.0000 in each case.

One can therefore calculate the total probabilities of 1, 2, 3 or 4 genomes of A, B, C and D being represented in the 8 Ab2(b) vials examined.

Probability of 1 in all Ab2(b) vials

$$\begin{aligned}
 &= 0.0625 \\
 &+ 0.2500 [0.0033 + (0.1269 \times 0.5) + (0.5233 \times 0.25)] \\
 &+ 0.2500 [0.0033 + (0.1269 \times 0.3333)] \\
 &+ 0.3750 [0.0033 + (0.1269 \times 0.1667)] \\
 &+ 0.0625 [0.0033] \\
 &= 0.1326
 \end{aligned}$$

Probability of 2 in all Ab2(b) vials

$$\begin{aligned}
 &= 0.2500 [(0.1269 \times 0.5) + (0.5233 \times 0.75) + 0.3466] \\
 &+ 0.2500 [(0.1269 \times 0.6667) + 0.5233 + 0.3466] \\
 &+ 0.3750 [(0.1269 \times 0.8333) + (0.5233 \times 0.5)] \\
 &+ 0.0625 [0.1269] \\
 &= 0.5850
 \end{aligned}$$

Probability of 3 in all Ab2(b) vials

$$\begin{aligned}
 &= 0.3750 [(0.5233 \times 0.5) + 0.3466] \\
 &+ 0.0625 [0.5233] \\
 &= 0.2608
 \end{aligned}$$

Probability of 4 in all Ab2(b) vials

$$\begin{aligned}
 &= 0.0625 [0.3466] \\
 &= 0.0217
 \end{aligned}$$

The expected number of genomes examined in the 8 Ab2(b) vials can thus be calculated to be:

$$0.1326 + (2 \times 0.5850) + (3 \times 0.2608) + (4 \times 0.0217) \\ = \underline{2.172}$$

Line Ab2(b) (for the P element, where only 2 of the Ab2(b)III lines (Ab2(b)IIIX and Y) were examined)

The calculation follows that used for line Ab2(b) above with 8 vials examined, except that new probabilities of representation have to be determined for the Ab2(b)III subline. The probability of the F4 generation genomes a, b, c and d being represented in the final Ab2(b)III vials can be calculated using F2 probabilities from table 4 and a sample size of 2 from table 5.

$$\begin{aligned} \text{Probability of 1 genome} &= [0.0625 \times 1.0000] \\ &+ [0.2500 \times 0.6250] \\ &+ [0.2500 \times 0.5000] \\ &+ [0.3750 \times 0.3750] \\ &+ [0.0625 \times 0.2500] \\ &= 0.5000 \end{aligned}$$

$$\begin{aligned} \text{Probability of 2 genomes} &= [0.2500 \times 0.3750] \\ &+ [0.2500 \times 0.5000] \\ &+ [0.3750 \times 0.6250] \\ &+ [0.0625 \times 0.7500] \\ &= 0.5000 \end{aligned}$$

One can combine these probabilities with those calculated for sublines Ab2(b)I and II to determine the probabilities that 1, 2, 3 or 4 of the genomes a, b, c and d are present in all 7 of the Ab2(b) vials examined.

Ab2(b)I and III can be initially considered:

If 1 genome is represented in the Ab2(b)I vials (at a probability of 0.2969):

Probability of 1 in all Ab2(b)I and III vials
= $0.5000/4 \times 0.2969$
= 0.0371

Probability of 2 in all Ab2(b)I and III vials
= $[(0.5000 \times 0.75) + 0.5000/2] \times 0.2969$
= 0.1856

Probability of 3 in all Ab2(b)I and III vials
= $0.5000/2 \times 0.2969$
= 0.0742

If 2 genomes are represented in the Ab2(b)I vials (at a probability of 0.6094):

Probability of 2 in all Ab2(b)I and III vials
= $[0.5000/2 + 0.5000/6] \times 0.6094$
= 0.2031

Probability of 3 in all Ab2(b)I and III vials
= $[0.5000/2 + (0.5000 \times 0.6667)] \times 0.6094$
= 0.3555

Probability of 4 in all Ab2(b)I and III vials

$$= 0.5000/6 \times 0.6094$$

$$= 0.0508$$

If 3 genomes are represented in the Ab2(b)I vials (at a probability of 0.0937):

Probability of 3 in all Ab2(b)I and III vials

$$= [(0.5000 \times 0.75) + 0.5000/2] \times 0.0937$$

$$= 0.0586$$

Probability of 4 in all Ab2(b)I and III vials

$$= [0.5000/4 + 0.5000/2] \times 0.0937$$

$$= 0.0351$$

The total probabilities of 1, 2, 3 or 4 of the a, b, c and d genomes being represented in the Ab2(b)I and III vials are thus 0.0371, (0.1856 + 0.2031 =) 0.3887, (0.0742 + 0.3555 + 0.0586 =) 0.4883 and (0.0508 + 0.0351 =) 0.0859 respectively.

One can calculate the probabilities that 1, 2, 3 or 4 of the genomes are present in all 7 Ab2(b) vials by combining the above probabilities with those calculated for Ab2(b)II alone.

If 1 genome is represented in the Ab2(b)I and III vials (at a probability of 0.0371):

Probability of 1 in all Ab2(b) vials

$$= 0.0938/4 \times 0.0371$$

$$= 0.0055$$

.....

Probability of 2 in all Ab2(b) vials

$$\begin{aligned} &= [(0.5938 \times 0.75) + 0.4062/2] \times 0.0371 \\ &= 0.0241 \end{aligned}$$

Probability of 3 in all Ab2(b) vials

$$\begin{aligned} &= 0.4062/2 \times 0.0371 \\ &= 0.0075 \end{aligned}$$

If 2 genomes are represented in the Ab2(b)I and III vials (at a probability of 0.3887):

Probability of 2 in all Ab2(b) vials

$$\begin{aligned} &= [0.5938/2 + 0.4062/6] \times 0.3887 \\ &= 0.1417 \end{aligned}$$

Probability of 3 in all Ab2(b) vials

$$\begin{aligned} &= [0.5938/2 + (0.4062 \times 0.6667)] \times 0.3887 \\ &= 0.2207 \end{aligned}$$

Probability of 4 in all Ab2(b) vials

$$\begin{aligned} &= 0.4062/6 \times 0.3887 \\ &= 0.0263 \end{aligned}$$

If 3 genomes are represented in the Ab2(b)I and III vials (at a probability of 0.4883):

Probability of 3 in all Ab2(b) vials

$$\begin{aligned} &= [(0.5938 \times 0.75) + 0.4062/2] \times 0.4883 \\ &= 0.3166 \end{aligned}$$

Probability of 4 in all Ab2(b) vials

$$\begin{aligned} &= [0.5938/4 + 0.4062/2] \times 0.4883 \\ &= 0.1717 \end{aligned}$$

.....

If 4 genomes are represented in the Ab2(b)I and III vials (at a probability of 0.0859):

Probability of 4 in all Ab2(b) vials
= 0.0859

The total probabilities of 1, 2, 3 or 4 of the a, b, c and d genomes being represented in all 7 of the Ab2(b) vials are thus 0.0055, (0.0241 + 0.1417 =) 0.1658, (0.0075 + 0.2207 + 0.3166 =) 0.5448 and (0.0263 + 0.1717 + 0.0859 =) 0.2839 respectively.

The probabilities that 1, 2, 3 or 4 of the F2 generation A, B, C and D genomes are represented in the 7 vials can be calculated using the above probabilities and table 4. The method used is the same as that with all 8 Ab2(b) vials in the previous calculation.

Probability of 1 in all 7 Ab2(b) vials
= 0.0625
+ 0.2500 [0.0055 + (0.1658 x 0.5) + (0.5448 x 0.25)]
+ 0.2500 [0.0055 + (0.1658 x 0.3333)]
+ 0.3750 [0.0055 + (0.1658 x 0.1667)]
+ 0.0625 [0.0055]
= 0.1466

Probability of 2 in all 7 Ab2(b) vials

$$\begin{aligned} &= 0.2500 [(0.1658 \times 0.5) + (0.5448 \times 0.75) + 0.2839] \\ &+ 0.2500 [(0.1658 \times 0.6667) + 0.5448 + 0.2839] \\ &+ 0.3750 [(0.1658 \times 0.8333) + (0.5448 \times 0.5)] \\ &+ 0.0625 [0.1658] \\ &= 0.5923 \end{aligned}$$

Probability of 3 in all 7 Ab2(b) vials

$$\begin{aligned} &= 0.3750 [(0.5448 \times 0.5) + 0.2839] \\ &+ 0.0625 [0.5448] \\ &= 0.2427 \end{aligned}$$

Probability of 4 in all 7 Ab2(b) vials

$$\begin{aligned} &= 0.0625 [0.2839] \\ &= 0.0177 \end{aligned}$$

The expected number of genomes examined in the 7 Ab2(b) vials can thus be calculated to be:

$$\begin{aligned} &0.1466 + (2 \times 0.5923) + (3 \times 0.2427) + (4 \times 0.0177) \\ &= \underline{2.130} \end{aligned}$$

Line Ab3

Only 1 line was examined.

Line Ac1

This is similar to line Aa, the expected number of genomes examined therefore being 1.75.

Line Ac2

The F3 vial was established from a single F2 fertilized female with genomes which can be called A, B, C and D, and then 2 females from these taken to form 2 F4 vials, Ac2(a) and Ac2(b). The Ac2(b) subline was further divided by taking 2 females from the F12 generation to produce Ac2(b)I and II. The F13 generation vials are equivalent to sampling 2 gametes from the F11 fertilized female which gave rise to the F12 offspring. The F2 ABCD fertilized female can be considered as the parental generation, and thus the F11 female as the F9 generation.

One can calculate the probability of 1 or 2 of the A, B, C and D genomes being represented in the Ac2(b) sublines using the probabilities of the different classes of fertilized female occurring in the F9 generation (table 4) and the probabilities of receiving 1 or 2 genome types for the classes in a sample size of 2 (table 5).

$$\begin{aligned}\text{Probability of 1 genome} &= [0.7355 \times 1.0000] \\ &+ [0.1516 \times 0.6250] \\ &+ [0.1091 \times 0.5000] \\ &+ [0.0040 \times 0.3750] \\ &= 0.8862\end{aligned}$$

$$\begin{aligned}\text{Probability of 2 genomes} &= [0.1516 \times 0.3750] \\ &+ [0.1091 \times 0.5000] \\ &+ [0.0040 \times 0.6250] \\ &= 0.1138\end{aligned}$$

The Ac2(a) subline represents an independent sample to Ac2(b) taken from ABCD. If there is 1 genome type in Ac2(b) (with a probability of 0.8862), the probability of there being the same genome type in Ac2(a) is 0.25, and of being a different type, 0.75. If there are 2 genome types in Ac2(b) (with a probability of 0.1138), the probability of there being the same or different genome types in Ac2(a) is 0.5 in both cases. Thus the total probabilities of having 1, 2 or 3 genomes of A, B, C and D in the 3 Ac2 lines examined can be calculated.

Probability of 1 in all Ac2 vials

$$= (0.8862 \times 0.25)$$

$$= 0.2215$$

Probability of 2 in all Ac2 vials

$$= (0.8862 \times 0.75)$$

$$+ (0.1138 \times 0.5)$$

$$= 0.7216$$

Probability of 3 in all Ac2 vials

$$= (0.1138 \times 0.5)$$

$$= 0.0569$$

The expected number of genomes examined in the 3 Ac2 vials can thus be calculated to be:

$$0.2215 + (2 \times 0.7216) + (3 \times 0.0569)$$

$$= \underline{1.835}$$

Line Ac3 (for the transposable elements 412, copia and F where both of the sublines Ac3(a) and (b) were examined)

The F3 vial was established from a single F2 fertilized female with genomes which can be called A, B, C and D, and then single females taken to form subsequent generations. The line was divided into 2 sublines Ac3(a) and (b) by taking 2 females from the F8 generation. The F9 generation vials are equivalent to sampling 2 gametes from the F7 fertilized female which gave rise to the F8 offspring. The F2 ABCD female can be considered as the parental generation, and thus the F7 female as the F5 generation.

The probabilities of 1 or 2 of the A, B, C, and D genomes being represented in the sublines can be calculated using the probabilities of the different fertilized female classes occurring in the F5 generation (table 4), and the probabilities of receiving 1 or 2 genome types for the classes in a sample size of 2 (table 5).

$$\begin{aligned} \text{Probability of 1 genome} &= [0.4092 \times 1.0000] \\ &+ [0.3008 \times 0.6250] \\ &+ [0.2286 \times 0.5000] \\ &+ [0.0606 \times 0.3750] \\ &+ [0.0010 \times 0.2500] \\ &= 0.7343 \end{aligned}$$

$$\begin{aligned}
\text{Probability of 2 genomes} &= [0.3008 \times 0.3750] \\
&+ [0.2286 \times 0.5000] \\
&+ [0.0606 \times 0.6250] \\
&+ [0.0010 \times 0.7500] \\
&= 0.2657
\end{aligned}$$

The expected number of genomes examined in the 2 Ac3 vials can thus be calculated to be:

$$0.7343 + (2 \times 0.2657) = \underline{1.266}$$

Line Ac3 (for the P element where Ac3(b) only was examined)

Only 1 line was examined.

Line Ac4

The F3 vial was established from a single F2 fertilized female with 4 haploid genomes A, B, C and D, and then single fertilized females taken until generation F5, at which 2 sublines Ac4(a) and (b) were produced. The F6 generation vials are equivalent to sampling 2 gametes from the F4 fertilized female which gave rise to the F5 offspring. The F2 ABCD female can be considered as the parental generation, and thus the F4 female as generation F2.

The probabilities of 1 or 2 of the A, B, C and D genomes being represented in the sublines can be calculated using the probabilities of the different classes of fertilized female

occurring in the F2 generation (table 4), and the probabilities of receiving 1 or 2 genome types for the classes in a sample size of 2 (table 5).

$$\begin{aligned}
 \text{Probability of 1 genome} &= [0.0625 \times 1.0000] \\
 &+ [0.2500 \times 0.6250] \\
 &+ [0.2500 \times 0.5000] \\
 &+ [0.3750 \times 0.3750] \\
 &+ [0.0625 \times 0.7500] \\
 &= 0.5000
 \end{aligned}$$

$$\begin{aligned}
 \text{Probability of 2 genomes} &= [0.2500 \times 0.3750] \\
 &+ [0.2500 \times 0.5000] \\
 &+ [0.3750 \times 0.6250] \\
 &+ [0.0625 \times 0.7500] \\
 &= 0.5000
 \end{aligned}$$

The expected number of genomes examined in the 2 Ac4 vials can thus be calculated to be:

$$0.5000 + (2 \times 0.5000) = \underline{1.500}$$

The expected number of genomes examined in the different lines can be summated to determine the total expected number in the analysis.

1) All 27 lines examined using the transposable elements 412,
copia and F.

Aa	:	1.750
Ab1	:	2.465
Ab2(a)	:	1.174
Ab2(b)	:	2.174
Ab3	:	1.000
Ac1	:	1.750
Ac2	:	1.835
Ac3	:	1.266
Ac4	:	1.500

TOTAL : 14.914

2) The 25 lines examined using the P element.

Aa	:	1.750
Ab1	:	2.465
Ab2(a)	:	1.174
Ab2(b)	:	2.130
Ab3	:	1.000
Ac1	:	1.750
Ac2	:	1.835
Ac3	:	1.000
Ac4	:	1.500

TOTAL : 14.604

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These figures are expected numbers, and the expected variance in the number of new sites present in the dysgenic lines will include a component due to the sampling variance in the number of genomes examined, in addition to the Poisson variance of transpositions into the genomes. This incremental variance, however, will be small.

Chapter 7 : References

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