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Somatic reversion of P transposable element insertion mutations in the *singed* locus of *Drosophila melanogaster* requiring specific P insertions and a *trans*- acting factor

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Summary

Destabilization in somatic cells of P-element insertions in the X-linked *singed* gene of *Drosophila melanogaster* has been studied. We have shown that some but not all unstable P-element insertions in *singed* can form mosaics. The cause of this variation is not clear from studies of the restriction maps of the mutations tested. The transposable element movements occur early in development and require, in addition to an appropriate P-element insertion in *singed*, a *trans*-acting maternal effect component. Movements appear to occur preferentially in attached-X stocks. However, the maternal effect component maps to the central region of chromosome 2.

Introduction

The phenomenon of P-M hybrid dysgenesis has been shown to be due to a family of transposable genetic elements called P-elements (Engels, 1983, 1986). P-factors are autonomous P-elements capable of producing a transposase protein which can mobilize both themselves and the smaller, deleted, P-elements. The transposable elements are mobilized at high frequencies in P-M hybrid dysgenesis, generating chromosome breaks, insertion mutations, and reversions of pre-existing P insertions. P-M hybrid dysgenesis occurs in the F1 of a cross between males containing P factors (P-strain males) and females from strains lacking such factors (M-strains). Hybrid dysgenesis does not occur in the reciprocal cross, nor does it occur in crosses between P strains. Thus P-factors are active only in the cytoplasm contributed by M-strain mothers (the M-cytotype) but not in the cytoplasm contributed by P-strain mothers (or P-cytotype). Some mutations revert at particularly high frequencies. The mutation *singed*^{weak} (*sn*^{w}), for example, reverts, in dysgenic conditions, either to an almost wild-type phenotype or to a much more extreme *singed* phenotype, at a combined rate of around 50%.

With such a high rate of movement, it might be expected that such flies would also be mosaics, with areas of *singed*-extreme or wild-type tissue resulting from *sn*^{w} reversions in somatic cells. However, somatic P movements are very rare. Furthermore, McElwain (1986) has shown that, even though male recombination in germ cells in P-M hybrid dysgenesis is mitotic, somatic recombination frequencies are not enhanced by hybrid dysgenesis. The basis of this restriction is now understood. It has been shown that, while all four long open reading frames (open reading frames 0, 1, 2 and 3) of the P-factor are required for transposase activity, the most abundant mRNA species seen in somatic cells still retains the intron between open reading frames 2 and 3 and, if translated, would yield a truncated protein of 66 kiloDaltons lacking amino acids encoded by open reading frame 3 (Karess & Rubin, 1984). These data imply that the limitation of P transposase activity to germ cells in hybrid dysgenesis is the result of a germ-cell specific splicing event removing an intron between open reading frames 2 and 3 from the P-factor transcript. This idea has been confirmed by a demonstration that a genetically engineered P-factor, with the intron between ORFs 2 and 3 removed, will, when introduced into flies by P-element-mediated transformation, produce transposase activity throughout the fly (Laski, Rio & Rubin, 1986). This can be seen by the generation of mosaics for unstable P element mutations. Furthermore, if the engineered P factor lacking this intron is expressed in *D. melanogaster* tissue culture cells, an 87 kiloDalton protein is produced using information from all four open reading frames. In cells with this

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protein expressed excision of nonautonomous P-elements can occur (Rio, Laski & Rubin, 1986).

It would be expected that a parasitic transposable element which replicates by transposition should restrict its movements to germ cells (Charlesworth & Langley, 1986). This, however, is a weak argument when applied to the P-factor, which has evolved mainly in genomes other than *D. melanogaster* (Brookfield, Montgomery & Langley, 1984; Daniels *et al.*, 1984; Daniels & Strausbough, 1986; Lansman *et al.*, 1985; 1987; Stacey *et al.* 1986). Furthermore, it is not known what molecular difference between germ cells and somatic cells causes this affect. Neither is it known which part of the P transcript controls the difference in splicing. P-elements in hybrid dysgenesis move so much more frequently than most other animal transposable elements which have been studied in detail that it is still not clear whether their restriction of movements to germ cells is typical of animal transposable elements. The *Caenorhabditis elegans Tcl* element (Emmons & Yesner, 1984; Ruan & Emmons, 1987; Moerman, Benion & Waterston, 1986) and the *Drosophila mauritiana mariner* element (Jacobson, Medhora & Hartl, 1986; Bryan, Jacobson & Hartl, 1987) move somatically, as do plant transposable elements (Nevers, Shepherd & Saedler, 1986), although in plants the distinction of the germ line is not clear.

In this paper we describe results derived using a strain of *D. melanogaster* in which somatic reversions occur of a P element insertion in the X-chromosomal *singed* gene. In the course of maintenance of a series of dysgenesis-induced mutations (Brookfield & Mitchell, 1985) a mutant strain, *loua* sn^4, maintained as *singed* males and C(1)DX*yf* females, yielded four male flies with areas of cuticle wild-type for *singed*. This occurred three generations after the isolation of the mutation. Figure 1 shows a fly of this type. These males were crossed to unrelated C(1)DX*yf* M strain females, and, while none yielded visibly mosaic F1 males, in three lines the mosaicism reappeared in the F2 males. The mosaicism phenotype has been maintained for more than 50 generations by crossing mosaic males to their C(1)DX*yf* sisters. If selection is relaxed, and random males are used from the mosaic line, the mosaicism phenotype is rapidly lost, although the *loua* sn^4 mutation persists. Here we describe experiments designed to elucidate the basis of this phenomenon.

Materials and Methods

Flies were kept on standard oatmeal and molasses media at uncontrolled temperatures generally around

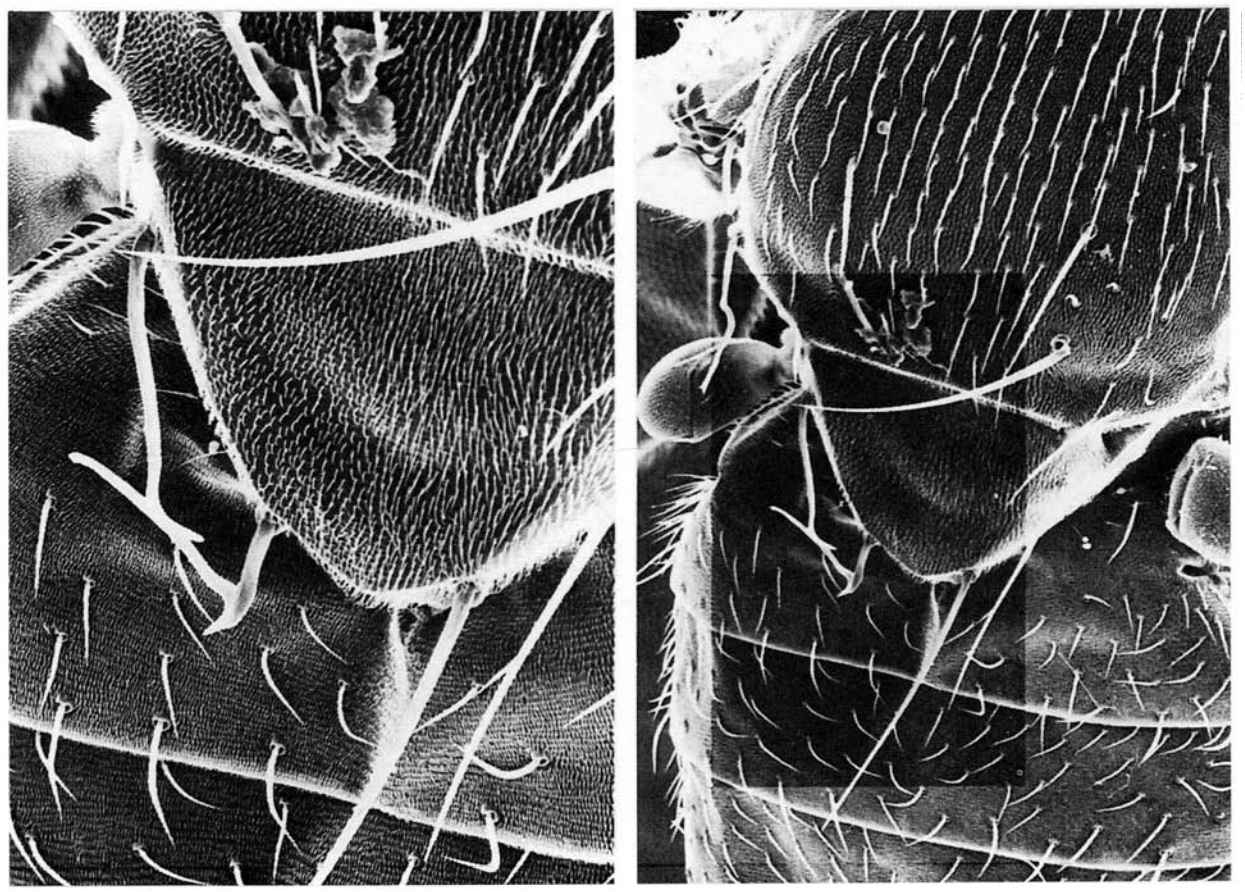

Fig. 1. *loua* sn^4 mosaic. Scanning electron micrograph of the posterior mesothorax of a *loua* sn^4 mosaic male. There is a region of wild-type macrochaetae on the right hand side of the fly. The shaded area in the right-hand micrograph is enlarged in the left-hand micrograph.

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22 °C. In the crosses used to map the *trans*-acting component the flies were maintained at 22 °C precisely. Mosaicism was maintained in the lines by crossing mosaic males to their virgin sisters. Mosaicism was operationally defined in terms of the thoracic macrochaetae. In each fly the major sternopleural, humeral and mesonotal macrochaetae on each side were examined, and if some were wild-type in a *singed* fly, the fly was defined as mosaic.

Fly stocks used in the experiments are shown on Table 1. In one early cross a partial germ cell revertant of *loua* sn^6 was discovered, which we call $singed^{verywweak}$ (sn^{vw}). This was one of a number of revertants of *loua* sn^4 and *loua* sn^6 isolated which had wild-type or nearly wild-type phenotypes, but showed patches of *singed* bristles. sn^{vw} could also revert to a strong *singed* phenotype in germ cells. Figures 2(a) and 2(b) show mosaic sn^{vw} flies. Lines with sn^{vw} males and C(1)DX*yf* females were established, and selection for mosaicism raised the frequency of mosaic males to around 20%. Males from sn^{vw} lines were used in the crosses to map the *trans*-acting component. With sn^{vw} it was the presence of a *singed* patch which defined mosaicism.

A number of *singed* mutations were analysed at the restriction map level. Adult fly DNA was prepared as described by Bingham, Levis and Rubin (1981), and Southern blotted (Southern, 1975) with the modification that nylon filters (Hybond-N, Amersham)

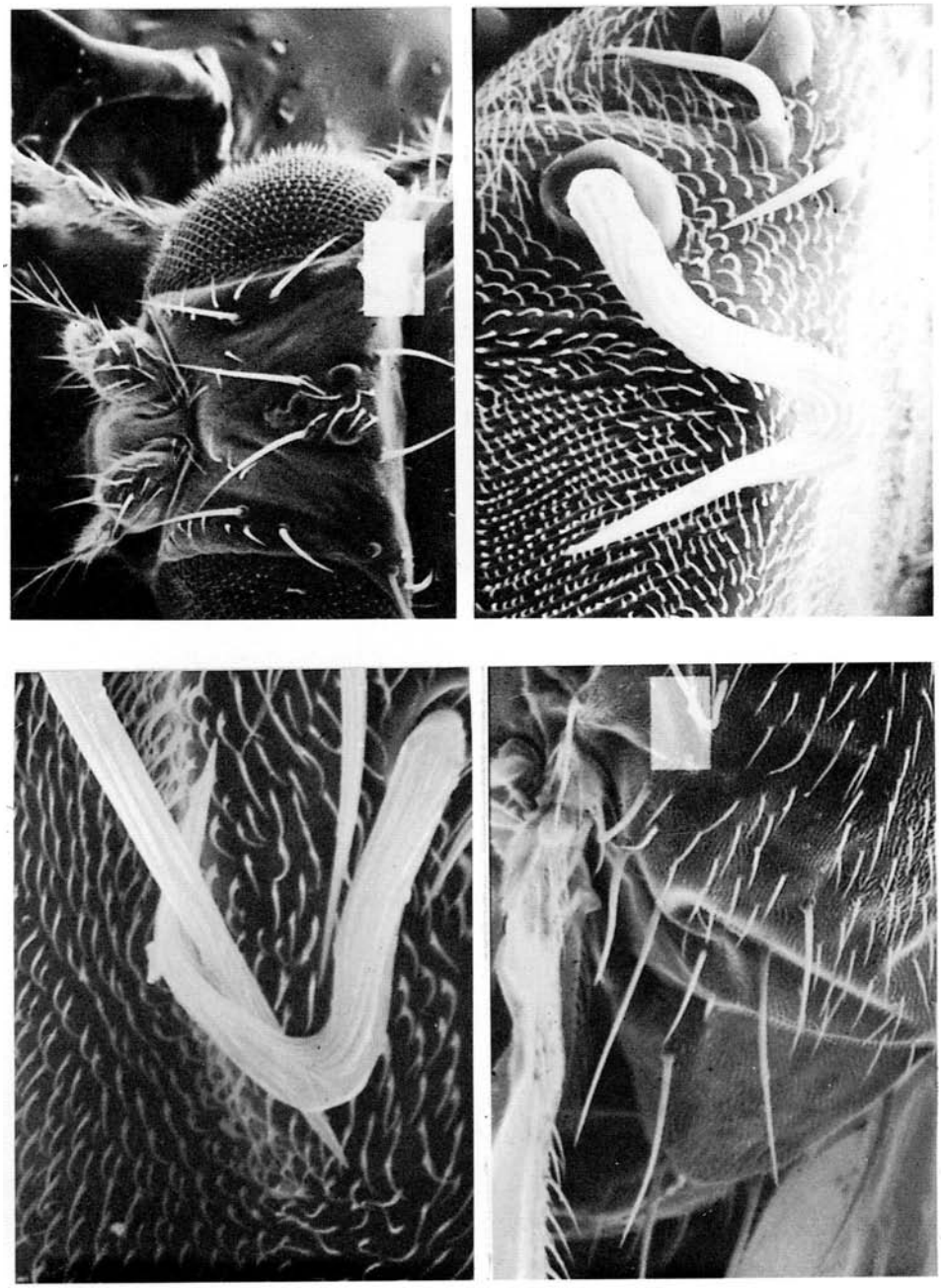

Fig. 2(a) and (b): sn^{vw} mosaic. Scanning electron micrographs of two sn^{vw} mosaic males. In 2(a) there is a region of *singed* bristles affecting the orbital bristles and in 2(b) the right presutural and anterior supraalar bristles are *singed*. The shaded areas in left-hand part of (a) and the right-hand part of (b) have been enlarged.

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Table 1. *Strains used in the experiments*

M and P represent M and P-cytotype

1. M C(1)DX*yf* ♀; *shi*^{ts} ♂. From R. Whittle, Univ. of Sussex.
2. M Muller-5. In(1)*sc*^{S1L} *sc*^{8R} + S,*sc*^{S1}*sc*^{8}*w*^{a}*B*. From B. Burnet, Sheffield
3. M Canton S. From B. Burnet.
4. P *π* 2. From M. Shelton, M. Ashburner, Cambridge.
5. P Loua 83. From G. Periquet, Tours (Anxolabéhère *et al.* 1984).
6. M *y sn*^{w}; Y*y*$^{+}$; *bw*; *st*. From W. R. Engels, Madison.
7. P *y*^{P}. P insertion in *y*. From W. R. Engels.
8. M *y*. From C. P. Kyriacou, Leicester.
9. M C(1)DX*yf* ♀s; *ysn*^{w} ♂s; Y*y*$^{+}$; *bw*; *st*. Produced by crossing strain 1 (above) ♀s to strain 6♂s, crossing F1 ♀s to strain 6♂s, and keeping F2 *bw*; *st* ♂s + ♀s.

10.	*loua sn*^{1}.		21. *loua sd*^{1}.	
11.	*loua sn*^{2}.		22. *π 2 sn*^{1}.	
12.	*loua sn*^{3}		23. *π 2 sn*^{2}.	
13.	*loua sn*^{4}.	M	24. *π 2 sn*^{3}.	M
14.	*loua sn*^{5}.		25. *π 2 sn*^{4}	
15.	*loua sn*^{6}.	M	26. *π 2 sn*^{5}.	
16.	*loua sn*^{8}.		27. *π 2 sn*^{6}.	
17.	*loua sn*^{9}.		28. *π 2 sn*^{7}.	
18.	*loua sn*^{10}.		29. *π 2 sn*^{8}	
19.	*loua sn*^{11}.		30. *π 2 sn*^{10}.	
20.	*loua y*^{1}.		31. *π 2 w*^{1}.	

Mutations 10–31 were generated in a Canton S *X*-chromosome in a hybrid dysgenic cross (Brookfield & Mitchell, 1985). They were subsequently maintained as ♂s with C(1)DX*yf* ♀s, or as strains with mutant ♂s and ♀s. The strains mentioned as M were tested for cytotype using the high temperature gonadal dysgenesis criterion (Engels, 1983).

32. *sn*^{vw}. Partial revertant of *loua sn*^{6}. They were maintained as ♂s in a C(1)DX*yf* strain produced by crossing *sn*^{vw} ♂s to strain 1 ♀s.
33. *y sn*^{vw} ♂s. These were generated in the F2 of a cross between strain 32 ♂s and strain 8 ♀s. These ♂s were crossed to C(1)DX*yf* ♀s from a line in which *sn*^{vw} was showing high frequencies of somatic destabilization. The line generated was the selected for mosaicism.
34. C(1)DX*yf* ♀s; *y sn*^{vw} ♂s; T(1,Y)*y*$^{+}$; *bw*; *st*. These were derived from crosses involving strains 9 and 33 above, sibmating non-mosaic F4s of these crosses to give a homozygous line. This was used in Crosses II(a) and II(b). Lines of corresponding genotype but selected for mosaicism were used to provide the mosaic males in the first generations of these crosses.
35. C(1)DX*yf* ♀s; T(1,Y)*y*$^{+}$; *sp px pr*; *st*. This strain, which has a wild-type *X*-chromosome in males, was derived from a strain with the *All* 2nd chromosome, (*sp px c pr b dp al*) over SM1(In(2LR)SM1, *al*^{2}*Cy cn*^{2}*sp*^{2}) supplied by A. E. Shrimpton (Edinburgh). Males were crossed to strain 9 ♀s above, and *Cy*$^{+}$ F1 ♂s and ♀s crossed. *sp px pr*; *st* F2 ♂s and ♀s were crossed, and selected in subsequent generations for *y*$^{+}$,*c*$^{+}$, *b*$^{+}$, *dp*$^{+}$, *al*$^{+}$ and *bw*$^{+}$.
36. C(1)DX*yf* ♀s; *y sn*^{vw} ♂s; T(1,Y)*y*$^{+}$; *b dp*. This strain was also derived from the *All* chromosome above, in the same cross, with the exception that F2 ♂s and ♀s which were *b* and *dp* were selected, and, over a series of generations, selected for *sp*$^{+}$, *px*$^{+}$, *c*$^{+}$, *pr*$^{+}$, *al*$^{+}$, *bw*$^{+}$, *st*$^{+}$, and *y*$^{+}$. The resulting strain was crossed to strain 34 ♂s, and *b dp bw*$^{+}$; *st*$^{+}$ ♂s and ♀s selected from the F2 of this cross.

were used instead of nitrocellulose. The *sn9* probe (kindly supplied by G. M. Rubin and K. O'Hare) (Roiha, Rubin & O'Hare 1988) was oligolabelled (Feinberg & Vogelstein, 1984) and hybridized to the filters following Reed & Mann (1985) with the exception that 6–12% PEG 8000 was used in place of dextran sulphate (Renz & Kurz, 1984).

A library of DNA from strain 32 males partially digested with Sau3A was constructed in λ L47·1 using packaging extracts prepared following an adapted protocol of Scalenghe *et al.* (1981). Preparation of λ phage arms and genomic DNA, ligation, *in vitro* packaging, and screening of the library with the ^{32}P-labelled *sn9* probe were performed following Maniatis *et al.* (1982).

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Cross I-requirements in cis for mosaicism

C(1)DX*yf* females from mosaic lines were crossed to males from 24 lines with separate P-M hybrid dysgenesis-generated X-linked mutations (20 at *sn*, 2 at *y*, 1 at *w* and 1 at *sd* (strains 6, 7 and 10–31)). Those mutant lines tested (*loua* sn^4, *loua* sn^6, π *2* sn^3, sn^w) were of M cytotype. Mosaicism appeared in the F1, or did not appear at all. The mutants used were generated as described elsewhere (Brookfield & Mitchell, 1985) except for *y* and sn^w which were kindly supplied by W. R. Engels. The F1 and F2 generations of the cross were sib mated. Males in the F1, F2 and F3 of the cross were scored for mosaicism, and the percentages of mosaics for these three generations are shown in Table 2 (see Fig. 3). The restriction maps of a number of these *singed* mutations were obtained, and are shown in Fig. 4.

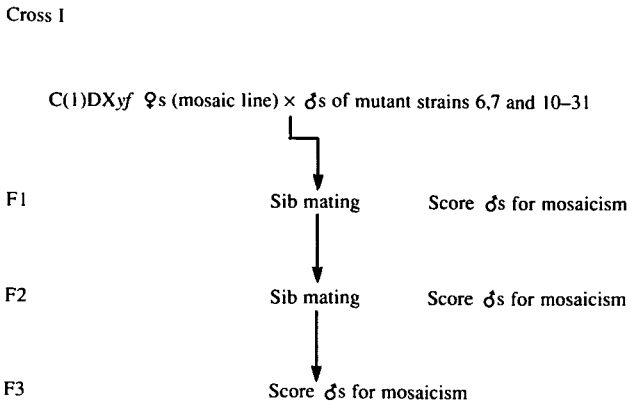

Fig. 3. Cross I.

Cross II-requirements in trans for mosaicism

These crosses were designed to map the mosaicism gene on chromosome 2. Early crosses had shown

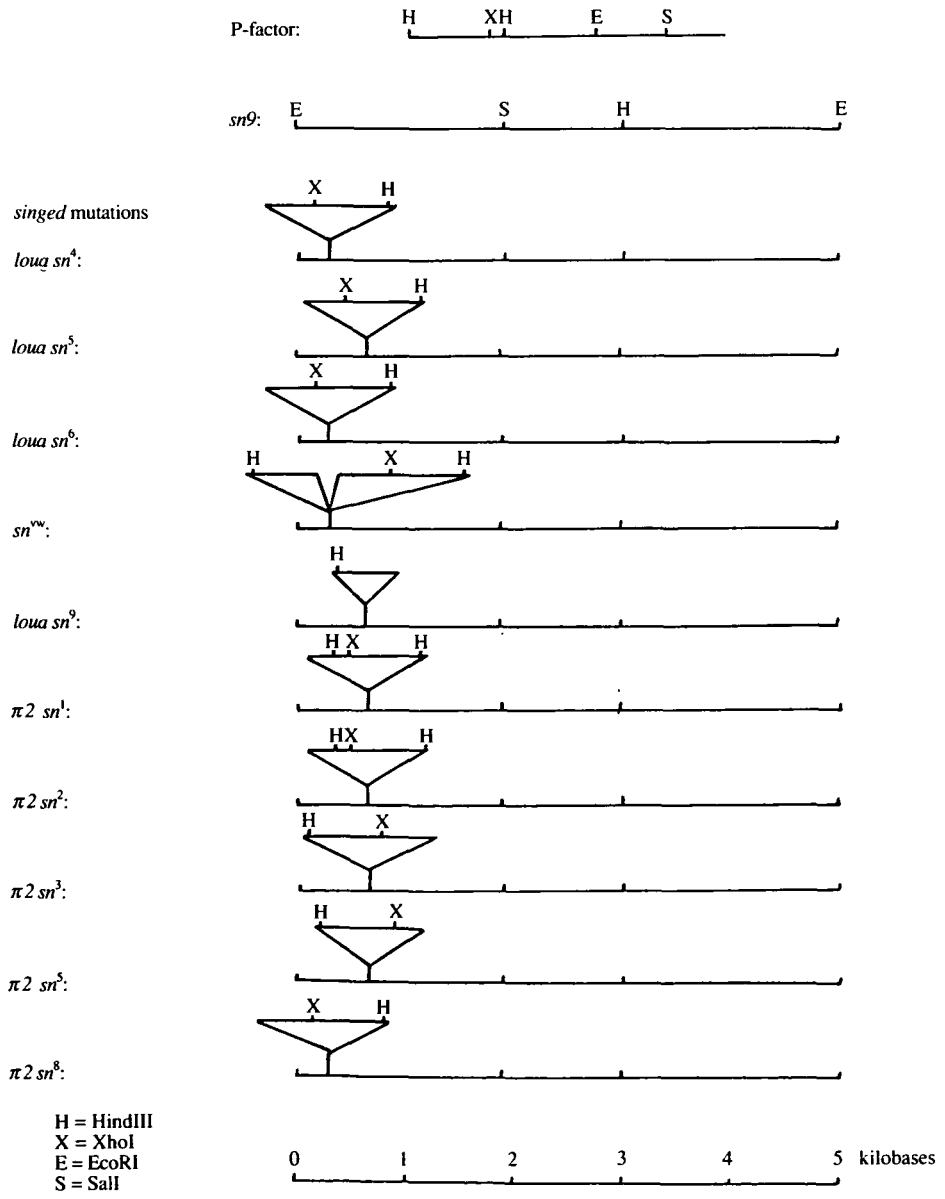

Fig. 4. Restriction maps of the complete P-factor (O'Hare & Rubin 1983) and the Canton S *singed9* region (Roiha, O'Hare & Rubin 1988), along with 10 *singed* mutations.

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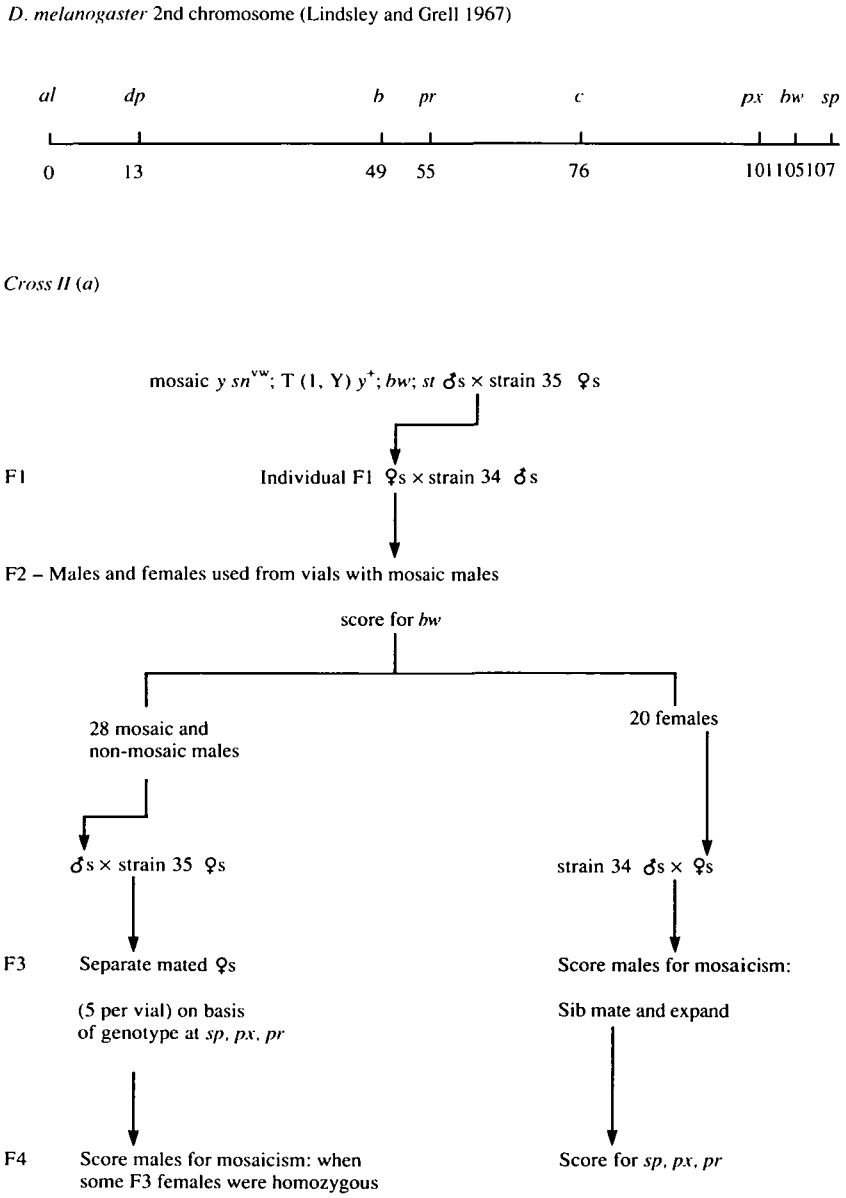
compelling evidence for a dominant maternal-effect mutation on this chromosome.

Cross II(a)

In the first cross mosaic *y* sn^{vw}; T(1,Y)y^+; *bw*; *st* ♂s were crossed to C(1)DX *yf*; T(1,Y)y^+; *sp px pr*; *st* ♀s(strain 35). The F1 females were crossed individually to *y* sn^{vw}; T(1,Y)y^+; *bw*; *st* ♂s from a non-mosaic strain (strain 34). In some vials mosaicism reappeared in the F1 males of this cross. If so, males, both mosaic and non-mosaic, were crossed to more strain 35 ♀s. The F1 females of this latter cross showed the recessive phenotypes of any 2nd chromosome mutations they received from their great grandmothers. The fertilized F1 ♀ s were separated on the basis of these markers, and their sons examined. Thus these females were tested for the presence of a *trans*-acting mosaicism gene and also for the regions of the second chromosome that they had received from their mosaic great-grandfathers. Seven mosaic males and 21 of their brothers which were not visibly mosaic were tested in this way. In addition, 20 F2 females were crossed to strain 34 males. In some of these lines, mosaicism appeared in the F1 and persisted into the F2. By expansion of these lines so that 100–200 F2 flies were scored by homozygosity of the chromosome 2 markers, it was possible to infer which, if any, of the chromosome 2 markers were present in the heterozygous state in these females (Fig. 5).

Cross II(b)

Mosaic *bw st* males were crossed to C(1)DX*y* *f* T(1,Y)y^+; *b dp* females (strain 36). The F1 females were crossed individually to strain 34 males. In some



for *sp*, *px*, or *pr*, any mosaics appeared only in the vial derived from these females.

Fig. 5. Linkage map of chromosome 2 and Cross II(a).

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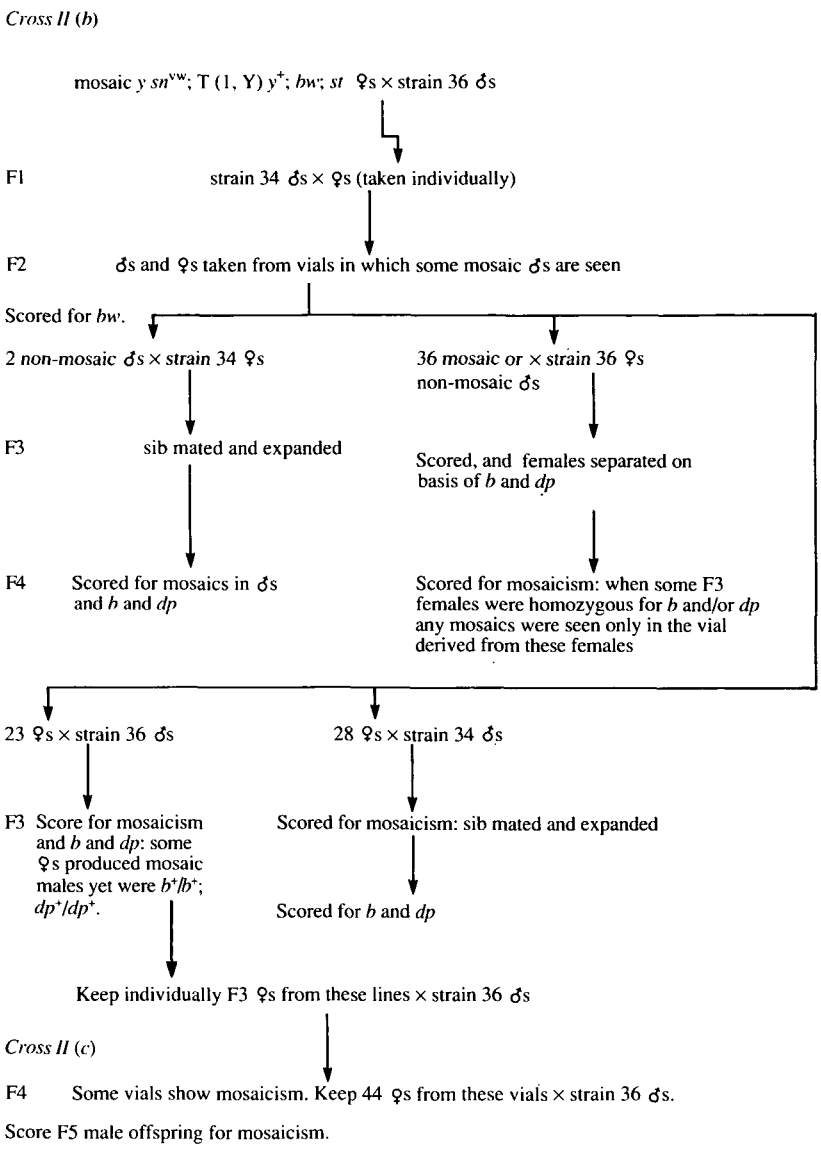

Fig. 6. Crosses II(b) and II(c).

of the F2s mosaics were seen. 16 mosaic males from the F2s and 20 of their non-mosaic brothers were crossed to strain 36 females. In addition, 2 non-mosaic brothers of mosaic males were crossed to strain 34 females. In the first two crosses the assessment of the flies' genotypes with respect to the *b* and *dp* loci was possible in the F1 and assessment with respect to mosaicism possible in the F2. In the second cross assessment of mosaicism and of *b* and *dp* only became possible in the F2. In addition, 23 sisters of mosaic males were crossed to strain 36 males, and 28 such females crossed to strain 34 females. In the first set of these crosses, the *b* and *dp* loci and mosaicism are assessed in the F1. In the second set, mosaicism is assessed in the F1 but the *b* and *dp* genotypes are assessed in the F2 (Fig. 6).

Cross II(c)

Some F2 females in Cross II(b) above which were tested for mosaicism by crossing to strain 36 males produced F1s which contained mosaics and which showed the females to be homozygous for b^+ and dp^+. F1 females (the sisters of such mosaic males) which were thus heterozygous for *b* and *dp*, were crossed to further strain 36 males. Some such females produced mosaics among their sons. These females must thus have also been heterozygous for the mosaicism gene, linked to b^+ and dp^+. 44 daughters of such females were then crossed to further strain 36 males, and their sons examined for mosaicism (Fig. 6). Thus, in cross II(a), 48 second chromosomes were scored for recombinants between mosaicism, *sp*, *px*, and *pr*. In Cross II(b) 89 second chromosomes, and, in Cross II(c), a further 44 chromosomes, were scored for recombinants between mosaicism and *b* and *dp*.

Results

Early characterization of the phenomenon

When crossed to C(1)DX*yf* females from M strain non-mosaic lines, mosaic males give non-mosaic F1

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males which are nevertheless *singed* (apart from occasional germ cell revertants to wild-type or weaker *singed* alleles). The mosaicism may reappear in the F2 males. If C(1)DX*yf* females from mosaic lines are crossed to males from *loua sn*^{4} lines with no history of mosaicism, then the mosaic phenotype may appear in the F1 males. These results demonstrate that the somatic instability of the *loua sn*^{4} mutation requires a factor or factors not on the *X*-chromosome and that this factor is neither simply dominant nor simply recessive in its effects. These data are consistent with a mutation with a maternal effect, a mutation on the *Y* chromosome, or a mutation with both these properties. If Muller-5 females are crossed to mosaic males, and their F1s sib-mated, then when the *sn* mutation reappears in the F2 the mosaicism frequency is much lower (by 5-10-fold) than if attached-*X* females were used. If non-mosaic F2 *sn* males from this cross are crossed to attached-*X* females, then high mosaicism rates are again seen in the F2 of this latter cross. These results, which were found with *loua sn*^{4}, *loua sn*^{6}, and *sn*^{vw}, suggest that, for unknown reasons, mosaicism rates are higher in attached-X crosses. By crossing mosaic males to *bw*; *st* attached-*X* females it was possible, using logic similar to that of Cross II, to look for linkage between the *trans*-acting determinant of mosaicism, which we shall henceforth refer to as *Mo*, and these markers. These experiments used strains 9, 33 and 34 (Table 1). It was found that the data were consistent with the *trans*-acting determinant of mosaicism being a single dominant maternal-effect mutation on the second chromosome, which was not tightly linked to *bw* (26 recombinants in 65 second chromosomes examined).

The developmental timing of the reversion events could be partially established by studies of the size distribution of the clonal patches observed. Using data from 2578 mosaics generated using *loua sn*^{4}, *loua sn*^{6} and *loua sn*^{8}, fate maps were constructed. These confirmed earlier fate maps of mesonotal macrochaetae based on smaller data sets (Murphy and Tokunaga, 1970). They further showed that element movements, while not occurring in the first few nuclear divisions following fertilization, were completed in the first 24 hours following egg laying. Over 5000 *sn*^{vw} mosaics have also been examined, and the distribution of clone size again indicates the same timing as for *loua sn*^{4}, *loua sn*^{6}, and *loua sn*^{8}, although now the process appears to continue until a little later.

Molecular biology

The *singed* mutations *loua sn*^{4}, *loua sn*^{5}, *loua sn*^{6}, *loua sn*^{9}, *π 2 sn*^{1}, *π 2 sn*^{2}, *π 2 sn*^{3}, *π 2 sn*^{5} and *π 2 sn*^{8} were shown to differ from wild-type by insertions of P elements into *singed*. For the structures of the *singed* gene in these mutants, see Fig. 4. The *sn*^{vw} mutation was shown to differ from the *loua sn*^{6} mutation by the addition of an extra 0·65 kb of DNA very close to the P element at *singed* in *loua sn*^{6} (Fig. 4). The restriction map of this DNA is consistent with it being a P-element, although the only site seen was the HindIII site near the end (O'Hare and Rubin, 1983). It is not known why this DNA causes a partial reversion of the *singed* phenotype, but we interpret the somatic reversion process of this mutant, in which strongly *singed* patches are generated, as being due to the loss of this extra DNA. Germ cell revertants of *sn*^{vw} to *singed* occur frequently, particularly in the crosses in which mosaics are formed. Such revertants can subsequently be destabilized in germ cells and somatic cells to a wild-type phenotype, suggesting that the *loua sn*^{6} structure may have been restored. In mosaic-forming crosses, phenotypically wild-type germline revertants are also found, although at lower frequencies than strong *singed* revertants. These can subsequently generate singed mutations in hybrid dysgenesis at elevated rates compared to a Canton S *singed* allele (P. Corish, pers. comm.). Somatic mutations to singed from these wild-type revertants have not been seen, but the data set is small. The phenotypic similarity between *sn*^{vw} and its wild type revertant product would make it impossible to detect any somatic reversions of *sn*^{vw} to wild-type. All these properties are reminiscent of the *singed*^{w} allele, which also has two inverted P-elements inserted at *singed*, although they are in a different relative orientation to those in *sn*^{vw}, and they have a different insertion site. *sn*^{w} reverts to strongly *singed* and wild-type phenotypes by excision of one or other of these elements (Roiha, Rubin and O'Hare, 1988; Engels, 1981). There are other examples also of the formation of double P-element insertions in *singed* (Hawley et al., 1988). For more details of these mutations, see Lewis (1987).

P element insertions differ in their capacities for somatic destabilization

Cross I described above was designed to test the destabilization rates of different hybrid dysgenesis-generated mutations. The results are shown in Table 2. Various *singed* mutations can be destabilized somatically, but the method was unsuccessful with mutations at *white*, *scalloped* and *yellow*. The *singed* mutations differ in their capacity for producing mosaics. The mutant strains used were not isogenic and part of the variance may be due to genetic background. The *loua sn*^{4}, *sn*^{6} and *sn*^{8} mutations had been isolated from the same bottle in a hybrid dysgenesis X-chromosomal mutation screen (Brookfield & Mitchell, 1985), and probably had the same mutation caused by a single premeiotic insertion appearing in siblings. *loua sn*^{6} and *loua sn*^{4} are identical in their restriction maps. *loua sn*^{8} was not examined at the molecular level. The somatic instabilities of the mutations in this assay were not well correlated with their germline instabilities in dysgenic conditions. In particular, *sn*^{w} yielded only one fly with

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Table 2. *Frequencies of mosaic males in the first three generations of cross I*

Mutant strain	F1	F2	F3	Total %
loua sn^{1} (10)	0/90	0/362	0/401	*0·00* – 0·35
loua sn^{2} (11)	1/169	0/1015	0/607	0·00 – *0·06* – 0·28
loua sn^{3} (12)	0/201	0/501	0/204	*0·00* – 0·33
loua sn^{4} (13)	11/453	25/1594	14/643	1·34 – *1·85* – 2·36
loua sn^{5} (14)	0/68	0/382	0/162	*0·00* – 0·49
loua sn^{6} (15)	8/337	3/1540	4/811	0·28 – *0·56* – 0·84
loua sn^{8} (16)	1/94	7/349	5/162	0·99 – *2·15* – 3·31
loua sn^{9} (17)	13/159	8/646	0/160	1·26 – *2·18* – 3·10
loua sn^{10} (18)	0/113	0/729	0/268	*0·00* – 0·27
loua sn^{11} (19)	0/122	1/468	0/257	0·01 – *0·12* – 0·59
loua y^{1} (20)	0/131	0/840	0/529	*0·00* – 0·20
loua sd^{1} (21)	0/9	0/161	0/116	*0·00* – 1·04
π 2 sn^{1} (22)	0/73	0/379	0/340	*0·00* – 0·38
π 2 sn^{2} (23)	0/136	0/582	0/175	*0·00* – 0·33
π 2 sn^{3} (24)	7/808	2/2787	5/1599	0·13 – *0·27* – 0·41
π 2 sn^{4} (25)	0/77	0/514	0/292	*0·00* – 0·34
π 2 sn^{5} (26)	4/201	1/633	1/386	0·21 – *0·49* – 1·00
π 2 sn^{6} (27)	0/80	0/254	0/183	*0·00* – 0·58
π 2 sn^{7} (28)	0/115	0/480	0/390	*0·00* – 0·30
π 2 sn^{8} (29)	2/146	2/418	1/171	0·26 – *0·68* – 1·48
π 2 sn^{10} (30)	0/113	1/360	0/149	0·01 – *0·16* – 0·81
π 2 w^{1} (31)	0/230	0/853	0/589	*0·00* – 0·18
y^{w} (7)	0/148	0/129	0/68	*0·00* – 0·90
sn^{w} (6)	1/492	0/912	0/168	0·01 – *0·06* – 0·32

The numbers shown are the mosaics seen and the numbers of mutant flies examined in each of the three generations. The mosaic with *sn*^{w} represents a fly with a *singed-* extreme patch. A wild-type patch would not have been seen. The total percentages (underlined) are preceded and followed by 95% confidence limis. These are based upon binomial expectations and a probably inaccurate assumption of independence of flies within any mutant line.

a *singed*$^{-extreme}$ patch in 1572 flies examined, despite its very high germline reversion rate. Thus, the movements are not simply due to the extension of normal P factor transposase activity to somatic cells.

It was not clear from the molecular data what is the necessary condition for somatic destabilization of the mutations. Roiha, Rubin and O'Hare (1988) in a study of a wide range of P element insertions in *singed*, found that all the insertions fell into a cluster of target sites spearated by less than 100 base pairs of DNA. In our mutations, three (*loua sn*^{4}, *loua sn*^{6} and *π 2 sn*^{8}) had a site of insertion around 400 base pairs away from this region. It was not clear if these insertions were at the same base pair, or whether they were at different sites too close to distinguish by restriction mapping. Probably, *loua sn*^{4} and *loua sn*^{6} are the same mutation. These mutations also all showed somatic mutation, as did *sn*^{vw}, which, of course, also had its P elements inserted at the same site. However, *loua sn*^{9}, which also showed a high frequency of somatic reversion, has a site of insertion located in the cluster of insertion sites described by Roiha, Rubin & O'Hare (1988). There was no significant correlation between somatic reversion frequency and the size or orientation of the inserts. All the mutations which showed appreciable somatic reversion (0·2% +) either used the new insertion site (*loua sn*^{4}, *loua sn*^{6}, *π 2 sn*^{8}, *sn*^{vw}) or had P-insertions with their 5′ ends towards the *EcoRI* site in Canton S *singed* (*π 2 sn*^{3}, *π 2 sn*^{5}, *loua sn*^{9}), but the data set was far too small to establish whether these properties constituted either a necessary or sufficient condition for somatic revertability. Certainly, excision of the P-element in *sn*^{w} which was in the appropriate orientation would give the *sn*^{e} product, which would be somatically distinguishable and yet was only seen once. (It is probable that the *sn*^{w} to *sn*$^{(+)}$ process would not be distinguishable in small clonal patches.)

In screening a genomic *sn*^{vw} library with the *sn9* probe, four positive plaques were isolated, but none turned out to include the transposable element insertion site. This difficulty in cloning *sn*^{vw}, which parallels that with *sn*^{w} (Roiha, O'Hare & Rubin, 1988) is consistent with the second DNA insertion in this mutant being an inverted P-element.

Preliminary attempts to map the trans-acting component of the system

Cross II represented a preliminary attempt to map the *trans*-acting component of the system. The results of earlier crosses had shown this component to be a long way from the *bw* locus. The results of Cross II(a) (Table 3) show that while there is no evidence for

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Table 3.

Cross II(a)-recombination between Mo, and sp, px and pr
It is possible to score each second chromosome scored as parental or recombinant with respect to *Mo* and each of the other markers.

	Recombinants	Parentals
sp-Mo	21	27
px-Mo	18	30
pr-Mo	8	40

There is thus significant evidence for linkage between *pr* and *Mo*.

Cross II(b)-recombination between Mo, and b and dp
As above

	Recombinants	Parentals
b-Mo	11	78
dp-Mo	31	58

Cross II(c)-recombination between Mo, and b and dp
As above

	Recombinants	Parentals
b-Mo	7	37
dp-Mo	14	30

Pooled data from crosses II(b) and II(c)

	Recombinants	Parentals
b-Mo	18	115
dp-Mo	45	88

There was no significant evidence for linkage to *bw* in these data sets. The significant linkage estimates are *b-Mo*: 13%, *pr-Mo*: 17%, and *dp-Mo*: 34%. There were 43 recombinants out of 133 between *b* and *dp* so it is impossible to say on which side of *b* *Mo* is located.

linkage to *speck* or *plexus* (as would be expected from their closeness to *bw*), there was highly significant evidence for linkage to *purple* (8 recombinants in 48 tested chromosomes). The most likely explanation of these data would be for the mosaicism gene to lie leftwards of *purple*. If it was to the right it would probably show a tighter linkage to *px* and *bw*. The data from Crosses II(b) and II(c) using *b* and *dp* are also shown in Table 3. There is significant evidence for linkage to both *b* and *dp*, but the map distance from *b* is much less (18 recombinants out of 133) than for *dp* (45 recombinants out of 133). There is still no significant evidence distinguishing which side of *b* and *pr* the mosaicism gene lies. Further experiments simultaneously examining *b*, *pr*, and *curved* (2R:76) are under way.

A consistent finding is that only about half of the male offspring of females which have mosaic sons are themselves capable of passing on the mosaicism, and that this proportion is independent of whether the males are themselves visibly mosaic. This implies that the mosaicism gene is not required in the zygote for a mosaic phenotype. Another data set which is relevant to this conclusion is of the mosaic males generated in the final generation of Cross II(c) from mothers which were themselves heterozygous for *b* or *dp*. If the mosaicism gene increased the frequency of mosaicism in the zygote, then males which received the b^+ allele from their mothers will almost always receive the mosaicism allele also. However, these males do not have a significantly higher frequency of mosaicism (35 out of 168) than their brothers which received the *b* allele (33 of 136). Of course, if the presence of the mosaicism allele in the zygote increased the frequency of mosaicism by 10–20% then this effect would not be visible in the data. The data can also be examined for variations in the frequency of mosaicism. There are a number of data sets in which a collection of females were examined for the numbers of mosaics present in their sons. In some data sets all females producing mosaic sons can be inferred to be heterozygous for *Mo*. In all sets of such females, the proportions of mosaic sons varied from 21·7% to 35·1%. Even among females heterozygous for *Mo*, there was significant heterogeneity in the proportions of mosaic males in their offspring. It is not clear whether this significant variation represents genetic variation at other loci between these females or between their offspring, or whether it is an environmentally-induced difference between the females. If mosaic males from such females are examined, it is found that mosaic males from females with large numbers of mosaics among their offspring males (30+%) have significantly larger numbers of bristles *singed* in phenotype than mosaic males from females which produce fewer mosaics (< 30%).

Discussion

We interpret the data presented here as implying that some P-element insertions in *singed* can revert somatically when acted on by a *trans*-acting component with a maternal effect. We further hypothesize that, while this *trans*-acting component appears to be most effective in attached-*X* crosses, the genetic determinant is a dominant mutation on the 2nd chromosome, approximately 10–20 map units away from *pr* and *b*.

Two types of hypotheses could be produced as to the nature of this *trans*-acting component. One would be that it is a factor which is involved in the cell type-specificity of P factor transcript splicing. The hypothesis would thus be that correct splicing of the P factor transcript has been extended beyond fertilization and egg laying due to a mutation in the system normally limiting the process to germ cells. Two arguments can be raised against this hypothesis. Firstly, it requires that, in addition to the *trans*-acting mosaicism mutation the mosaic lines also possess at least one intact P-factor to generate the transcript to

be spliced. It is possible that the chromosomal position of this P factor could give anomalous results in the above mapping experiment. More tellingly, such a prediction would envisage that P element insertions with high rates of movement in P-M hybrid dysgenesis would also have the highest somatic destabilization rates. However this is not the case. The second possible hypothesis is that the *trans*-acting factor is itself a P-factor, but one mutated in such a way that it can produce an active transposase (one for which the requirements in *cis* for P element movements are slightly changed). The prediction of this hypothesis is that the mutated P factor transcript can now be spliced, and the product inherited into the egg. This could be by a process analogous to the inheritance of P-cytotype. One theory of the nature of P transposase repression in P-cytotype is that repression is brought about by the 66 kiloDalton protein generated by the translation of P-factor transcripts in which the intron between ORFs 2 and 3 is retained (Rio, Laski and Rubin, 1986; O'Hare, 1985). Such a model hypothesizes that not only does the 66 kiloDalton protein compete for P element ends with the active 87 kiloDalton transposase protein and thereby acts as a transposition repressor, but that it also positively feedbacks with the P-factor splicing system, favouring the retention of the intron between ORFs 2 and 3 and the production of more of itself. One assumption of this model of P-cytotype is that the 66 kiloDalton protein has a half-life in the egg long enough for it to influence the splicing of transcripts from P factors in the zygotic germ cells. Such a long half-life for a P protein would be long enough to explain the mosaicism seen in our data. The data would not require any somatic expression of active transposase genes, merely that the expression of the active transposase in the mother continues so late in germ cell development that the zygote still contains an active transposase protein. A heterogeneous distribution of this protein in the embryo, in which the protein was distributed around the periphery, could explain the timing of the reversion events. It could be that P element reversions at *singed* only start when nuclei migrate to the surface of the embryo.

If this second hypothesis is true, and the *trans*-acting component on chromosome 2 is a modified P-factor, then one prediction is that the factor itself may be unstable. Indeed, one hypothesis for the necessity of selection for mosaicism, would be that selection is being opposed by excision. It could, indeed, be suggested that the consistency of chromosomal position of this factor, in experiments separated by years, itself argues against it being a P-element.

In a general sense, it is not at all clear what the relationship is between the phenomena described here and hybrid dysgenesis. The mosaic phenotype is maintained by selection at the phenotypic level. If a condition of hybrid dysgenesis is required in order for the *singed* somatic reversions to occur, the selection will also select for the continuance of hybrid dysgenesis. There is a tendency, even among the selected lines, for the rate of mosaicism to drop slowly, despite the continuance of both sn^{vw} and *Mo* in the lines. The mosaicism frequency can be restored by crossing males from the lines to M-cytotype females. The cause of this phenomenon, which we have not precisely documented, could be the buildup in the lines of P-cytotype, or of zygotic P-repressors of the type documented by Black *et al.* (1987).

A more accurate position for *Mo* will be established using multiply marked second chromosomes. This will allow it to be established (by *in situ* hybridization) whether *Mo* corresponds to a site of P factor homology. We will also be using balancer chromosomes to investigate possible differences between *Mo* homozygotes and heterozygotes. Male *Mo* homozygotes are certainly viable and fertile, and females appear to be also. There is some weak evidence that the mosaicism rate in the sons of homozygous females may be elevated relative to the sons of heterozygotes, showing dominance to be incomplete. It appears from our data that only a few unstable P-element insertion mutations are capable of responding to the action of the *Mo* mutation. It may indeed be that flies showing the *Mo* phenotype may be quite common in wild populations and that this could be assessed using crosses to sn^{vw}.

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