Genet. Res., Camb. (1996), **67**, *pp.* 109–121 *With 7 text-figures* Copyright © 1996 Cambridge University Press

Natural Repressors of P-induced hybrid dysgenesis in *Drosophila melanogaster*: a model for repressor evolution

P. CORISH[1], D. M. BLACK[2], D. W. FEATHERSTON[3], J. MERRIAM[4] AND G. A. DOVER[1]*
[1] *Department of Genetics, Adrian Building, University of Leicester, University Road, Leicester LE1 7RH, UK*
[2] *Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearden, Glasgow, Scotland*
[3] *Katholieke Universiteit, 6525 ED, Nijmegen, Netherlands*
[4] *Department of Biology, University of California, Los Angeles, 405 Hilgard Avenue, CA 90024, USA*

(*Received 5 June 1995 and in revised form 21 September 1995*)

Summary

Type I repressors control P element transposition and comprise full length elements and elements with small 3′ deletions in the final exon. Using a sensitive assay for measuring the strength of repression of P element transposition in somatic and germline tissues, we have isolated and characterized a naturally occurring type I repressor element from a Q population of *Drosophila melanogaster*. We demonstrate that the almost complete repression of transposition in this population is a mixture of KP elements with intermediate levels of repression, and the strong contribution of a single 2·6 kb P element deletion derivative, which we call SR (Strong Repressor). A deletion in the final intron of SR allows for the constitutive production of a putative 75 kDa repressor protein in germline tissues in addition to the production of the 66 kDa repressor in the soma, which would result in a biparental mode of inheritance of repression. Based on the four observed classes of natural Q populations, we propose a model in which populations containing SR-like elements, capable of producing strong type I repressor constitutively, have a selective advantage over populations which rely either on maternally transmitted P cytotype or on KP-induced weak levels of repression. Such populations may subsequently spread and constitute an evolutionary stable strategy for the repression of hybrid dysgenesis in *Drosophila melanogaster*.

1. Introduction

The spread of transposable P elements throughout the species *Drosophila melanogaster* during the last half century (Kidwell, 1992) has been a remarkable achievement given the ability of intact, autonomous elements to induce a variety of severely disadvantageous phenomena known as 'hybrid dysgenesis' (Kidwell, 1977; reviewed by Engels, 1989). This has been achieved through the coevolution of at least two distinct systems that repress the dysgenic effects of mobile P elements, (Rio, 1990). The first system to be analysed in detail was termed 'P cytotype' (Engels, 1989), indicating by this name that repression of dysgenesis is inherited only through the females of P populations harbouring intact P elements. The second system of repression was shown to be mediated by a specific P element deletion-derivative, termed the KP element (Black *et al.* 1987; Jackson *et al.* 1988), in M′ populations that exhibit a range of intermediate levels of repression (Kidwell 1983). KP-mediated repression is not as strong as P cytotype but differs in that it is transmitted through either sex, indicating a chromosomally transmitted genetic system. Recent evidence suggests that KP polypeptide interferes with transposase (Andrews & Gloor, 1995) in accordance with our original proposal (Black *et al.* 1987). KP-mediated repression was first revealed through the amelioration of ovary under-development (gonadal dysgenesis). Other bioassays, capable of measuring the repression of P element transposition or transcription, confirmed our original findings of biparentally-transmitted effects of KP elements (Lemaitre *et al.* 1993; Rasmusson *et al.* 1993).

It has been proposed (Gloor *et al.* 1993), that elements capable of repressing P element mobility and hybrid dysgenesis can be classified into at least two structural types: type I elements (either complete elements or elements with specifically located small deletions) and type II elements (having more extensive deletions, of which KP is an example). Complete P elements are 2907 bp in length and contain four exons

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

(originally numbered 0, 1, 2 and 3) required for the transposase enzyme. Elements belonging to the type I repressor class have at least the entire sequence of exons 0, 1 and 2 and the first 9 bp of the third intron (IVS3), whereas KP, a type II repressor, is deleted for most of exon 1, all of exon 2 and some of exon 3.

Detailed molecular studies of P cytotype repression (Misra & Rio, 1990; Laski, 1986) indicate that failure to excise the IVS3 produces a 66 kDa protein, in place of the usual 87 kDa transposase protein encoded by all four exons after removal of all three introns. The 66 kDa protein acts as a repressor of P transposition in somatic tissues (Misra & Rio, 1990); and the accumulation of P cytotype 66 kDa repressor is believed to be a result of low levels of third intron splicing in the female germline. Hence, type I elements require sequences for their repressor function that type II elements lack (Gloor *et al.* 1993). Interestingly, *in vitro* modified P elements with lesions affecting exon 3 and resulting in a truncated protein have been shown to have repressor activities that 'mimic P cytotype effects' (Robertson & Engels, 1989); except that, unlike P cytotype, the repression is biparentally transmitted. On this basis, it is not yet possible to define type I and type II repressors on the basis of their mode of inheritance (female only or biparental); but only according to their structural properties that may, in turn, influence the specific molecular mechanism through which repression takes place.

Whilst many of the questions regarding repression will be solved through direct molecular analysis, it is necessary to complement *in vitro* studies with an examination of the range, structures and mechanisms of repression (if any) of naturally-occurring elements. For example, in an analysis of the strong P cytotype strain, π_2, there are a range of structures containing a variety of large deletions (some similar to KP), as well as complete P elements (O'Hare *et al.* 1992). Although the experimental methods employed in the survey could not have detected small type I element deletions, as defined, it is clear that type II elements are relatively abundant. A screen for naturally occurring type I repressors by Gloor *et al.* (1993) yielded only one element deleted in the appropriate way and several independent cases of complete P elements. The type I deletion was considered to have arisen *de novo* during the experimental genetic crosses prior to its isolation (Gloor *et al.* 1993).

From all such surveys, including our own (Black *et al.* 1987) several key questions need to be asked. Do type I repressors, other than complete P elements exist in nature? Is there a natural balance between type I and type II repressors? Do they represent two different mechanisms of repression, also distinguishable by different modes of inheritance? Is there a natural evolved succession of repression leading to one type, or balance of types, as an evolutionary stable strategy? For example, what might be the significance of Q populations which, by definition, strongly repress dysgenesis yet fail to significantly induce it in the appropriate genetic crosses (Kidwell, 1983). Are the genotypically distinct classes of Q populations (Jackson *et al.* 1988) transition stages to one stable, efficient, system of repression?

In order to begin answering such questions, we have isolated and characterized a single naturally occurring type II (KP) and a single naturally occurring type I (designated SR, for strong repressor) elements from English M′ and Q populations respectively. The strength of somatic and germline repression of the KP elements has been measured using a sensitive eye-colour assay and the same assay, applied to the Q population, has permitted the isolation of one particular element with a strong repressor effect in both somatic and germline tissue. Molecular characterization of this SR element shows that it can be classified as a type I repressor, but with some additional and unusual features. The occurrence of such type I and type II elements in M′ and Q populations is discussed in relation to the evolution of repression in P-induced hybrid dysgenesis in natural populations, leading to a model in which Q populations with strong, biparentally transmitted repression, represent a final stable strategy.

2. Materials and Methods

(i) *Fly strains used*

For a full description of mutants and balancers see Lindsley & Zimm (1992).

A4-4. A P-element transformed line of w^{1118} carrying a wild type copy of the *white* gene at cytological position 100F (Hazelrigg *et al.* 1984). The heterochromatic location of this insertion results in under-expression of the *white* gene and the flies consequently have pale orange eyes rather than the normal red. It contains no other P elements.

w; Sb e Δ2-3/TM6. A constitutive producer of P element transposase, in both somatic and germline tissues, from the Δ2-3(99B) transposon insertion on a dominantly marked third chromosome (Robertson *et al.* 1988).

I-27. A strain which is homozygous for a single KP element insertion on chromosome 3 in a w^{1118} background. It was obtained from a breeding scheme designed to give varying KP copy numbers from the Q strain, Cambridge, in combination with chromosomes from the standard P strain, Harwich. (Jackson *et al.* 1988). *I-27* contains no Harwich-derived P chromosomes and no other P elements as determined by Southern blot analysis.

North Wootten. A strain derived from approximately 30 adults caught in 1987 from a vineyard in Somerset,

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

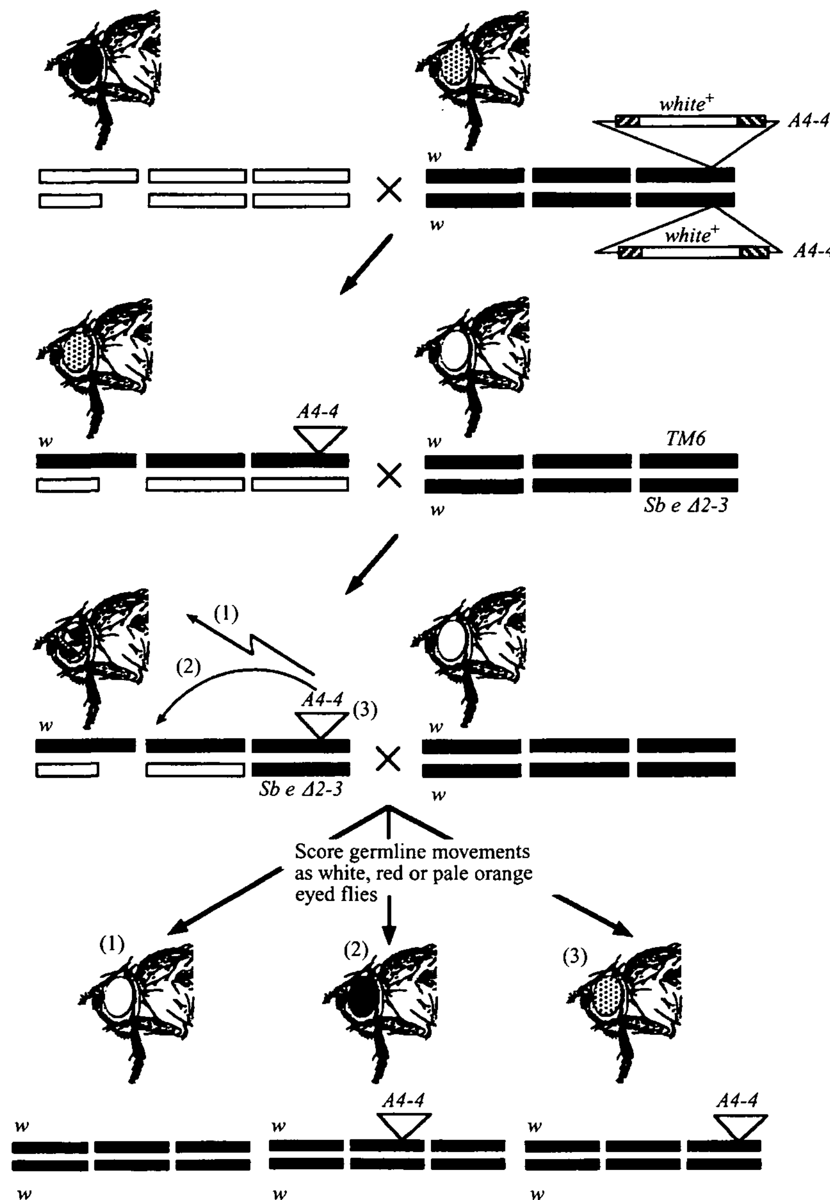


Fig. 1. The *A4-4* eye colour mosaic assay. Wild type red eye colour shown as filled black, and *A4-4* pale-orange phenotype shown shaded. White eye mutants shown as open white. The *A4-4* phenotype is caused by the insertion of the *white*⁺transgene (P element ends shown as hatched boxes) into the subtelomeric heterochromatin of chromosome 3. The combination of the transgene with the dominantly-marked Δ2-3 mobilizer chromosome leads to three possible outcomes (1) Excision and loss, resulting in white patches in the eye. (2) Re-insertion into euchromatin and full expression (red patches) or (3) no movement of *A4-4* (pale-orange patches). Germline transposition rates are obtained by crossing mosaic-eyed male flies to *white* females and scoring flies with white, red and pale-orange eyes.

England. The strain behaved as a Q strain in the P–M hybrid dysgenesis system giving less than 5% GD ovarian sterility in the A and A* tests (Kidwell, Kimura & Black 1988). This population exists alongside other local populations, which exhibit biparental inheritance of repression. The strain was kept as a large mass breeding population and has consistently been tested as Q.

(ii) *A4-4 eye colour mosaicism assay for repression*

One of the main problems in the study of the control and repression of P elements in natural populations has been that the most important phenotypic consequences of hybrid dysgenesis, namely, gonadal dysgenesis (GD) and increased mutation rate, are not the most amenable to simple genetic analysis. In particular, GD sterility assays are invasive and involve destroying sample flies. Several groups have, therefore, used alternative genetic measures of the transposition of P elements, many of which involve easily scorable visible markers such as *singed*^{weak} (Engels, 1984) and *vestigial* wings (Williams, 1988). With the increased use of P-element-mediated transformation of *Drosophila* using the wild type allele of the *white* gene as a selectable marker, it is now possible to use eye

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

colour as another assay for P transposition. We assume that these transgenes behave as normal P elements when supplied with a suitable transposase source and the *white* marker they contain serves as a convenient marker for the movement of the P element within the genome.

Figure 1 is a schematic representation of the assay developed to measure transposition and its repression. Male flies to be tested for their ability to repress P transposition are crossed to *A4-4* females to give orange-eyed flies heterozygous for the *A4-4* insertion. Male progeny from this cross are then mated to *w; Sb e Δ2-3 / TM6* females as a source of P transposase. F2 flies resulting from this cross show one of two eye phenotypes depending on the presence of repressors in the original male fly. If there is no repression then flies have mosaic eyes, consisting of red and white patches. This results from the somatic transposition of the *A4-4* element and its reinsertion into euchromatin or its loss by excision respectively. However, any repression of transposition of the *A4-4* element gives eyes in which the predominant colour is orange with red and white flecks. This phenotype is distinguishable from the normal *A4-4* phenotype as it is slightly darker and we have termed it 'flecked' to distinguished it from the 'mosaic' phenotype. While the eye phenotype gives an indication of repression, the assay is quantifiable by measuring the germline transposition rate. For this, mosaic or flecked male F2 flies are backcrossed to w^{1118} females and the F3 progeny are scored for red, white or orange eyes. Therefore, a higher level of repression results in more orange-eyed F3 progeny.

(iii) *Genomic* DNA *extraction and Southern blot analysis*

DNA was extracted from 100–200 adult flies by the method of Mian and Dover (1990). For Southern analysis, 2–5 μg of DNA was restricted according to the suppliers' instructions and electrophoresed in 1% agarose, before alkali capillary blotting onto Hybond N^+ (Amersham).

Filters were probed either with a 1 kb *Xho* I-*Eco* RI or a 0·7 kb Pst I gel-isolated fragment derived from a complete P element and labelled by the random prime method.

(iv) *Genomic library construction, sub-cloning and sequencing*

A genomic library of *Sau3a*-digested North Wootten DNA was made in the lambda replacement vector λ2001 (Karn *et al.* 1984) according to standard techniques (Sambrook, 1989). DNA fragments were sub-cloned into the pBluescript KS+ plasmid vector (Stratagene Inc) and double stranded sequencing performed following standard alkaline lysis DNA preparations using the Sequenase (USB) protocol.

3. Results

While an assay for P element transposition that utilizes eye colour mosaicism has already been described (Misra & Rio, 1990) the method described here benefits from the unique properties of the *A4-4* mutant. Under-expression of the *A4-4 white* gene in this strain, due to its heterochromatic location, results in a pale orange eye colour. Transposition followed by re-insertion of the element into euchromatin, or loss of the element by excision, is visible in this assay as these events give rise to red or white eyes respectively. Therefore, transposition events which are followed by re-insertion of the element, which could be incorrectly classed as repressed in previously described eye colour assays, are correctly classed with the *A4-4* assay. Despite its heterochromatic position, however, the *A4-4* element is fully mobilizable, giving mosaic eyes at a high frequency when the transposase source is the Δ2-3(99B) element.

(i) *A single* KP *element can repress the transposition of the* A4-4 *element*

We determined the mobility of the *A4-4* element in the eye colour mosaicism assay and the effects of the KP element repressor on transposition.

Using strain *I-27* as a source of a single KP element we constructed an experimental line that contained KP in *cis* with the *A4-4* reporter (Fig. 2). Pale orange eyed females of the genotype *w; A4-4* were crossed to white eyed *I-27* males producing F1 females that carried KP in *trans* with *A4-4*. Crossing these females to *white* males gave some F2 progeny that were recombinant for the third chromosome and therefore carried KP in *cis* with *A4-4* but were indistinguishable from flies containing *A4-4* alone. However, mating these F2 males to *w; Sb e Δ2-3(99B)* females to mobilize the *A4-4* insert resulted in *Sb* F3 flies with either 'flecked' or 'mosaic' eye phenotypes. These phenotypic differences were presumed to be due to the action of the KP element in *cis* with the *A4-4* element and repressing its transposition somatically. Table 1 shows that when the F3 male flies with mosaic eyes were crossed to *white* females, to quantify the germline movement of *A4-4*, 72·5% of the progeny had red or white eyes. F3 males with flecked eyes, however, gave 30·6% red or white-eyed progeny indicating that levels of repression were higher in these males. Obtaining these two discrete classes of repression ability strongly supported the assumption that flecked-eyed males carried the KP element in *cis* with *A4-4* (confirmed by Southern blotting of the F3 males after mating) and that KP was responsible for the decrease in transposition.

This repression ability was completely stable and heritable and the effect on the mobilization of the *A4-4* element was consistent. Mating non-stubble sibling flies of the original F3 mosaic and flecked

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

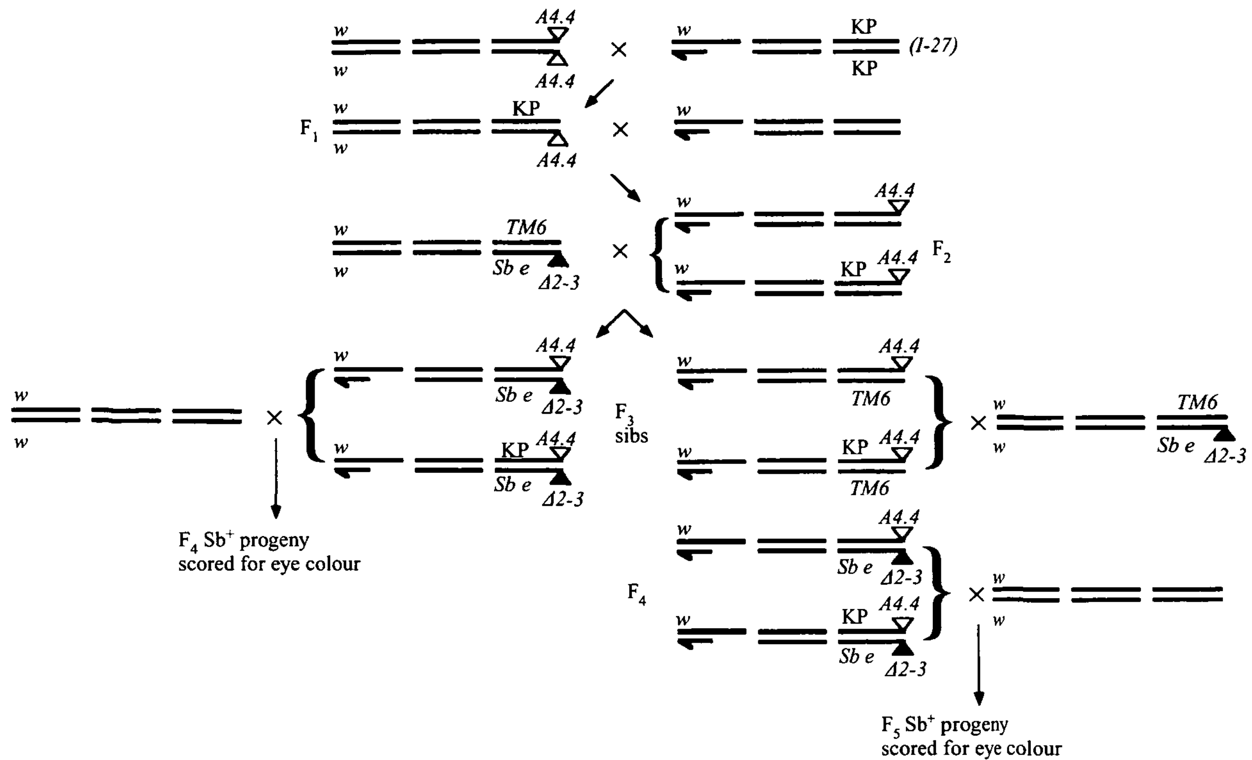


Fig. 2. Breeding scheme used to generate lines with and without the single KP element repressor, from strain *I-27*, in *cis* with *A4-4* marker locus, (see text).

Table 1. *Germline transposition rates of the* A4-4 *transgene in presence or absence of the KP repressor. Data obtained from single male crosses (either F3 or F4 males with either mosaic or flecked eye phenotypes) with* white *females. Percentage excision/insertion frequency calculated from the total number of red and white-eyed flies scored from all non-Stubble progeny.*

Male genotype	Male eye phenotype	Total number of excisions and insertions	Total number of Sb+ progeny	Percentage excision and insertion frequency
F3 males				
w; A4-4/Δ2-3	Mosaic	1162	1602	72·5 ± 1·1
w; KP A4-4/Δ2-3	Flecked	430	1404	30·6 ± 1·2
F4 males				
w; A4-4/Δ2-3	Mosaic	159	243	65·4 ± 3·1
		140	182	76·9 ± 3·1
w; KP A4-4/Δ2-3	Flecked	83	264	31·4 ± 2·8
		87	321	27·1 ± 2·4
Pooled data				
w; A4-4/Δ2-3	Mosaic	1461	2027	72·1 ± 0·9
w; KP A4-4/Δ2-3	Flecked	600	1989	30·2 ± 1·0

males (whose genotypes were *w; A4-4/TM6* and *w; KP A4-4/TM6* respectively) to *w; Sb e Δ2-3(99B)* females resulted in only flecked eyed flies from *w; KP A4-4/TM6* males and only mosaic eyed flies from *w; A4-4/TM6* males. Table 1 shows that in these F4 males, germline movement of the *A4-4* element, from two replicates, was 27·2% and 33·7% for lines containing KP and 65·4% and 76·9% for lines only carrying *A4-4*.

If the KP element is responsible for the decrease in mobility of the *A4-4* element in these experiments, then this demonstrates that the eye colour mosaicism assay is extremely sensitive as a measure of transposition repression. The KP element from *I-27* was only capable of producing 4–10% increased fertility in a gonadal dysgenesis test (Jackson *et al.* 1988) and is clearly not a strong repressor element, and this leaves the prospect that the *A4-4* assay is capable of detecting elements that have a much weaker effect on transposition, or indeed the effect of multiple elements.

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

Fig. 3. Breeding schemes used to quantify the chromosome 2 and chromosome 3 components of repression in the North Wootten Q strain. North Wootten chromosomes shown as open bars, and recombinant chromosomes are shown as open and filled mosaics. *Scheme A*. Isolation of North Wootten chromosome 2 repression ability. Two discrete classes of F2 male, differing in their repression ability, were obtained depending on whether they had or had not inherited the North Wootten second chromosome. *Scheme B*. Construction of experimental lines with and without sections of the third North Wootten chromosome in *cis* with the *A4-4* marker locus. Ten independent lines were set up from F3 males which had shown exclusive chromosome 3 repression and did not carry recombinant second chromosomes.

(ii) *Repression of* A4-4 *mobility by natural* Q *chromosomes*

Typically, natural Q strains have high levels of repression, restoring 90% fertility in gonadal dysgenesis tests (Kidwell, 1983), and it is possible that this is due to the cumulative effects of the multiple copies of KP that these strains often contain, with each element contributing the intermediate repression levels observed for the *I-27* element. However, as some Q strains possess few if any KP elements there may be an additional or completely separate chromosomal component to Q repression.

We have used the *A4-4* assay to investigate the chromosomal basis for the repression observed in the North Wootten Q strain, which has a high KP copy number (20–30 per genome) and which also exhibits a high repression of gonadal dysgenesis, comparable to P cytotype strains. The method we have used, shown in Fig. 3 (schemes A and B), involves the separation of repression caused by chromosome 2 from that caused by chromosome 3.

Chromosome 2 (Fig. 3, scheme A) was isolated by crossing *A4-4* homozygous females to North Wootten males, After mating pale orange eyed F1 males to *w;Sb e Δ2-3/TM6* females all *Sb* male F2 progeny gave either a flecked or mosaic eye phenotype, constituting a heterogenous sample of flies that either carry the North Wootten chromosome or do not. The F2 males were individually mated to *white* females and the non-*Sb* F3 progeny scored for the germline transposition rate. Following mating, individual F2 males were Southern blotted and probed with the 1 kb *Xho* I/*Eco* RI fragment to detect which flies had inherited the North Wootten chromosome 2 and which only carried the *A4-4* second chromosome. The latter, which carried no other P elements than the *A4-4* itself and had a mosaic eye phenotype, had a germline transposition rate of 71·5% (Fig. 4, column 1). Transposition in male germlines bearing the North Wootten chromosome, which corresponded to having the flecked eye phenotype, was only 7·9% (Fig. 4, column 3).

Analysis of chromosome 3 was complicated by the fact that both the reporter (*A4-4*) and the mobilizer (Δ2-3) are also on the third chromosome. Figure 3, scheme B, shows the cross scheme used to generate recombinant third chromosomes to test different

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

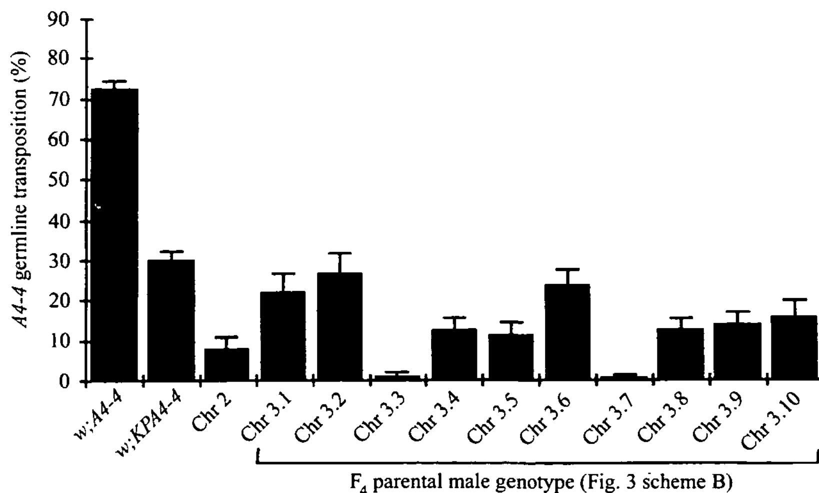


Fig. 4. Germline transposition rates, with 95% confidence limits, of the *A4-4* marker locus using experimental lines containing North Wootten Q chromosome as the source of repressor. Intervals 1 and 2 show results from table 1 for KP and non-KP containing lines. Experimental lines with North Wootten chromosome 2 (Chr 2) show higher levels of repression than a single KP element. Experimental lines with recombinant third chromosomes (Chr 3.1 to Chr 3.10) give intermediate levels of repression except for Chr 3.3 and Chr 3.7 which give less than 1% transposition of *A4-4*.

regions of this chromosome for the presence of repressors.

F1 females, resulting from a cross between *w;A4-4* females and North Wootten males, were mated to *white* males to produce pale orange eyed F2 males potentially recombinant for all chromosomes. When fifty of these F2 males were mass mated to *w; Sb e Δ2-3 / TM6* females, three classes of *Sb* F3 progeny were obtained. Those with white eyes had not inherited the *A4-4* from their fathers. Those that had inherited the *A4-4* had either mosaic or flecked eye phenotypes depending on any inherited repression from the North Wootten chromosomes. F3 males with flecked eyes could have inherited the repression in *trans* from the second chromosome or in *cis* from a section of the third. To distinguish between these possibilities, these males were crossed to *white* females again and the *A4-4* in the male progeny mobilized by crossing to *Sb Δ2-3* (not shown in Fig. 3B). If repression was determined exclusively by chromosome 2 then offspring of this second mobilization cross would segregate for both mosaic (unrepressed) and flecked (repressed) eyes. If, however, repression was linked to regions of chromosome 3, then all flies would have the flecked phenotype.

We established ten lines (Chr 3.1 to Chr 3.10) from *w; A4-4/TM6* males, sibling flies of *w; A4-4/Sb eΔ2-3* males that had shown only chromosome 3 inheritance of repression in *cis* with *A4-4*. Individual males from these lines were then assayed for their germline *A4-4* transposition by crossing to the *Δ2-3* mobiliser strain and mating the resulting *Sb* flecked eyed males to *white* females. As shown in Fig. 4, eight of the lines had levels of repression that were lower than the observed levels for chromosome 2 (the *A4-4* element transposing at frequencies between 11·1% to 26·6%). This repression may be mediated by multiple KP elements, the reduced levels being indicative of intrinsic differences in KP copy number between chromosomes 2 and 3 in North Wootten, or of a lower KP copy number because of the recombinant nature of these chromosomes.

However, two lines, Chr 3.3 and Chr 3.7, exhibited extremely high levels of repression of transposition with red or white eyed flies constituting less than 1% of the progeny. This is comparable to the total repression observed in the North Wootten strain and so we investigated the molecular basis for this repression.

(iii) *Molecular characterization of the strong repressor of transposition* SR

Figure 5A shows a Southern blot analysis, using the internal 1 kb *Xho* I/*Eco* RI P fragment as a probe, of lines Chr 3.3 and Chr 3.7 and includes the original North Wootten and chromosome 2 lines (Chr 2). With *Eco* RV, which has no restriction sites within the canonical P element sequence, Chr 3.3 and Chr 3.7 gave a single strongly hybridising band at 5·0 kb (lanes 3 and 4) which suggested that both these lines contained the same region of chromosome 3 with a single element inserted into it. It appears to be the only P element in these strains, although there is weak hybridization for smaller sized bands. This band was present in the original North Wootten strain (lane 1) but was absent from the Chr 2 line (lane 2), which nevertheless contained several other P elements. Further restriction mapping of these lines using *Pvu* II and *Pst* I (lanes 5–12) confirmed that Chr 3.3 and Chr 3.7 contained the same element with shared bands at 0·9 kb and 2·0 kb for *Pvu* II and 0·7 kb and 2·5 kb for *Pst* I. The strong bands of hybridization at 0·9 kb and 0·7 kb indicated that the central and 5′

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

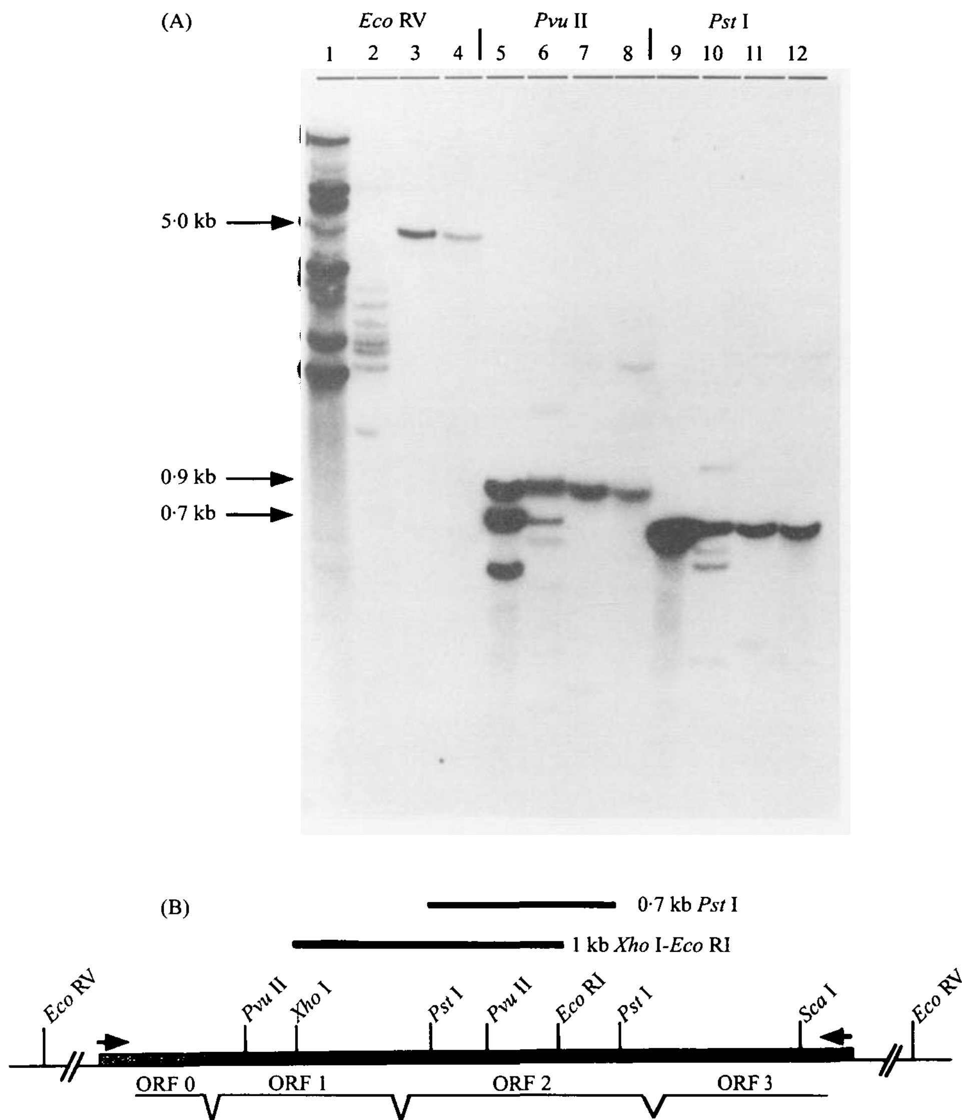


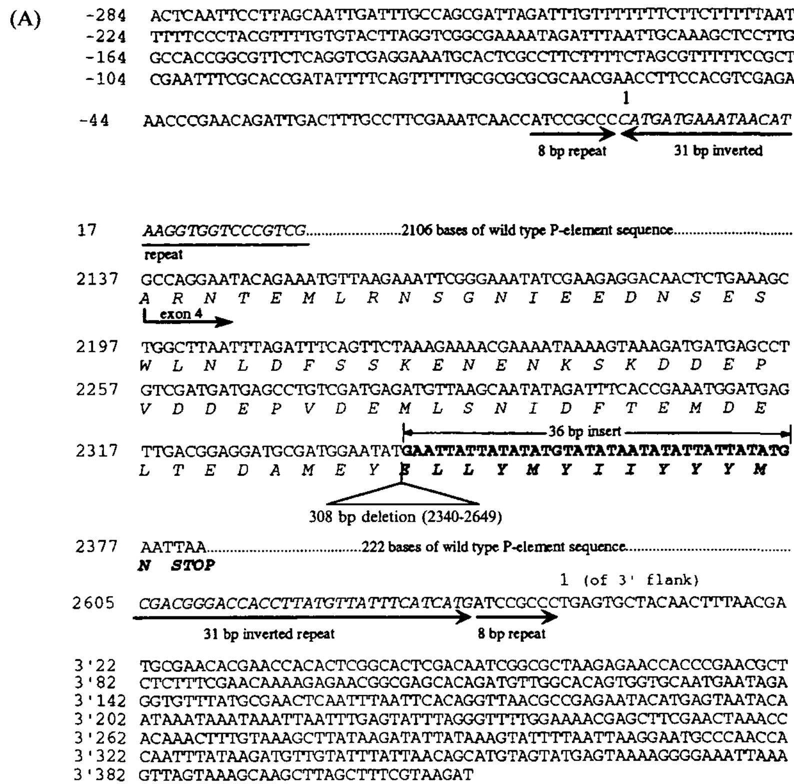
Fig. 5. Autoradiogram showing Southern blotted genomic DNA probed with a 1 kb *Xho* I/*Eco* RI internal fragment from a complete P element. Approximately 5 μg of DNA was loaded for each sample. Lane order is as follows: (1) North wootten digested with *Eco* RV. (2) Line Chr 2 digested with *Eco* RV. (3) Line Chr 3.3 digested with *Eco* RV. (4) Line Chr 3.7 digested with *Eco* RV. (5)–(8) As for lanes (1)–(4) but digested with *Pvu* II. (9)–(12) As for lanes (1)–(4) but digested with *Pst* I. A 5·0 kb band (indicated by arrow) is shared between Chr 3.3 and Chr 3.7 and North Wootten but absent from Chr 2. (B) Restriction map of complete P element showing location of probes used for the Southern analysis and for the North Wootten λ library screen.

regions of the element were intact and that the element was either a complete P element or an element with a comparatively small internal deletion in the 3′ half of the element.

In order to clone this P element, we screened a North Wootten genomic λ library, using a 0·7 kb *Pst* I fragment as a probe, and isolated 20 hybridizing plaques. As this fragment comprises the central portion of the complete P element sequence it does not hybridize to KP elements which are deleted for this region. Two of the twenty clones gave the same restriction pattern as the Chr 3.3 and Chr 3.7 lines when digested with *Pvu* II and *Pst* I and probed with the *Xho* I/*Eco* RI fragment. Further restriction mapping of these λ clones showed them to contain the same P element and that it lacked the *Sca* I restriction site at position 2627 of the complete P element sequence. This strong repressor, or SR element, was subcloned as a 3·3 kb *Eco* RV/*Sca* I fragment into pBluescript and the sequence of the entire insert determined as described in Materials and Methods.

The clone contains 704 bp of flanking DNA (Fig. 6A, B) including an 8 bp direct repeat target site duplication typically associated with P element insertions, which has a weak homology to the consensus sequence GGCCAGAC (O'Hare & Rubin, 1983). The SR element is a deleted P element, 2635 bp in length, and can be derived from the complete P element sequence by a 308 bp deletion extending from position 2341 to 2649. At the site of this breakpoint,

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press



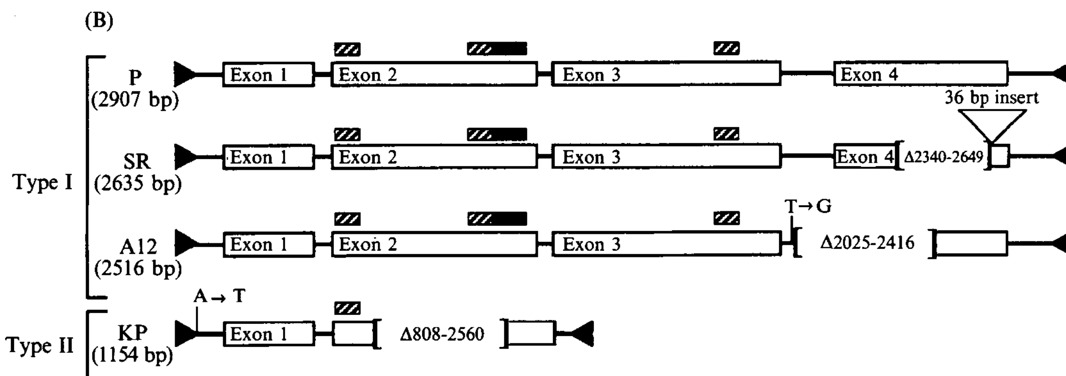


Fig. 6. (A) Complete sequence of the 2635 bp SR element and immediate flanking DNA from North Wootten. The 36 bp insert, unique to SR, is shown in bold. (B) Structural comparisons of three type I elements and one type II element. Terminal inverted 31 bp repeats shown by filled triangles. Protein motifs shared by both elements are shown. The putative transposase DNA binding domain is shown as a filled box and the three leucine zippers by striped boxes. P element and A12 structures taken from gloor *et al.* (1993).

there is a 36 bp insertion of a simple A.T. rich sequence which causes a frame shift resulting in an additional 13 unique amino acids before a premature TAA stop codon. Therefore, the SR element has a coding capacity for a 642 amino acid protein with a calculated size of 75 kDa. However, due to the position of the deletion breakpoint in SR, the final 2-3 intron (IVS3) is maintained and therefore, the SR element is also capable of producing the identical 66 kDa protein that is produced by complete P elements somatically. If the germline specific splicing its applied to SR RNA, then SR will produce a truncated 75 kDa protein in the germline and 66 kDa repressor protein somatically.

4. Discussion

(i) *Type* I *and type* II *repressor elements*

It is the prevailing view that complete P elements in *Drosophila melanogaster* catalyse their own transposition via a self-encoded 87 kDa transposase, and auto-regulate their transposition via the truncated 66 kDa repressor protein produced by incomplete splicing of the final, third IVS3 intron (Rio, 1990; Engels, 1989).

This pivotal role for the complete P element, based on studies of regulation in P cytotype strains which predominate in North America, is a consequence of

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

the demonstrated repressor ability of the 66 kDa product (Misra & Rio, 1990; Ronserray *et al.* 1991) and the failure to-date to isolate a deletion-derivative representing a Type I repressor from a naturally occurring P strain (O'Hare *et al.* 1992; Gloor *et al.* 1993). Having identified the 66 kDa product as the possible mediator of repression in P strains, questions still remain as to how the strict maternal inheritance of cytotype is brought about, and whether and how inaccurate splicing takes place in germline tissues. Any explanatory model needs to take into account the gradual acquisition of P cytotype (Preston & Engels, 1989). For example, Lemaitre and colleagues have proposed a model, derived from an earlier proposal of O'Hare *et al.* 1992, in which the strength of repression is related to the decreased efficiency of IVS3 splicing (Lemaitre *et al.* 1993). This model has experimental support from the discovery of an RNA-binding protein, PSI, which is strictly limited to somatic tissues and which inhibits IVS3 splicing (Siebel *et al.* 1995). More recently, it has been shown that P transcripts retaining the third intron, and therefore capable of producing 66 kDa repressor, are present at low levels in dysgenic conditions and the level of unspliced transcripts increases as repression increases in a positive feedback loop (Roche *et al.* 1995).

(ii) *The* Q *phenotype and a stable strategy for repression*

Whatever the precise molecular mechanism of repressor production might be, it is probable that the balance between the production of the 87 kDa and 66 kDa proteins determined the rate at which the P cytotype becomes established. If, however, the production of the 66 kDa, or a '66 kDa-like', protein switches from being dependent on intron splicing efficiencies to being the only product of a permanently altered type I repressor element, then a strong, biparentally transmitted, repression might ensue. This has the added advantage that no significant levels of hybrid dysgenesis would be induced by autonomous P elements inherited through the males. In other words, the evolution of the Q phenotype would have occurred; that is, populations with strong repressor activity but less than 10% induction activity in gonadal dysgenesis tests despite the presence of autonomous elements (Kidwell, 1983). It is against this background that we sought and found a strong type I repressor, the SR element, in the North Wootten Q population. To our knowledge, the SR element is the first naturally-occurring deletion-derivative, tested to be functionally serving as a type I repressor, to be isolated. What is the significance, if any, of such an element being found in a Q population?

The current situation in *D. melanogaster*, having P, Q and M′ populations may reflect the recent invasion of the P family of elements into this species and the initial demand on populations to coevolve a repression system, in whatever form, in order to survive the damaging consequences of hybrid dysgenesis. As long as some degree of protection is achieved, then there is a short term, but possibly transistory, benefit. The final resting place for *D. melanogaster* will depend on the long term selective differences between the type I and type II systems. We argue below that the SR element may, over evolutionary time, give Q strains a selective advantage over M′ and P strains and that the Q phenotype class is an evolutionary stable strategy (Maynard Smith, 1982).

First, the SR element effectively reduces transposition levels to zero, whereas the relatively weak KP-mediated repression in M′ strains gives variable levels of hybrid dysgenesis (Kidwell, 1983). This is particularly evident in intrastrain sterility tests where M′ strains give higher levels of infertility compared to P and Q (Jackson *et al.* 1988; Kidwell, 1988).

Secondly, the SR element operates biparentally (as does the KP element) and there is no delay between the introduction of the repressor element into the genome and the establishment of effective repression. For P strains, repression can be temporarily lost in a situation in which a P male mates with an unrepressed female.

Thirdly, the putative 75 kDa SR product contains the same protein motifs as the 66 kDa repressor with three protein-binding leucine zippers and the DNA-binding domain. The mode of action of P element repressors, whether via transcription blocking, protein poisoning or steric hindrance at termini is not known; however, all the structural features that allow the 66 kDa protein to act as a strong repressor are also present in the SR protein. If the repressor works by poisoning of a transposase multimer, then the increased length of the SR product may even enhance repression by increasing the stability of the interaction with the 87 kDa subunit. However, the situation is more complex due to the variety of known Q populations.

(iii) *A variety of* Q *populations*

To our knowledge there are four genotypically distinct Q populations that divide into two phenotypic classes: those with maternally transmitted repression (here termed Q_{mat}) and those with a biparental mode of inheritance (Q_{bip}). In Fig. 7 we depict the four genotypes. The Q_{mat} class contains two distinct genotypes, one without type II (KP) repressor elements and one with type II repressor elements. The former has a much more pronounced maternal transmission of repression than the latter: [see for example assays of Q phenotypes in Jackson, 1988, table 4 – please note that due to a printer's error the headings in the last two columns, Cross 1 and Cross 2, should be reversed]. Q_{mat} without KP elements (see Q1. in Fig. 7) exhibits exclusive cytotype repression, probably de-

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

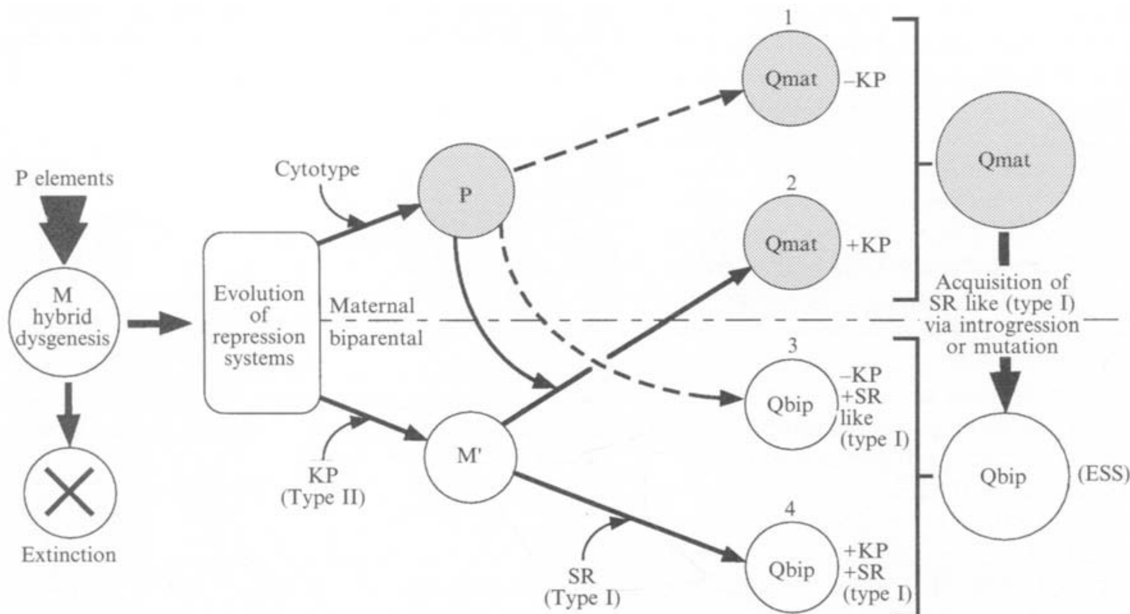


Fig. 7. A schematic outline of the possible course of evolution of repression of P-induced hybrid dysgenesis. *Step A*: P elements from other *Drosophila* species invade M populations of *D. melanogaster* and cause hybrid dysgenesis. Some populations go extinct. *Step B*: Survival depends on evolution of repression systems, of which there are two types defined by their mode of transmission (maternal or biparental). Maternal transmission characterizes P cytotype repression in P populations. Biparental transmission characterizes M′ populations with the *de novo* origin of type II (KP) elements. *Step C*: Evolution of four known classes of Q populations (Q populations repress but do not induce hybrid dysgenesis). (1) Q_{mat} (−KP). This has strong, exclusively maternally transmitted repression and does not contain KP elements. This is represented by the Hacateppe (Turkey) population. (2) Q_{mat} (+KP). Predominantly maternally transmitted repression, despite the presence of KP elements which give a moderate level of paternal transmission. This class probably evolved from crosses between P and M′ populations. (3) Q_{bip} (−KP; +SR-like type I?). This class has biparentally transmitted repression but has no KP elements, and is represented by the Hunter Valley (Australian) population. We postulate the existence of SR-like elements and its probable evolution from P populations. (4) Q_{bip} (+KP; +SR-like type I). This class, represented by North Wootten, has biparentally transmitted, strong repression due to its SR and KP elements. Probably evolved from an M′ population. *Step D*: Evolution of Q_{mat} populations into Q_{bip}, with permanent, biparentally inherited strong type I repression as a consequence of the introgression or *de novo* origins of SR-like elements producing 66 kDa-like repressors (see text). *Note*: The genotypes and phenotypes of classes 1, 2 and 3 are described in Jackson, 1988, table 4, excepting that, due to a printer's error the headings of the last two columns, Cross 1 and Cross 2, should have been in the reverse order.

pendant on differential germline splicing of IVS3 as in P populations and might have evolved from P populations. The Q_{mat} with KP elements has a degree of paternal transmission in an otherwise cytotype-based repression system. This Q genotype could have evolved from natural crossing between P and M′ populations (see Q2. in Fig. 7).

By way of contrast, the Q_{bip} class has roughly equal degrees of repression transmitted through both sexes. Like the Q_{mat} class, there are two distinct genotypes based on the presence and absence of type II (KP) repressors. A Q population derived from Hunter Valley (Australia) is an example of a Q_{mat} genotype with no KP elements but with strong biparentally transmitted repression (Jackson *et al.* 1988). We predict that this population will be shown to contain an SR-like (type I) repressor; that is, an element producing a 66 kDa-like repressor constitutively and which is transmittable through both sexes (see Q3. in Fig. 7). The North Wootten Q population is an example of the second Q_{bip} genotype: a mixture of strong type I (SR) repression and intermediate type II (KP) repression (see Q4. in Fig. 7), the latter certainly giving biparental transmission in keeping with all previous studies (Jackson, 1988).

We propose below that the Hunter Valley Q_{bip} genotype, lacking KP elements, might have evolved from P populations, whereas the North Wootten Q_{bip} with KP elements might have evolved from M′ populations with the acquisition of the SR (type I) element.

While the SR element is probably the main agent of repression in the North Wootten Q_{bip} population, we do not know the precise mechanism of its repression or the significance, if any, of its insertion site on the third chromosome as position effects have been shown to be critical in determining the strength of repression (Misra *et al.* 1993).

At present, we have no evidence for the presence of the SR element in other Q strains. We believe that the SR element would be strongly favoured by selection, given its strong repression ability, but other Q strains may simply have similar but not identical 'SR-like' elements. These would perform the same function, producing type I repressor protein directly rather than relying on the occasional mis-splicing of IVS3 from

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

complete P elements. Using 3′ deletions generated *in vitro*, it has been shown that Type I repressors required the first 1956 bp of the P element sequence (Gloor *et al.* 1993), and P element deletion-derivatives, having structures characteristic of Type I repressors, have been isolated from several Japanese Q strains (Nitasaka *et al.* 1987; Sakoyama *et al.* 1985). The element described in Sakoyama *et al.* has a deletion breakpoint precisely located in the region identified to be critical for type I repressor function. However, this element has not yet been shown to be functionally active as a repressor.

Whatever the fine detailed structures of 'SR-like' elements might be, the benefits they confer on Q_{bip} strains implies that this method of repression could be the long term outcome for *D. melanogaster*. Chromosomes carrying SR-like elements could spread, due to the potential ability of Q_{bip} populations to interbreed with M′ and P populations with no loss of fitness to the offspring. Q_{bip} populations mating with M′ populations would not induce hybrid dysgenesis and the stronger type I-mediated repression would be likely to replace the weaker KP-mediated system. The transition from P to Q_{bip} could occur by one of two methods (Fig. 7). Either *de novo* generation of a type I repressor from a complete P element, or the introgression of SR-like repressor-carrying chromosomes from the Q population. Whatever the mode of entry, the presence of strong biparentally effective SR-like type I elements might lead to the eventual decay of complete elements.

Studies on many other *Drosophila* species, in which P elements have been present for a longer time than in *D. melanogaster*, have not revealed complete, functional P elements. Instead, some contain tandem repeats of elements with degenerate fourth exons which might encode some 66 kDa-like repressor activity (Miller *et al.* 1992; Paricio *et al.* 1991; Paricio *et al.* 1994). In *Drosophila nebulosa*, a complete element has been isolated but contains many base changes in all four exons and is non-functional (Lansman *et al.* 1987). If these species had been invaded by complete elements as *D. melanogaster* has been, then this could be additional suggestive evidence that cytotype repression, mediated by complete P elements, was not the most stable mode of repression.

The prediction of our model is that Q_{bip} populations are the stable end state for both M′, P and Q_{mat} populations. We are currently testing this model, by screening for SR-like elements and examining the spread of Q_{bip} repression, using natural populations systematically collected along the east coast of Australia. A north-south cline of repression ability has been demonstrated in this area, with discrete regions of P in the north, through Q, to M′ in the south (Boussy *et al.* 1988). An extension of the range of the Q_{bip} phenotype, with SR or SR-like elements, would indicate the potential selective advantage of populations possessing such elements.

The grant support of the Natural Environmental Research Council (NERC, UK) is gratefully received and judiciously spent.

References

Andrews, J. D. & Gloor, G. B. (1995). A role for the KP leucine zipper in regulating P element transposition in *Drosophila melanogaster*. *Genetics* **141**, 587–594.

Black, D. M., Jackson, M. S., Kidwell, M. G. & Dover, G. A. (1987). KP elements repress P-induced hybrid dysgenesis in *Drosophila melanogaster*. *EMBO Journal* **6**, 4125–4135.

Boussy, I. A., Healy, M. J., Oakeshott, J. G. & Kidwell, M. G. (1988). Molecular analysis of the P, M gonadal dysgenesis cline in eastern Australian *Drosophila* melanogaster. *Genetics* **119**, 889–902.

Engels, W. R. (1984). A trans-acting product needed for P factor transposition in *Drosophila*. *Science* **226**, 1194–1196.

Engels, W. R. (1989). P elements in *Drosophila melanogaster*. In '*Mobile DNA*'. (ed. D. E. Berg and M. M. Howe). 437–484.

Engels, W. R., Johnsonschlitz, D. M., Eggleston, W. B. & Sved, J. (1990). High-frequency P element loss in *Drosophila* is homolog dependent. *Cell* **62**, 515–525.

Gloor, G. B., Preston, C. R., Johnsonschlitz, D. M. *et al.* (1993). Type I repressors of P element mobility. *Genetics* **135**, 81–95.

Hazelrigg, T., Levis, R. & Rubin, G. M. (1984). Transformation of white locus DNA in *Drosophila* – dosage compensation, zeste interaction, and position effects. *Cell* **36**, 469–481.

Jackson, M. S., Black, D. M. and Dover, G. A. (1988). Amplification of KP elements associated with the repression of hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **120**, 1003–1013.

Karn, J., Matthes, H. W. D., Gait, M. J. & Brenner, S. (1984). A new selective phage cloning vector, lambda-2001, with sites for *Xba* I, *Bam* HI, *Hind* III, *Eco* RI, *Sst* I and *Xho* I. *Gene* **32**, 217–224.

Kidwell, M. G., Kidwell, J. F. & Sved, J. A. (1977). Hybrid dysgenesis in *Drosophila melanogaster*. A syndrome of aberrant traits including mutation, sterility and male recombination. *Genetics* **36**, 813–833.

Kidwell, M. G. (1983). Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster*. *Proceedings of The National Academy of Sciences of The United States of America Biological Sciences* **80**, 1655–1659.

Kidwell, M. G., Kimura, K. & Black, D. M. (1988). Evolution of hybrid dysgenesis potential following P element contamination in *Drosophila melanogaster*. *Genetic* **119**, 815–828.

Kidwell, M. G. (1992). Horizontal transfer of P-elements and other short inverted repeat transposons. *Genetica* **86**, 275–286.

Lansman, R. A., Shade, R. O., Grigliatti, T. A. & Brock, H. W. (1987). Evolution of P transposable elements-sequences of *Drosophila nebulosa* P elements. *Proceedings of The National Academy of Sciences of The United States of America* **84**, 6491–6495.

Laski, F. A., Rio, D. C. & Rubin, G. M. (1986). Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. *Cell* **44**, 7–19.

Lemaitre, B. & Coen, D. (1991). P-regulatory products repress *in vivo* the P promoter activity in P-*lacz* fusion genes. *Proceedings of The National Academy of Sciences of The United States of America* **88**, 4419–4423.

Lemaitre, B., Ronsseray, S. & Coen, D. (1993). Maternal repression of the P element promoter in the germline of

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press

Drosophila melanogaster – a model for the P cytotype. *Genetics* **135**, 149–160.
Lindsley, D. L. & Zimm, G. G. (1992). 'The Genome of *Drosophila melanogaster*.' Academic Press.
Maynard Smith, J. (1982). 'Evolution and the Theory of Games.' Cambridge University Press.
Mian, A. & Dover, G. A. (1990). Promoter variation in the ribosomal-RNA genes in *Drosophila melanogaster*. *Nucleic Acids Research* **18**, 3795–3801.
Miller, W. J., Hagemann, S., Reiter, E. & Pinsker, W. (1992). P element homologous sequences are tandemly repeated in the genome of *Drosophila guanche*. *Proceedings of The National Academy of Sciences of The United States of America* **89**, 4018–4022.
Misra, S., Buratowski, R. M., Ohkawa, T. & Rio, D. C. (1993). Cytotype control of *Drosophila melanogaster* P element transposition – genomic position determines maternal repression. *Genetics* **135**, 785–800.
Misra, S. & Rio, D. C. (1990). Cytotype control of *Drosophila* P element transposition – the 66 kDa protein is a repressor of transposase activity. *Cell* **62**, 269–284.
Nitasaka, E., Mukai, T. & Yamazaki, T. (1987). Repressor of P elements in *Drosophila melanogaster* – cytotype determination by a defective element carrying only open reading frames 0 through 2. *Proceedings of The National Academy of Sciences of The United States of America* **84**, 7605–7608.
O'Hare, K., Driver, A., Mcgrath, S. & Johnsonschiltz, D. M. (1992). Distribution and structure of cloned P elements from the *Drosophila melanogaster* P strain PI. *Genetical Research* **60**, 33–41.
O'Hare, K. & Rubin, G. M. (1983). Structures of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**, 25–35.
Paricio, N., Perezalonso, M., Martinezsebastian, M. J. & Defrutos, R. (1991). P sequences of *Drosophila subobscura* lack exon-3 and may encode a 66-kDa repressor-like protein. *Nucleic Acids Research* **19**, 6713–6718.
Paricio, N., Martinezsebastian, M. J. & Defrutos, R. (1994). A heterochromatic P sequence in the *D. subobscura* genome. *Genetica* **92**, 177–186.
Preston, C. R. & Engels, W. R. (1989). Spread of P transposable elements in inbred lines of *Drosophila melanogaster*. *Progress in Nucleic Acid Research and Molecular Biology* **36**, 71–85.
Rasmusson, K. E., Raymond, J. D. & Simmons, M. J. (1993). Repression of hybrid dysgenesis in *Drosophila melanogaster* by individual naturally-occurring P elements. *Genetics* **133**, 605–622.
Rio, D. C. (1990). Molecular mechanisms regulating *Drosophila* P element transposition. *Annual Review of Genetics* **24**, 543–578.
Robertson, H. M. & Engels, W. R. (1989). Modified P elements that mimic the P cytotype in *Drosophila melanogaster*. *Genetics* **123**, 815–824.
Robertson, H. M., Preston, C. R., Phillis, R. W. *et al.* (1988). A stable genomic source of P element transposase in *Drosophila melanogaster*. *Genetics* **118**, 461–470.
Roche, S. E., Schiff, M. & Rio, D. C (1995). P-element repressor autoregulation involves germ-line transcriptional repression and reduction of third intron splicing. *Genes & Development* **9**, 1278–1288.
Ronserray, S., Lehmann, M. & Anxolabehere, D. (1991). The maternally inherited regulation of P elements in *Drosophila melanogaster* can be elicited by 2 P copies at cytological site 1A on the X-chromosome. *Genetics* **129**, 501–512.
Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). 'Molecular Cloning – A Laboratory Manual.' Cold Spring Harbor Laboratory Press.
Sakoyama, Y., Tod, T., Isiwachigusa, S. *et al.* (1985). Structures of defective P transposable elements prevalent in natural Q and Q-derived M-strains of *Drosophila melanogaster*. *Proceedings of The National Academy of Sciences of The United States of America* **82**, 6236–6239.
Siebel, C. W., Admon, A. & Rio, D. C. (1995). Soma-specific expression and cloning of PSI, a negative regulator of P element pre-messenger-RNA splicing. *Genes & Development* **9**, 269–283.
Williams, J. A., Pappu, S. S. & Bell, J. B. (1988). Suppressible P element alleles of the *vestigial* locus in *Drosophila melanogaster*. *Molecular and General Genetics* **212**, 370–374.

https://doi.org/10.1017/S0016672300033577 Published online by Cambridge University Press