A novel repressor of P element transposition in *Drosophila melanogaster*

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**Summary**

We have discovered, in an inbred line (Loua) of *Drosophila melanogaster* from Zaire, a third chromosome showing unusual P element repression. Repression of P element transposition by this chromosome, named Loua3, is dominant zygotic and has three unusual properties. Firstly, its repression of the gonadal dysgenesis caused by a strong P haplotype is strongly temperature-dependent, being most evident at higher rearing temperatures. Secondly, subdivision of Loua3 by recombination abolishes repression: the effect is apparently a function of the intact chromosome. Finally, Loua3 also diminishes somatic lethality when chromosomes carrying many ‘ammunition’ elements (Birmingham2) are exposed to the constitutive transposase source Δ2-3(99B). The chromosome has 17 P elements, none full-length, located in at least 12 dispersed positions.

1. **Introduction**

Unregulated transposition of the P transposable element within the germline of *Drosophila melanogaster* can result in sterility. When germline tissues survive, the progeny show aberrant recombination, chromosome transmission abnormalities, and high frequencies of mutation and chromosome rearrangement—a phenomenon known as hybrid dysgenesis (Kidwell *et al.*, 1977). Unregulated transposition, which results in hybrid dysgenesis, contrasts with the many levels at which transposition is regulated. Transposition is limited to the germline by the requirement for germline-specific splicing of the transposase mRNA. In the soma, alternative splicing of the P transcript results in the production of a 66 kDa transcriptional repressor (Laski *et al.*, 1986). In strains where the intact (2907 bp) P element has become established (P strains) germline transposition is absent due to the maternal inheritance of a cellular state (P cytotype) characterized by low levels of P transcription (Roche *et al.*, 1995), probably mediated by the 66 kDa protein (Misra & Rio, 1990). In addition, a number of repression systems apparently mediated by internally deleted copies of the P element are known. The KP element is the best characterized repressor element. At high copy numbers, this element can repress gonadal dysgenesis (Black *et al.*, 1987). The mechanism may involve the ‘poisoning’ of heteromeric transposase complexes via the leucine zipper domain retained in the product of the KP element (Andrews & Gloor, 1995). However, since the KP protein product will also, unlike P transposase, bind to multiple sites at the termini of P elements, including the 31 base inverted repeats, it may repress through protein–DNA interactions (Lee *et al.*, 1996).

The KP element and the less well characterized SP element have been classified as type II repressor elements by Gloor *et al.* (1993). Type I elements retain the first three P element exons and encode a protein similar to the 66 kDa repressor (Gloor *et al.*, 1993). Both type I and type II repressor elements can be produced by internal deletion during transposition and thus occur stochastically within transpositionally active genomes. Repressors can be divided into those that show their effect maternally, and those repressing through expression in the zygote. Among the former are the strong repressors discovered at site 1A on the X chromosome in numerous wild populations (Ronsseray *et al.*, 1991), and the repressive effects studied by Rasmusson *et al.* (1993), some of which were generated by KP elements. Higuert *et al.* (1992) report a KP element that generates strong P repression, acting through a reduction of P transcription, but the
recessivity of this repression suggests a mechanism involving loss of a host function required for P transcription, with the KP element’s involvement in generating the mutation being coincidental.

Here we report the characterization of a repressive effect associated with a wild-type chromosome with a number of novel attributes. The Loua3 chromosome was identified in a screen of genetically diverse P-bearing wild-type strains for zygotic repression of snw destabilization. The extracted chromosome was also shown to repress gonadal dysgenesis. This repression is strongly temperature-dependent. Subdivision of the Loua3 chromosome by recombination resulted in the loss of repression, suggesting that the effect is associated with two or more loci dispersed across the chromosome. The chromosome is also able to repress the somatic lethality caused by transposition of P elements in the presence of the constitutive transposase source (Δ2-3)99B (Robertson et al., 1988). These data are discussed in the context of the mechanism of action of this novel repression system.

2. Materials and methods
(i) Drosophila strains

Fly stocks are listed below, with their genotype, origin and stock number (where applicable).

Loua. A wild-type strain derived from an isofemale line (the kind gift of P. Capy, CNRS) collected in Zaïre in the mid-1980s. The line was inbred for six generations and subsequently maintained in quarter-pint milk bottles (50–200 individuals/bottle).

Harwich. A wild-type strong P strain collected from Rhode Island, obtained from M. Ashburner, Cambridge. This strain was regularly tested for the ability to induce hybrid dysgenesis, and the maintenance of P cytotype.

CantonS/Harwich. A stock resulting from the crossing of the M wild-type strain Canton S with Harwich, and its subsequent maintenance in mass culture for a number of generations.

C23a. In (2R) Cy sp5/In (2LR) Pm(bw0) dR3/b dp; In (3LR) D, cxF ru h/Sb. A balancer strain carrying multiple inversions on the Pm(bw0) marked second chromosome and the D marked third chromosome used for extraction of the Loua major autosomes.

A balanced lethal system maintains the inverted chromosomes in a heterozygous state.

Attached X (bw st). C(1)DXy f1/y snw; T(X;Y)y+; bw p b dp; st. A strain used to extract potentially revertant X chromosomes. The recessive markers bw and st reveal the presence of similarly marked control chromosomes.

snw assay stock y snw; T(X;Y)y+; bw; st. The M’ stock carrying the snw assay insertion.

ruca. ru h th st sr e’ cu ca. A strain with a multiply

2 h light

Selected F1 y/y+ snw; Pm

y/y+ snw; D

y/y+ snw

Score F1 for snw destabilization and eye colour

Fig. 1. Crossing scheme to test the effect of Loua second and third chromosomes upon snw destabilization induced by the Harwich haplotype.

recessively marked third chromosome used for extracting defined sections of the Loua3 chromosome. Bowling Green Stock Center (no. 2569).

1798. w;ry506; Sb [ry+P A2-3(99B)]/TM6. A strain carrying a constitutive transposase source, the insertion of a ry+ P A2-3 element at 99B. Bowling Green Stock Center (no. 1798).

2538. Birm2; ry506. A standard ‘ammunition’ strain carrying the second chromosome from the Birmingham strain (an M’ strain with approximately 60 deleted P elements). Bowling Green Stock Center (no. 2538).

(ii) Culture conditions

All stocks were maintained on maize meal/molasses medium seeded with dried baker’s yeast in plastic vials or half-pint milk bottles bunged with cotton wool. Unless stated otherwise standard growth conditions were at 22–24 °C in a controlled-temperature room with 12 h light/dark cycling. Flies for high temperature gonadal dysgenesis assays (29–31 °C) were cultured in incubators with 12 h light/dark cycling or were floated in water baths under ambient lighting conditions.

(iii) snw destabilization assays

Fig. 1 illustrates the crossing scheme used to assay the effect of the Loua major autosomes on snw destabilization by the major autosomes of the strong P strain Harwich. Under hybrid dysgenic conditions, snw reverts to more extreme (sn”) and pseudowild-type (sn”) phenotypes, by excision of one or other of the two deleted elements inserted at this locus. At (a), Loua males are crossed to a strain carrying snw”. The resultant male progeny, heterozygous for the Loua second and third chromosomes over recessively
marked M strain major autosomes, are crossed to the balancer strain C23a to generate female flies carrying snw and all possible combinations of the Loua and recessively marked second and third chromosomes. At (b), Harwich females are crossed to C23a males and male progeny carrying the dominantly marked Cy and Sb chromosomes selected. At (c), these males are crossed to the females selected from cross (a). The release from P cytotype via paternal transmission enables transposition of P elements carried by the Harwich autosomes and, in trans, the mobilization of the deleted P elements at snw. The final cross (d) reveals the rate of germline snw destabilization in the progeny of cross (c) by crossing these males to an attached-X strain. Both extreme and pseudowild-type revertants can be scored in this background. The presence of the same recessive markers (bw and st) in the attached-X strain allows the inference of the father’s genotype: i.e. whether he carried the Loua second or third chromosome, both or neither. This discriminatory cross allows the assignment of rates of snw destabilization to particular genotypes, and thus particular chromosomes or their combinations.

(iv) Gonadal dysgenesis assays

The crossing scheme used to construct lines containing only the Loua third chromosome is illustrated in Fig. 2. Cross (a) yields male flies with the Loua autosomes in a heterozygous state. Crossing these flies (b) to C23a females enables the selection of males carrying all the dominantly marked C23a chromosomes, with the exception of Sb, which is replaced by Loua3. This cross also excludes the Loua X chromosome. Crossing these males to C23a again (c) produces male and female flies carrying Pm/Cy; D, which can then be used to establish stable lines (d). These lines are maintained by selection on the dominant markers, retaining the Loua third chromosome in a balanced heterozygous state. A strain bearing the extracted third chromosome was used in the Southern hybridization described below.

Females from these extraction lines were mated to males of the strong P strain Harwich at room temperature for 1–2 hr (Fig. 3a). The females were then transferred to fresh vials at the assay temperature (b) and transferred again to fresh vials at the appearance of L1 larvae. F1 females were classified into control and experimental groups (c) on the basis of the presence or absence of the Dichaete marked balancer chromosome. They were then aged at room temperature for 2 d (d) and dissected to reveal their ovarian phenotype(s). Flies with either unilateral or bilateral dysgenic ovaries were classified as ‘dysgenic’. Only flies with two phenotypically normal ovaries were classified as ‘normal’.

(v) Construction of recombinant lines

Lines carrying third chromosomes recombinant for the Loua third and the multiply recessively marked ruca third chromosomes were constructed as illustrated in Fig. 4. A single Loua female was crossed to a male of the ruca strain (a). The female progeny were then backcrossed to ruca, to reveal the presence of recombinant third chromosomes (b). Males were selected on the absence of one to five colinear markers of the ruca chromosome, and were crossed to the balancer strain C23a (c). The selected male progeny of this cross may carry the recombinant third chromosome in a balanced heterozygous state. These males are crossed to both C23a and ruca females (d) to complete the extraction and confirm the presence of
(a) \[ C23a-Pm/Cy; Sh/D \times 2538-Birm2 ; ry^{506} \]
Select \( Pm; D \)

(b) \[ C23a-Pm/Cy; Sh/D \times Selected Pm; D \]
Select \( Pm; Sh/D \) and \( D \)

(c) Selected \( Pm; Sh/D \times Selected Pm; Sh/D \)
Maintain stock by selection on \( Pm; Sh/D \) phenotype

**Assay cross**

(d) \( L_3 \) ext. line \( Pm/Cy; D \times Birm2 ext. stock \( Pm; Sh/D \)
Select \( F_1 Pm; D \)

(e) Selected \( Pm; D \times 1978 w \times ry^{506} \); \( Sh \)
\[ (fry + P.A2-3(99B))/TM6 \]
Score \( F_1 \) phenotypes (including pupal lethality)

**NB:** TM6 is a balancer chromosome marked with the Tubby (\( Tb \))
dominant mutation

Fig. 5. Crossing scheme for the construction of ammunition and test genotypes for the \( Loua3/99B/Birm2 \) interaction and assay cross.

The recombinant chromosome, respectively. Once established (e), these lines can be maintained by selection on the three dominant markers \( Pm/Cy; D \).

These lines were assayed for repression of gonadal dysgenesis in the same way as the \( Loua3 \) extraction lines.

(vi) \( 99B/Birm2 \) assay

The Birmingham second chromosome, which carries approximately 20 deleted \( P \) elements, was extracted from the 2538 stock as illustrated in Fig. 5a–c. This line, maintained by selection for the \( Pm; Sh/D \) phenotype, was crossed to four \( Loua3 \) extraction lines, and female flies carrying \( Loua3 \) and \( Birm2 \) over balancers selected (d). These were crossed to the transposase source strain 1798 (e). The \( F_1 \) were scored for their inheritance of the variously dominantly marked chromosomes and their expression of 99B-induced late pupal lethality. All the dominant phenotypes can be scored, even if eclosion fails (the \( Dichaete \) mutation can be scored by virtue of the associated deletion of the supra-alar bristles).

\( Sh \) and \( D/\overline{Sh} \) phenotypes are classified as experiment and control respectively, since they reflect the level of late pupal lethality in the presence and absence of \( Loua3 \), when the \( Birm2 \) and 99B chromosomes are combined.

(vii) In situ hybridization

In situ hybridizations to the polytene chromosomes of salivary glands from larvae of the \( Loua \) strain were carried out following Ashburner (1989). The probe was the pT125.1 plasmid, bearing a complete \( P \) element (O’Hare & Rubin, 1983) linearized by \( BanHI \) digestion. This was labelled, using nick translation, with digoxygenin-labelled uridine triphosphate following the manufacturer’s instructions (Boehringer). Visualization was through use of an anti-digoxygenin antibody conjugated to alkaline phosphatase; the substrate used for this was nitroblue tetrazolium and X-phosphate, yielding a dark blue precipitate at chromosomal sites labelled with digoxygenin (Boehringer DNA Labelling and Detection Kit, cat. no. 1093 657).

(viii) Southern blotting and hybridization

Genomic DNA was extracted from 20–30 adult flies using the Nucleon Phytopure kit (Scotlab), with an additional phenol/chloroform extraction. Five to ten micrograms of genomic DNA was digested overnight using appropriate restriction buffers and enzymes (Gibco BRL). Digested DNA was separated by electrophoresis on 0.6–0.8 % agarose gels, and transferred by capillary blotting to Magna nylon membrane (MSI). Transferred DNA was fixed to the membrane by UV crosslinking. \( P \) element probes were made from the pT125.1 clone by polymerase chain reaction (PCR) amplification using the primers JB6 and JB7. These primers bind 20 bp inside the \( P \) element termini, and yield a PCR product containing an almost complete \( P \) element sequence. The purified PCR product (Qiagüick–Qiagen) was labelled with \( ^{32}P \)dUTP by random priming. Blots were hybridized with the radiolabelled \( P \) element and molecular weight marker probes overnight in 7 % SDS, 0.5 M sodium dihydrogen phosphate, 0.1 mM- EDTA at 65 °C. Filters were washed in \( 0.1 \times \) SSC, 0.05 % SDS and were exposed for up to 4 d to a phosphorimager plate (Molecular Dynamics).

3. Results

(i) The \( Loua \) third chromosome represses \( sn^w \) destabilization

The results of the \( sn^w \) destabilization assay are reported in Table 1. Despite the presence of the major autosomes from a strong \( P \) strain (Harwich), an anomalously low rate of destabilization was observed in the control crosses (where pure \( M \) strain chromosomes are tested). This appears to be a function of the particular \( sn^w \) stock used (unpublished data) and produces some difficulties in the data analysis. The many families with no revertants give a large number of ties when non-parametric rank-based tests such as Mann–Whitney’s \( U \)-test are applied. These analyses lack power in this situation. As a result, the method of Engels (1979) was adopted, which uses parametric statistics incorporating the clustering of reversion events within a germline lineage. This analysis involves
Table 1. Effect of the Loua major autosomes on sn\(^{w}\) destabilization

<table>
<thead>
<tr>
<th>Strain</th>
<th>Chromosome (X)</th>
<th>Family no.</th>
<th>sn(^{w}) no.</th>
<th>Rev. no.</th>
<th>Stat.</th>
<th>U/W</th>
<th>t</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loua L (2)</td>
<td>17</td>
<td>245</td>
<td>4</td>
<td>U</td>
<td>0.1568</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loua L (3)</td>
<td>25</td>
<td>416</td>
<td>5</td>
<td>W</td>
<td>3.346</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loua L (2+3)</td>
<td>14</td>
<td>165</td>
<td>5</td>
<td>W</td>
<td>0.6643</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>74</td>
<td>986</td>
<td>37</td>
<td>W</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reported are the results of t-tests comparing the destabilization rate when either or both of the Loua major autosomes are present, and when they are absent. The column ‘Stat. U/W’ refers to the Unweighted or Weighted statistics used in calculating the modified t-statistic as suggested by Engels (1979). The significance levels refer to one-tailed t-tests between the control rate and reversion rate when Loua autosomes are present (*5%, **1%, ***0.1%).

Table 2. Ovarian dysgenesis in Loua3 extraction lines at 30 °C

<table>
<thead>
<tr>
<th>Line no.</th>
<th>Normal</th>
<th>Dysgenic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3</td>
<td>99</td>
<td>67</td>
<td>(1 \times 10^{-15}***)</td>
</tr>
<tr>
<td>4, 5, 6, 7</td>
<td>153</td>
<td>54</td>
<td>(1 \times 10^{-15}***)</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>290</td>
<td></td>
</tr>
</tbody>
</table>

Reported are the results of comparing the pooled rates of ovarian dysgenesis across Loua3 extraction lines with the pooled control rate, at 30 °C. The P value is the result of a one-tailed Fisher’s exact test, where the null hypothesis is that the presence of Loua3 does not reduce ovarian dysgenesis.

(iii) Temperature dependence

Six of the seven extraction lines (line no. 2 was lost) were assayed for repression of ovarian dysgenesis at

Table 3. Ovarian dysgenesis in Loua3 extraction lines at 29 °C and 31 °C

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Line no.</th>
<th>Normal</th>
<th>Dysgenic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 °C</td>
<td>1, 3, 4, 5, 6, 7</td>
<td>142</td>
<td>249</td>
<td>(1 \times 10^{-15}***)</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 °C</td>
<td>1, 3, 4, 5, 6, 7</td>
<td>100</td>
<td>3</td>
<td>(1 \times 10^{-15}***)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reported are the results of comparing the pooled rates of ovarian dysgenesis across Loua3 extraction lines with the pooled control rate, at two rearing temperatures. The P values are the result of a one-tailed Fisher’s exact test, where the null hypothesis is that the presence of Loua3 does not reduce ovarian dysgenesis.
29 and 31 °C. In these datasets all six lines were pooled since none showed significantly different rates of ovarian dysgenesis (two-tailed Fisher’s exact test). The data for each temperature treatment are shown in Table 3. In both cases the extracted lines significantly repress ovarian dysgenesis compared with the control rate (one-tailed Fisher’s exact test). Comparing rates of dysgenesis in experimental lines across temperatures showed that all differences between temperature treatments were significant at the 1% level (two-tailed Fisher’s exact tests). The trend is of reduced ovarian dysgenesis as temperature increases (64% dysgenic at 29 °C, 32% at 30 °C, and 3% at 31 °C).

(iv) Partitioning the Loua3 effect

Sixteen independent recombinant lines were generated with colinear arrays of selected third chromosome markers. These marker mutations (from the ruca marker chromosome) allow the inference of the section of Loua3 carried by a particular recombinant line, as illustrated in Fig. 6. Table 4 shows the results of ovarian dysgenesis assays for all 16 lines. The P values reported are for two-tailed Fisher’s exact tests comparing each line’s rate of ovarian dysgenesis with the control rate and also with the rate of ovarian dysgenesis associated with the intact chromosome.
Table 4. Rate of ovarian dysgenesis for 16 recombinant lines

<table>
<thead>
<tr>
<th>Line no.</th>
<th>Normal</th>
<th>Dysgenic</th>
<th>Line v. Loua3 (total)</th>
<th>Line v. control (total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>60</td>
<td>$1.563 \times 10^{-3}$</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>7</td>
<td>$7.096 \times 10^{-3}$</td>
<td>1.0</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>43</td>
<td>$1.563 \times 10^{-3}$</td>
<td>NS</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>360</td>
<td>0.0 (15 d.p.)</td>
<td>0.5665</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>12</td>
<td>$1.453 \times 10^{-2}$</td>
<td>NS</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>95</td>
<td>0.0 (15 d.p.)</td>
<td>NS</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>136</td>
<td>0.0 (15 d.p.)</td>
<td>0.3587</td>
</tr>
<tr>
<td>29</td>
<td>4</td>
<td>62</td>
<td>0.0 (15 d.p.)</td>
<td>0.5963</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>35</td>
<td>$2.651 \times 10^{-3}$</td>
<td>0.7286</td>
</tr>
<tr>
<td>33</td>
<td>6</td>
<td>255</td>
<td>0.0 (15 d.p.)</td>
<td>8.581 $\times 10^{-2}$</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>6</td>
<td>$2.604 \times 10^{-2}$</td>
<td>NS</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>10</td>
<td>$1.192 \times 10^{-2}$</td>
<td>1.0</td>
</tr>
<tr>
<td>102</td>
<td>1</td>
<td>6</td>
<td>$4.525 \times 10^{-2}$</td>
<td>0.9844</td>
</tr>
<tr>
<td>103</td>
<td>0</td>
<td>35</td>
<td>$1.563 \times 10^{-2}$</td>
<td>1.0</td>
</tr>
<tr>
<td>108</td>
<td>0</td>
<td>5</td>
<td>$6.115 \times 10^{-2}$</td>
<td>NS</td>
</tr>
<tr>
<td>112</td>
<td>0</td>
<td>8</td>
<td>$3.205 \times 10^{-2}$</td>
<td>NS</td>
</tr>
</tbody>
</table>

Loua3 (total) 276 196
Control (total) 2 773

Reported are the results of comparing each recombinant chromosome’s rate of ovarian dysgenesis with that of the intact chromosome, as well as with the control rate. $P$ values refer to two-tailed Fisher’s exact tests where the null hypothesis is that there is no difference between the two rates of ovarian dysgenesis tested.

None of the lines showed a rate of ovarian dysgenesis significantly different from the control rate. This suggests that none of the recombinant lines carries a locus responsible for the repressive effect of the intact chromosome.

(v) Loua3 suppresses somatic lethality associated with $\Delta 2-3 (99B)$

Three Loua3 extraction lines (1, 3, 7) were used to construct female flies carrying the Loua3 and Birm2 chromosomes in a heterozygous state, over balancers (Fig. 5). These flies were crossed to males of the 99B-carrying stock. 1798. Eclosed F1, were scored for the presence of the dominant markers Plum, Dichaete, Stubble and Tubby. F1 that failed to eclose were dissected from the pupal case and also scored for these markers. Pupal lethality was only observed in the D/Sb class, where 32 of 34 flies died as pupae. This class, and the Sb class, assay the effects of 99B and Birm2 in the absence and presence of the Loua3 chromosome, respectively. There were no dead pupae among 63 flies in the Sb class – a result significantly different from the above at the 0.01 % level (two-tailed Fisher’s exact test). The presence of the Loua3 chromosome eliminates pupal lethality associated with the 99B/Birm2 system.

(vi) The Loua3 chromosome has 17 P elements, none of which is full length

In situ hybridization to the Loua strain revealed that the third chromosome has sites of hybridization at 61C, 62B, 63B, 67E, 87D, 90E, 91B, 92C, 93B, 95F,
96F and 98C. Fig. 7a shows 17 bands of P hybridization from the extracted *Loua*3 chromosome in DNAs digested with *BamHI* (which does not cut within the P element), suggesting 17 P elements. Note that the control P strain *Harwich* has many more hybridizing fragments, and the M’ strain 3560, which has all P homology in a single long *BamHI* fragment bearing the snw allele, shows a single labelled fragment. Internal digestion with *DdeI* (Fig. 7b) shows approximately 14 bands of hybridization, indicating the number of different deletion derivatives present among the 17 elements. (Some bands may represent P elements that have lost a *DdeI* site, creating a fragment running from the remaining internal site to one in flanking genomic DNA.) The discrepancy between 12 sites in the *in situ* experiment and the 17 seen in the Southern may be because of sites in heterochromatin, visible only in the latter experiment, or possibly because more than one P element is located in the same chromosomal band. The 2-17 kb fragment expected from a full-length P element, and visible in the P strains in lanes 1 and 2, is not seen. However, the
strong band of hybridization at 0.42 kb is of the size expected from KP elements. The sn" strain 3560 shows two DdeI fragments, as expected.

4. Discussion

(i) The entire Loua3 chromosome appears to be required for repression

The third chromosome of the Loua strain was initially noted as a weak zygotic repressor of sn" destabilization, in a screen of recently derived wild-type stocks for such activities. At 30 °C, ovarian dysgenesis induced by the Harwich haplotype is reduced from 99.3 to 32% by the Loua3 chromosome. This effect is temperature-sensitive, showing 64% ovarian dysgenesis at 29 °C and 3% at 31 °C. The strength of the repressive effect prompted attempts to localize it to a recombinant region of the chromosome, but all regions failed, individually, to show the repressive effect. The chromosome also represses the late pupal somatic lethality associated with the 99B/Birm2 system.

The failure of the panel of recombinant chromosomes to localize the repressive effect, despite coverage of the whole chromosome (Fig. 6), indicates that there is not a single contiguous fraction of the intact chromosome that, in isolation, can repress ovarian dysgenesis. This is tested using a likelihood ratio approach. We calculate the probability of observing the dataset under a pair of alternative models. These are at map position x, there is a repressor locus with the effect of the intact chromosome, or alternatively, that there is no such locus at x (or anywhere else on Loua3). For all values of x we can assess the relative probabilities, under the two models, of the observed dataset. The likelihood ratio is plotted against map position of x in Fig. 8. For all 110 map units of Loua3, the likelihood ratio is negative, indicating that for the entire chromosome there is evidence against the hypothesis that there is such a repressor locus at this map position. This implies that at least two loci are responsible for the Loua3 effect, and that these were not combined in any chromosome in the recombinant panel.

There is a continuum of models to explain the apparent requirement for the whole chromosome for repression. The simplest model is of a pair of repressor loci that are ineffective in isolation but act synergistically, when combined, to produce effective repression. These loci are sufficiently physically distant that they were not combined in any of the recombinant lines constructed. This hypothesis could be tested by recombining pairs of recombinant lines, and assaying for the restoration of repression.

Other explanations involve many repressor loci, distributed across the intact chromosome, with individually small effects, but which repress when combined. Some P-containing strains that repress P mobilization contain high copy numbers of particular P deletion derivatives. The KP element, apparently present in Loua3, was first described as a repressor of P transposition, which acted in a copy number-dependent manner (Black et al., 1987). Studies (Andrews & Gloor, 1995) of the effect of constructs overexpressing the KP transcript in transformed flies have shown that mutation of the leucine zipper motif of KP polypeptide, which is implicated in transposase oligomerization, removes the ability of KP constructs to repress transposition. This result, if not simply due to a destabilizing of the polypeptide, is consistent with repression either through a protein–protein interaction between the KP product and the transposase polypeptide, or through a KP protein–DNA interaction requiring prior KP dimerization. Either way, it is possible that the relationship between the level of KP polypeptide production and strength of repression is non-linear, giving a synergism between multiple repressing loci.

![Fig. 8. Likelihood ratios for the unitary repressor hypothesis. The graph plots the natural logarithm of the likelihood ratio against the 110 map units of the third chromosome. The likelihood ratio compares the probability of observing the dataset under the models: (i) that a repressor locus with the full effect of the whole chromosome is present at a given map unit or (ii) that such a locus is absent (from the whole chromosome). The troughs in the plot coincide with the eight recessive markers of the rueca chromosome (th and st occupy a single trough).](https://doi.org/10.1017/S0016672397003066 Published online by Cambridge University Press)
(ii) Temperature dependence

The repression of ovarian dysgenesis by the intact *Lousa* third chromosome shows strong temperature dependence. All groups of extraction lines show rates of ovarian dysgenesis that are significantly different from the associated control rate. It is formally possible that the apparent temperature dependence could be due to a reduction in transposase activity with increasing temperature. However, Robertson *et al.* (1988) showed that the 99B transposase source is more active at higher temperatures (assayed by snr destabilization). Since there is no reason, *a priori*, to expect the transcription of this insertion to increase with temperature, it is probably increased activity of the transposase protein that is the cause of this observation.

Why should repressive activity be correlated with temperature? A consideration of the potential modes of action of repressors of transposition may be informative. Type I repressors (Gloor *et al.*, 1993) have in common the potential to encode a protein similar to the 66 kDa transcriptional repressor that is observed here. Since other assays indicate that 99B represses P element transposition, it seems most likely that repression of ovarian dysgenesis in *Drosophila melanogaster* is due to a reduction in transposase activity with increasing temperature. However, Robertson *et al.* (1988) showed that the 99B transposase source is more active at higher temperatures (assayed by snr destabilization). Since there is no reason, *a priori*, to expect the transcription of this insertion to increase with temperature, it is probably increased activity of the transposase protein that is the cause of this observation.

(iii) Abolition of somatic lethality

The *Lousa* third chromosome abolishes induction of somatic lethality by the constitutive transposase source Δ2-3(99B) in the small experiment reported above. Since other assays indicate that *Lousa* reduces the rate of P element transposition, it seems most likely that somatic lethality is avoided by a reduction in the transcription or activity of the 99B product. Again, a transcriptional interaction seems unlikely since the transcription of the Δ2-3 construct at 99B is under the control of a constitutive endogenous promoter (Robertson *et al.*, 1988). By default, since we can exclude splicing interactions, a protein–protein interaction mechanism seems most likely.

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References


