

A particular P-element insertion is correlated to the P-induced hybrid dysgenesis repression in *Drosophila melanogaster*

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Summary

Transposable P elements in *Drosophila melanogaster* cause hybrid dysgenesis if their mobility is not repressed. The ability to regulate the dysgenic activity of the P elements depends on several mechanisms, one of which hypothesized that a particular deleted P element (the KP element) results in a non-susceptibility which is biparentally transmitted. In this study totally non-susceptible lines, and susceptible lines containing exclusively KP elements (IINS2 line and IIS2 line) were isolated from a M' strain. We show that non-susceptibility is correlated with a particular insertion of one KP element located at the cytological site 47D1. The repression ability of the GD sterility is determined by a recessive chromosomal factor, and cannot be due to the KP-element number. Here the repression of the P mobility is associated with reduction of the P transcripts and the inhibition of P promoter activity.

1. Introduction

P–M hybrid dysgenesis in *Drosophila melanogaster* is a syndrome of germline abnormalities associated with mobilization of the P family of transposable elements (reviewed by Engels, 1989). Populations and strains of *Drosophila* may be characterized on the basis of two properties related to the phenotypic effects of their P elements. One, the P-activity potential, is the ability to mobilize P elements when these are in an unregulated state; the other, called P susceptibility, is the ability to regulate or suppress the dysgenic activity of the P elements and includes the joint action of all mechanisms affecting P-element regulation. P strains manifest a P-activity potential and no P susceptibility; these strains contain both autonomous P elements and internally deleted P elements but transposition and excision are suppressed. M strains do not contain any P elements and manifest total P susceptibility. A third kind of strain, the M' strains, do not manifest any P activity potential but contain a variable number of P elements, many, if not all, of which are defective. These M' strains present an intermediate state of P susceptibility and either totally susceptible or totally non-susceptible lines can be isolated from them (Anxolabéhère *et al.* 1982).

Dysgenesis is due to the mobilization of the P

element in the germ cells of the progeny from the crosses between the females from M (or M') strains and males from P strains, but not in the reciprocal crosses.

The non-susceptibility (P-element repression) in P strains is a property different to the non-susceptibility occurring in the M' strains (Rio, 1990). In P strain, this property is defined by Engels (1979*a*) as P cytotype, and has been shown to depend on both genomic P elements and maternal inheritance; on the contrary, in M' strains it can be passed on by both males and females. Recently, some progress has been made to elucidate the mechanisms of suppression of P transposition. Genetic and molecular evidence has been presented to support three models. First, P cytotype can be conferred by defective P elements that have retained intact the expression of their first three exons and code a putative truncated transposase (Nitasaka *et al.* 1987). Robertson & Engels (1989) have produced genetic evidence showing that a truncated form of the transposase may mediate repression. Meanwhile Simmons & Bucholz (1985) have shown that autonomous and non-autonomous elements may compete for the transposase, which they refer to as transposase titration. A third possibility is that products generated by particular internally deleted P elements poison transposase function. One of these particular deleted P elements has been described by Black *et al.* (1987) and called KP element

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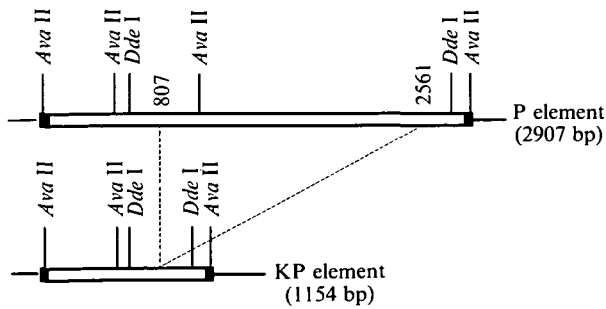


Fig. 1. The autonomous P element and a particular deleted P element: the KP element.

(Fig. 1), it has been found to confer some protection against the deleterious effects of P-element mobilization (Jackson *et al.* 1988). As many copies of this element are found principally in *M'* strains, this protection could be dependent on the maternal and paternal inheritance of the KP copy number (Jackson *et al.* 1988). P-element regulation could involve a patchwork of these mechanisms and others with different mutual importance in the diverse types of strains.

We report here an additional mechanism which suppresses P susceptibility in lines exclusively bearing KP elements located on the second chromosome. This is due to the repression of P-element transcription and is associated with a single KP element located at the cytological site 47D1.

2. Materials and methods

(i) Strains

Canton-S and *Harwich* strains were used as typical M and P strains respectively (Kidwell *et al.* 1977).

CyO, *Adh^{nB}*, *pr*, *cn*; *TM₃Sb*, *Ser*, *ry^K*/*T(2, 3) ap^{Xa}*: balancer stock of chromosomes II and III and called 190 (Deutsch, personal communication), devoid of any P element.

CyO, *Adh^{nB}*, *pr*, *cn*/*T(2, 3) ap^{Xa}*: balancer stock of chromosome II and derived from 190 stock, devoid of any P element.

Tm₃Sb, *Ser*, *ry^K*/*T(2, 3) ap^{Xa}*: balancer stock of chromosome III and derived from 190 stock, devoid of any P element.

Muller-5 balancer stock of chromosome I, devoid of any P element.

M5/sn^w; π^2 is a P strain with the genetic background of π^2 (a strong P reference strain). This strain has both the *Muller-5* (M5) balancer chromosome and an X chromosome with the P-element-insertion mutation *singed-weak* (*sn^w* allele) (Engels, 1979*b*; Engels, 1984; Roiha *et al.* 1988), which becomes unstable in the presence of the P transposase, with reversion towards either more extreme phenotype (*sn^e* allele) or to the wild type (*sn⁽⁺⁾* allele). However, in this stock, the *sn^w* mutation is stabilized by the P cytotypic.

w sn³, an M stock homologous for two recessive X-linked markers, including an extreme allele of the

singed (*sn³*) locus. The dominance relations of the *singed* alleles are $sn^+ > sn^w > sn^e = sn^3$ (Simmons, 1987).

BQ16, BC69, BA37 and R20A: four lines harbouring an insertion of a P-*lacZ* fusion gene: P[*lac*, *ry⁺*]A. This construct contains an in-frame translational fusion of the *E. coli* β -galactosidase gene (*lacZ*) to the second exon of the P transposase gene and the *rosy⁺* gene as a marker for transformation (O'Kane & Ghering, 1987). The P[*lac*, *ry⁺*]A insertions are located on the third chromosome for BQ16 and on the second chromosome for BC69, their *lacZ* activity is germ-line specific. The insertion is located on the second chromosome for BA37 and on the third chromosome for R20A, the *lacZ* activity is somatic tissue specific, in follicular cells of ovaries for BA37 (Couderc, personal communication) and in all somatic tissues at all stages for R20A (Lemaître & Coen, 1991).

(ii) Gonadal dysgenesis sterility

The diagnostic tests used were the standard tests for measuring gonadal (GD) sterility potential (Kidwell, 1983). *A** diagnostic cross: 5–15 females of each line or subline were mated *en masse* with males of a strong P strain (*Harwich*) and allowed to grow for 4–5 days at 29 °C; the laying flies were then discarded. Approximately 3 days after the onset of eclosion, F1 progeny were transferred to fresh culture and allowed to mature for 2 days at 25 °C. More than 30 F1 daughters were dissected and ovaries scored as unilateral (S1 type) or bilateral (S0 type) dysgenic ovaries (Schaefer *et al.* 1979). The frequency of dysgenic ovaries was calculated as:

$$\% \text{ GD} = \% \text{ SO} + \frac{1}{2} \% \text{ S1}.$$

A diagnostic cross: by the same way, GD sterility was estimated on F1 females from *Canton-S* females mated with males under test.

(iii) Suppression of hypermutability at the *singed* locus

Twenty to thirty tested females were crossed *en masse* to *sn^w* males, from *M5/sn^w*; π^2 strain, at 20 °C. Fifty F1 progeny virgin females were crossed *en masse* to 50 *w sn³* males at 25 °C and were allowed to lay eggs for 3 days. Only *sn^e* and *sn^w* phenotypes were scored in the F2 progeny of both sexes. Because it is impossible to distinguish *sn⁺* from the wild strain tested and *sn⁽⁺⁾* revertant, the hypermutability percentage was calculated as follows:

$$\% = (sn^e / (sn^e + sn^w)) \times 100.$$

(iv) DNA and RNA blot hybridization

Genomic DNA was extracted using the method described by Junakovic *et al.* (1984). Restriction

enzyme digestion of DNA was performed according to supplier's instructions. After gel electrophoresis, transfers were carried out on nylon membranes (Biodyne, Pall) which were subjected to hybridization. Filters were prehybridized 2 h at 65 °C in solution containing 5× Denhart, 5× SSPE (20× SSPE is 3.6 M-NaCl, 0.2 M-Na phosphate, pH 8.3, 0.02 M-EDTA), 0.2% SDS and 500 µg ml⁻¹ of denatured salmon sperm DNA. Filters were then hybridized over night at 65 °C, in the same solution, to which a DNA probe labelled with ³²P by nick translation had been added. Two post-hybridization washes of 30 min each were carried out in 5 mM-Na phosphate, pH 7, 1 mM-EDTA, 0.2% SDS.

Total RNA was phenol extracted from adult females and poly(A)⁺ RNA was purified by chromatography through an oligo(dT) column as described by Maniatis *et al.* (1982). Electrophoresis was carried out on 1% agarose gels containing formaldehyde (final concentration of 2.2 M formaldehyde) and transferred on nitrocellulose membrane. Hybridization and washing were performed under conditions recommended by the supplier (Schleicher & Schuell).

(v) In-situ hybridization

Larval salivary gland chromosomes were prepared by the method described by Pardue & Gall (1975), revised by Strobel *et al.* (1979). The number and location of P elements in the genomes were estimated using p π 25.1 [³H]DNA (O'Hare & Rubin, 1983) prepared by nick translation as probe. Particular KP element was located using biotinylated p3ICA as probe.

(vi) Cloning of the insertion site

The pUC19 BamH I-digested DNA library of the non-susceptible selected lines was constructed by the method of Hanahan (1983), and screening was done according to Maniatis *et al.* (1982) using the Hind III fragment of p π 25.7bwc as probe.

(vii) Quantitative measurement of β -galactosidase activity

Using BQ16, BC69 and BA37 P-lacZ strains, approximately three ovaries were homogenized in Z buffer (Miller, 1972) and centrifuged for 10 min at 10000 rpm (4 °C) to remove debris. β -galactosidase activity was measured as described by Miller (1972). The protein concentration was determined in each sample by the BioRad protein assay, using BSA as a standard. Results are given in nmol min⁻¹ mg⁻¹ protein. Using R20A P-lacZ strain, 3 larvae at third instar level were homogenized in Z buffer, then the same procedure as above was performed.

3. Results

(i) Characteristics of the ICA strain

The ICA strain is a long established laboratory strain (collected in 1959 in Peru). It must be classified as a M' type with an intermediate level of P susceptibility (70% of GD sterility measured by the A* diagnostic cross, and 0% of GD sterility measured by the A diagnostic cross), and bearing 13–15 sequences homologous to the P element, with an insertion site polymorphism detected by *in situ* hybridization. The genomic DNA analysis performed on this strain reveals, almost exclusively, the expected *Ava* II and *Dde* I restriction fragments characteristic of the KP element (data not shown), moreover, no P element of this strain hybridizes with the *Hind* III–*Sal* I restriction fragment of the complete P element (overlapping a part of exon1, exon2 and a part of exon3).

(ii) Selection on the P susceptibility in the ICA strain

To analyse the variability of P susceptibility in this strain, 29 isofemale lines were made, and their P susceptibility measured. Isofemale lines repressing P-induced sterility are found as well as isofemale lines with P susceptibility (Fig. 2a), but they do not differ in regard to their mean copy number of P elements. Therefore determinant of P susceptibility other than the number of P elements (Jackson *et al.* 1988) has been investigated using a bidirectional selection method applied to these two kinds of isofemale lines.

From one isofemale line repressing the P-induced sterility (I16; 0.5% of GD sterility measured by A* diagnostic cross), 30 sublines were initiated, each from a single-pair mating. At the following generation the P susceptibility of each subline was measured by the A* diagnostic cross and two sublines, one with the highest value of GD sterility and one with the lowest value were selected. Each was subdivided into 15–30 new sublines in order to initiate the two ways of selection (I16-H and I16-L). Selection was achieved in subsequent generations by choosing, in each way, the most extreme subline among the tested sublines to produce 15–30 new sublines. From one isofemale line with a high P susceptibility (I23; 99% of GD sterility by A* diagnostic cross) the same bidirectional selection was performed towards high and low P susceptibility (I23H and I23L, respectively). The selection procedure was maintained during five generations. A directional selection towards high P susceptibility was also applied on another P-susceptible isofemale line: I24; 98% of GD sterility by A* diagnostic cross (data not shown).

The results are shown in Fig. 2b. High and low P-susceptible selected sublines were obtained with the I23 isofemale line, revealing the occurrence of a polymorphism in P-susceptible determinants in this line. The characteristics of all selected sublines with regard to their P susceptibility was unchanged five

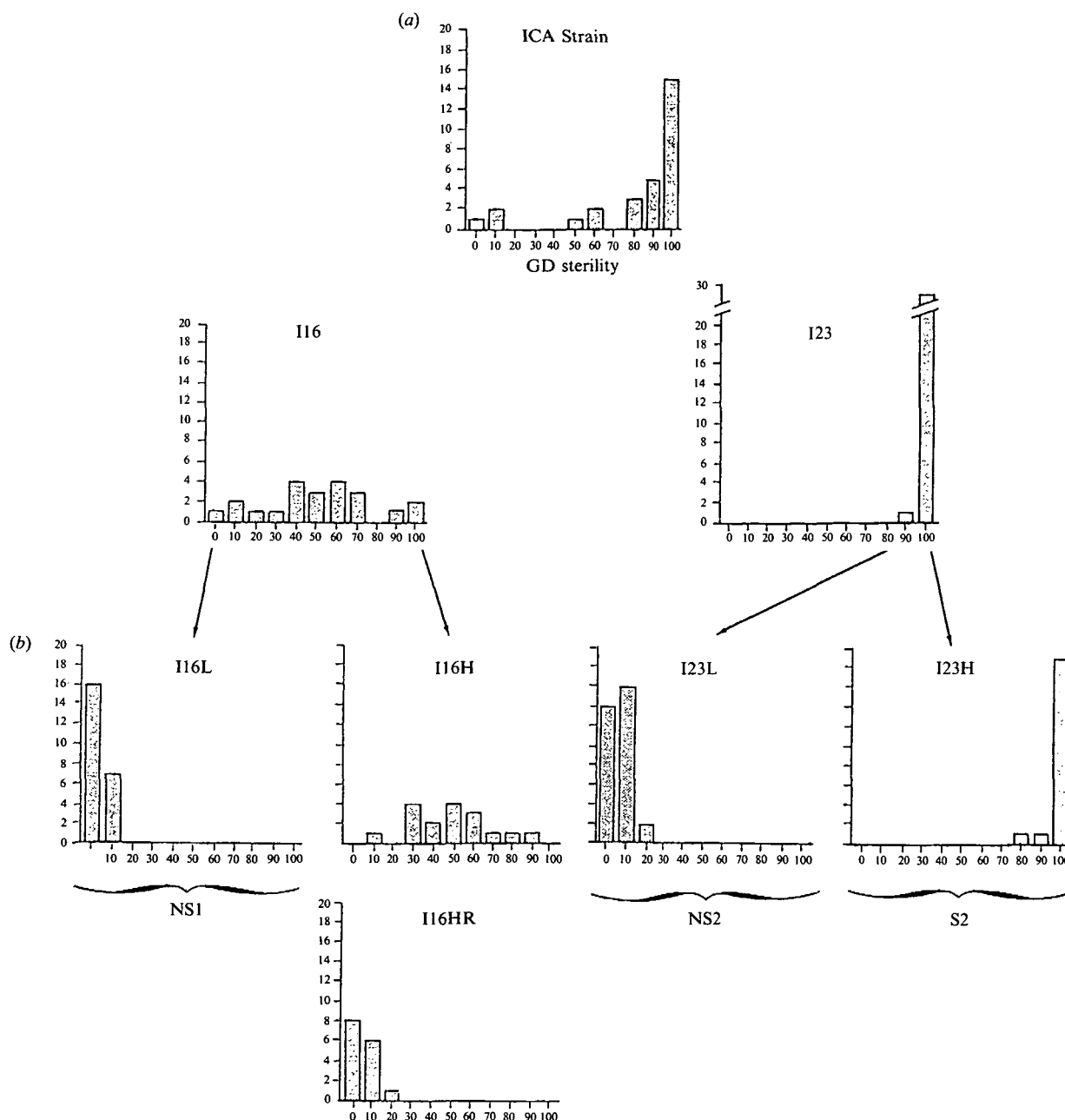


Fig. 2. P-susceptibility selection (a) Distribution (in number of tested lines) of GD sterility in A* diagnostic cross in 29 isofemale lines from the ICA strain. Fifteen to twenty females from each line were tested. One line with high level of GD sterility potential (I23) was used to perform a bidirectional selection towards high or low P-susceptibility. A bidirectional selection was also applied on a line with low level of GD sterility (I16). (b) Distribution (in number of tested sublines) of the P susceptibility after 5 generations of bidirectional selection. Five females from each subline were tested. The selection was successfully performed, except towards high P susceptibility with the I16 isofemale line (I16H sublines). Indeed, after one generation of relaxation of the selection, these last sublines regain a low P susceptibility (I16HR sublines).

generations after the end of the selection. The rapidity of the selection and the stability argue in favour of a small number of determinants, probably one (see below). The same stability was observed with I24 sublines selected towards the high P susceptibility (I24H). The selection applied on I16 isofemale line led to low P-susceptible sublines, but did not allow complete isolation of high P-susceptible sublines (I16H sublines, Fig. 2b). In fact, as soon as the selection was relaxed these selected sublines regained a repressing P-induced sterility state, like that expected when the

variability is not due to additive genetic variance (Fig. 2b, I16HR).

These results suggest that the I23 isofemale line is polymorphic for determinants repressing the P-induced sterility, while the I16 isofemale line is monomorphic for such determinants.

After five generations of relaxation, the selected sublines from each selection method were pooled to initiate susceptible and non-susceptible lines: the I16L sublines and I23L sublines gave the non-susceptible lines hereafter called NS1 and NS2 respectively; the

Table 1. *Hypermutable regulation test at the singed-weak locus (the scores of males and females are pooled), hypermutability percentage was calculated as $sn^e / (sn^e + sn^w) \times 100$*

Line under test	sn^e	sn^w	%
Harwich	5	2090	0.24
Canton-S	62	627	9.00
NS1	0	416	0.00
NS2	1	498	0.20
S2	34	361	8.61
S3	37	508	6.79

Table 2. *Hereditary transmission of P-susceptibility regulation. Given values are GD sterility measured on F1 females from A* diagnostic cross. The A* diagnostic cross was performed on female progeny of both reciprocal crosses between the lines under test (T) and a M reference strain: Canton-S (C-S). Number of dissected females in parentheses*

Line under test	Original cross	
	♀T × ♂C-S	♀C-S × ♂T
Harwich	20.8 (120)	46.5 (120)
NS1	100.0 (60)	100.0 (150)
NS2	100.0 (150)	100.0 (120)
S2	100.0 (90)	100.0 (60)
S3	100.0 (60)	100.0 (90)

I23H sublines and I24H sublines gave the susceptible lines called S2 and S3 respectively.

(iii) *Genetic analysis of the susceptible and non-susceptible lines*

Table 1 presents the results of the hypermutability assay at the *singed* locus. The repression of the P transposase activity measured by the suppression of hypermutability at the *singed* locus occurs in the NS1 and NS2 lines (defined as non-susceptible lines according to the GD sterility criterion) at the same level as the control test with the strong P strain, Harwich (Table 1). Conversely, the susceptible lines (S2 and S3), like the M reference strain, Canton-S, do not repress the P transposase activity. With respect to GD sterility and hypermutability tests, the non-susceptible and susceptible lines present the same characteristics as the P and M reference strains, respectively.

In order to analyse if the repression in the non-susceptible lines is due to P cytotypic, the hereditary transmission of P-susceptibility was measured by A*

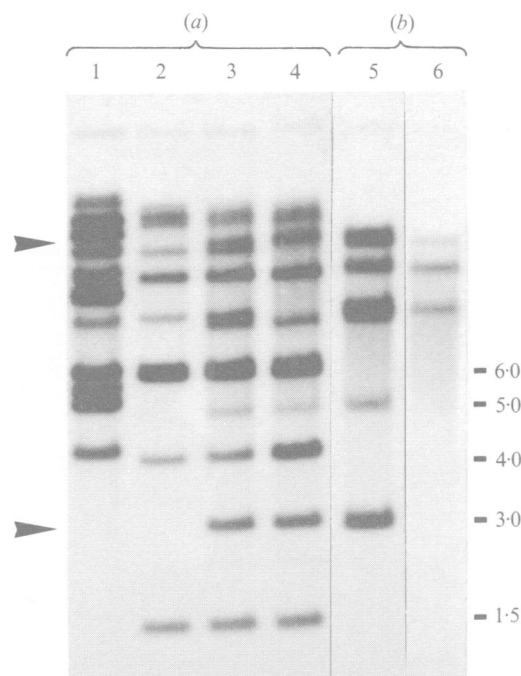


Fig. 3. Comparisons of insertion sites. Southern blot on genomic DNA digested by *Bam*H I endonuclease (no cut inside P element) and using pπ25.7bwc (O'Hare, personal communication) as probe. (a) DNA from selected lines (1) S3, (2) S2, (3) NS2 and (4) NS1. Two restriction fragments discriminate non-susceptible and susceptible lines at 3 and 16 kbp. (arrows). (b) DNA from isochromosomal lines (5) IINS2, with the second chromosome from NS2 line (lane 3) and (6) IIS2, line with second chromosome from S2 line (lane 2). Only the 3 kbp restriction fragment discriminates these two isochromosomal lines: the band at 5 kbp in IINS2 is also present in S3 line (lane 1) and the first and third bands from top in both lanes are doublets in IINS2 but each band of them is present in IIS2 or S3 lines (lanes 6 and 1).

diagnostic cross on female progeny of both reciprocal crosses, performed at 25 °C, between the lines under test (susceptible and non-susceptible) and a reference M strain: Canton-S. The hereditary transmission of the P-susceptibility regulation is not the same as the normal inheritance observed with the P cytotypic which is partially maternally transmitted (Table 2). As neither type of hybrid female with Canton-S strain showed any ability to repress gonadal dysgenesis in its offspring, this suggests a system in which repression potential, in regard to GD sterility criterion, is controlled by purely recessive chromosomal factors. Thus, the control of the repression of P transposase activity in our non-susceptible lines is different from that found with the P cytotypic. The results of A* diagnostic crosses performed with F1 females from NS1 and NS2 lines indicates that these two lines belong to the same complementation group. The procedure was to cross ten females from NS2 lines with twenty males from NS1 lines. The same procedure was used for the reciprocal cross. Three repetitions of these crosses were performed. At the following generation 30 females from each cross were tested

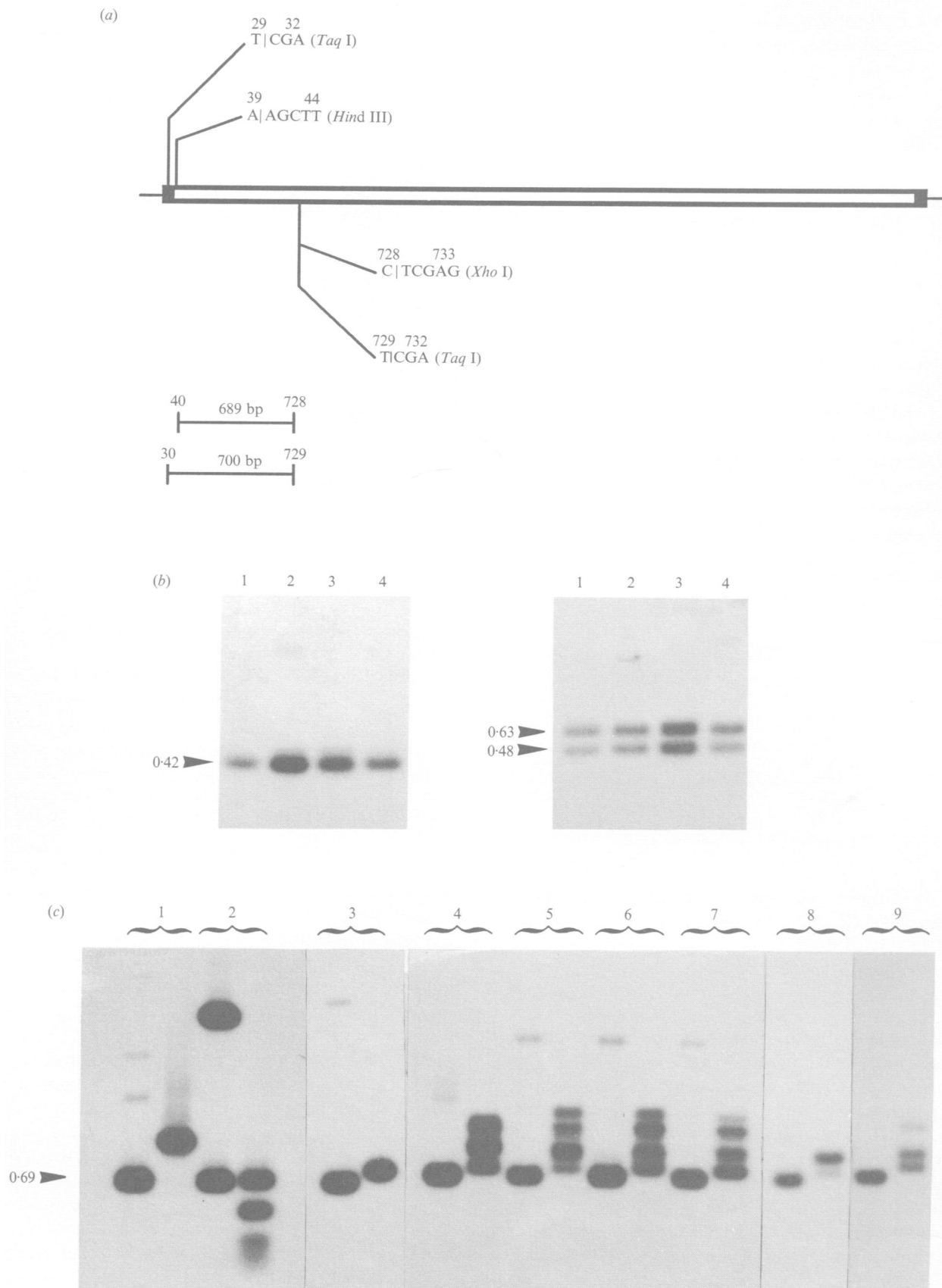


Fig. 4. The susceptible and non-susceptible lines selected from ICA strain harbour almost exclusively P elements with a nucleotide substitution between positions 29–32 and an internal deletion removing nucleotide 808–2560, as the KP element. (a) Diagram of the 5' side of the canonical P element showing the restriction sites pertinent to this analysis. A nucleotide substitution at position 32 in the P element results in the obliteration of the first *Taq I* recognition site. If this *Taq I* recognition sequence is present (as inside P element) the *Hind III/Xho I* and *Taq I/Taq I* fragments are nearly identical of size 689 and 700 pb respectively. When this *Taq I* recognition sequence is absent (as inside KP element) the

by A* diagnostic cross. The mean score on the 6 repetitions (300 dissected females) was 1.5% of GD sterility.

(iv) *Molecular analysis of susceptible and non-susceptible lines*

(a) *Number and structure of P elements.* The P copy number was estimated by Southern blot analysis performed on genomic DNAs digested with *Bam*H I endonuclease (no site inside P element) and probed with *p* π 25.7bwc (O'Hare, personal communication). The non-susceptible lines NS1 and NS2 show, respectively, 10 and 12 *Bam*H I restriction fragments hybridizing with the probe, and the susceptible lines S2 and S3, 8 and 11 (Fig. 3a). Thus the difference in P-susceptibility characteristics between the lines cannot be due to their P-element copy number but rather to the structure and/or localization of their respective P elements.

As already mentioned, the P elements present in the ICA strain are, almost exclusively, KP elements (data not shown). Therefore the P-susceptibility characteristics in the selected lines might be associated with the presence or absence of another particular P element. The existence of such a putative element has been investigated by reference to the molecular characteristics of the KP element. The KP element is derived from a 2.9 kb P factor by an internal deletion removing nucleotides 808–2560 (see Fig. 1). Consequently, specific fragments of 0.48 and 0.63 kbp in length are generated by the endonuclease *Ava* II, and of 0.42 kbp in length by the endonuclease *Dde* I. Moreover, in the KP element, the retained sequence shows complete homology to the P factor in *p* π 25.1 (O'Hare & Rubin, 1983), with the exception of a single replacement of an A nucleotide by a T at position 32 in the sequence (Black *et al.* 1987). The single base difference in the KP-element results in the obliteration of a *Taq* I recognition site. The absence of this site in all of the P elements in the lines under analysis can be monitored by the *Taq* I endonuclease assay described by Daniels *et al.* (1990) and presented in Fig. 4a. All the P elements of the selected lines show the expected fragments from the KP element when Southern blot analysis was performed on genomic DNA digested with *Ava* II, *Dde* I, *Hind* III and *Xho* I endonucleases (Fig. 4b, c), with the exception of one P element with a deletion greater than the internal deletion of the KP element and generating a heavier restriction fragment than expected with *Dde* I endonuclease. This deletion includes the *Xho* I site (at position 728) and the *Ava* II

site at position 502. As this particular element is present in S2 and NS2 line (Fig. 4b), it is not correlated with P susceptibility. The *Taq* I endonuclease assay, did not reveal the corresponding restriction site spanning base 32 either in the susceptible or the non-susceptible lines, attesting to, at least, one of the positions 29–32 is altered (Fig. 4c). In the absence of DNA sequencing it is not possible to be sure that the substitution occurs at position 32 as in the KP element. When, taken together, these results strongly suggest that there are almost exclusively KP elements, in the selected lines whatever their P-susceptibility characteristics.

(b) *Insertion site analysis.* The investigations on the correlation between P susceptibility and insertion sites have been performed using Southern blots on genomic DNA of selected lines digested with *Bam*H I endonuclease (no site inside the P sequences) and probed with *p* π 25.7bwc DNA. Two insertion sites at 3 and 16 kbp discriminate the susceptible lines S2 and S3 from the non-susceptible lines NS1 and NS2 (Fig. 3a).

In order to determine if both insertion sites are correlated with the non-susceptibility, each chromosomal pair from the NS2 and S2 lines has been isolated using the chromosomal substitution method with chromosomal balancer stocks devoid of any P sequences and described in the materials and methods section. Six isochromosomal sublines were built: two isochromosomal sublines including chromosome I from NS2 or S2 lines, other chromosomes were brought by the balancer stocks, and called INS2 and IS2, respectively. Similar isochromosomal sublines were built with chromosome II (IINS2 and IIS2 isochromosomal lines) or with chromosome III (IIINS2 and IIIS2 isochromosomal lines) from NS2 or S2 lines, respectively. The isochromosomal lines with chromosome I (INS2 and IS2) and with chromosome III (IIINS2 and IIIS2) are totally susceptible (100% of GD sterility by the A* diagnostic cross). Only the sublines including chromosome II from the NS2 line retain the non-susceptibility characteristics (0% of GD sterility in the A* diagnostic cross and less than 1% of *sn*^w destabilisation in the *sn*^w hypermutability assay). Southern blot analysis on genomic DNA from the IINS2 and IIS2 lines digested with *Bam*H I endonuclease demonstrate that the difference between susceptible and non-susceptible lines is correlated with the 3 kbp restriction fragment and not with the 16 kbp restriction fragment (Fig. 3b). Southern blot analysis on genomic DNA from the IINS2 and IIS2 lines digested with *Hind* III–*Xho* I and

Taq I/*Taq* I fragment disappears (Daniels *et al.* 1990). (b) Southern blot analysis of DNA from selected lines, digested with *Dde* I (on the left) and with *Ava* II (on the right) endonucleases. Samples are as follows: (1) S3, (2) S2, (3) NS2 and (4) NS1. (c) Southern blot analysis. Nine pairs of lanes are shown. In each pair, the lane on the left contains DNA digested with *Hind* III and *Xho* I and on the right the same DNA digested with *Taq* I. *Acc* I restriction fragment from *p* π 25.1 was used as a probe. Samples are as follows: (1) *p* π 45.1: plasmid containing the KP element (O'Hare, personal communication), (2) *p* π 25.1: plasmid containing the autonomous P element (O'Hare & Rubin, 1983), (3) p3ICA (see text), (4) S3, (5) S2, (6) NS2, (7) NS1, (8) IIS2 and (9) IINS2 (see text).

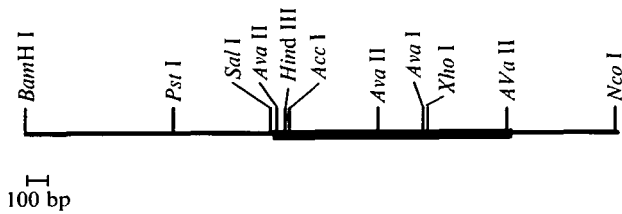


Fig. 5. Restriction map of the 3 kb *BamH I* fragment inside pUC19 and called p3ICA, the thick line corresponds to P-element insert showing the similar restriction sites that the KP element.

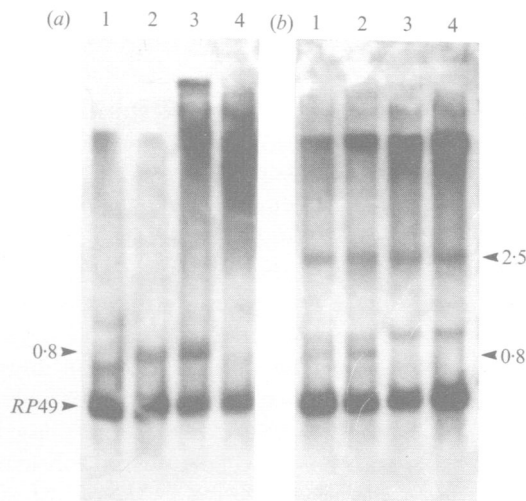


Fig. 6. Northern blot of P-element transcripts in adult females. Approximately 5 μ g of poly(A)⁺ RNA was loaded per lane and probed with the 0.84 kbp *Hind III* fragment of the P factor. To check for relative quantities of RNA on the blot, the same filter was rehybridized with RP49, a ribosomal protein cDNA (Vaslet *et al.* 1980). (a) RNA from selected adult lines: (1) NS1, (2) NS2, (3) S2 and (4) S3. The 0.8 kb KP-element transcript is observed only in the susceptible line (S2) and in the non-susceptible line (NS2), but it is reduced in the last. (b) RNA of hybrid adult female progeny from crosses between Harwich males and NS2 females (lane 1), or S2 females (lane 2), or Canton-S females (lane 3) and RNA from Harwich strain (lane 4). The 0.8 kb KP transcript is reduced in the non-susceptible hybrid (lane 1) compared to the susceptible hybrid (lane 2). The 2.5 kb P factor transcript (lane 4) is also reduced in non-susceptible ones (lane 1).

Taq I endonucleases (Fig. 4c) revealed the exclusive presence of a particular P element, probably the KP element. The cloning of the 3 kbp *BamH I* fragment has been performed inside pUC19 and called p3ICA. It presents a P element with a restriction map similar to the KP element (Fig. 5) and contains a substitution between positions 29–32 (Fig. 4c). *In situ* hybridization on polytene chromosomes of the salivary glands from the Gruta strain (containing no P elements) with p3ICA as probe demonstrates that the insertion site of the KP element included in the 3 kb *BamH I* restriction fragment has been localized on the right arm of the second chromosome in the 47D1 chromosomal bridges subdivisions (data not shown).

(c) *Northern blot analysis.* Differences in P element

transcription between susceptible and non-susceptible lines were investigated. As it has been shown that the P-element promoter is transcribed in adult somatic cells (Laski *et al.* 1986), the analysis was performed on the poly(A)⁺ RNA from adults. Using the *Hind III* restriction fragment of the P factor as probe, a single transcript similar in size to the KP-element transcript of 0.8 kb (Black *et al.* 1987) was detected in both the susceptible line S2 and in the non-susceptible line, NS2. However, the transcript level is reduced in non-susceptible line NS2 (Fig. 6a). In the susceptible line S3 and in the non-susceptible line NS1, the KP transcript is not detected. Here, the quantity of the KP transcript is not correlated to the susceptibility.

(v) *Analysis of the repression mechanism*

The lower level of KP transcript in NS2 line than in S2 line suggests that repression of the P-induced dysgenic traits could be due to reduction of P-element transcription. To check this hypothesis, we analysed the P transcripts on the poly(A)⁺ RNA in the hybrid adults from dysgenic and non-dysgenic crosses performed at 18 °C, between Harwich males and S2 females or NS2 females (Fig. 6b). The 2.5 kb major P-factor transcript is reduced in hybrids from non-susceptible mothers relative to both hybrids from susceptible mothers and from the control hybrids (progeny from cross between Harwich males and Canton-S females). This result suggests that repression acts on the transcription of the P element.

Lemaître & Coen (1991) have shown that P regulatory products repress the P-promoter activity in *P-lacZ* fusion genes in both somatic and germline tissues. In order to demonstrate that the P regression in our non-susceptible (NS) lines acts on the P transcription, we have assayed their ability to reduce the *P-lacZ* activity. The β -galactosidase activity was measured on the ovaries of the female progeny from crosses between females from IINS2 or IIS2 lines and males from three independent *P-lacZ* lines (Table 3). Two control crosses were done using a P reference strain (Harwich) and a M reference strain (Canton-S). The germline specific *P-lacZ* activities (BQ16 and BC69) are strongly reduced with the IINS2 line relative to those with IIS2 line ($t = 7.80$, 17 D.F., $P < 0.001$ for BQ16; $t = 9.19$, 15 D.F., $P < 0.001$ for BC69), in contrast the somatic specific *P-lacZ* activities (BA37 and R20A) are not significantly different between these lines ($t = 1.13$, 17 D.F. for BA37; $t = 2.56$, 5 D.F. for R20A). In germline tissue, with the BQ16 *P-lacZ* line, the repression of the P promoter observed with the IINS2 line occurs at the same level as with the Harwich strain, but the repression is weaker using the BC69 *P-lacZ* line. With the IIS2 line, compared to the Canton-S strain, the P promoter is repressed slightly using the both *P-lacZ* lines. In contrast in somatic tissue, both IINS2 and IIS2 lines repress β -galactosidase activity, but to a lesser extent

Table 3. Non-susceptibility acts through repression of P-promoter activity. The activity β -galactosidase is given in $\text{nmol min}^{-1} \text{mg}^{-1} \text{protein}$. Standard error is in parentheses and was estimated on several independent preparations (n = number of repetitions). Measures were performed on ovaries of the F1 females for BQ16, BC69 and BA37, and on larvae at third-instar larval for R20A, from crosses between females under test and males from P-lacZ lines

Females under test	P-lacZ lines			
	Germline		Somatic	
	BQ16	BC69	BA37	R20A
IINS2	0.774 (0.075) $n = 7$	1.514 (0.111) $n = 10$	1.481 (0.134) $n = 9$	2.122 (0.086) $n = 4$
IIS2	1.890 (0.100) $n = 12$	3.307 (0.171) $n = 7$	1.698 (0.136) $n = 10$	2.635 (0.206) $n = 3$
Canton-S	2.470 (0.074) $n = 8$	4.200 (0.164) $n = 8$	4.096 (0.198) $n = 6$	4.233 (0.236) $n = 2$
Harwich	0.609 (0.054) $n = 3$	0.358 (0.122) $n = 3$	1.009 (0.006) $n = 3$	1.181 (0.042) $n = 2$

than the Harwich strain (Table 3). These results suggest two mechanisms, the first present in the IINS2 and IIS2 lines occurring in somatic tissue and probably due to the KP-element product, the second occurring in germline tissue would be independent of the KP product and correlated to the alone difference between IINS2 and IIS2 lines, the insertion at 47D1.

Although no detectable effect has been observed upon somatic expression of β -galactosidase between susceptible and non-susceptible lines, a difference has been detected by northern blot method. This inconsistency could be due to the fact that extracted P RNAs from dysgenic females would not be exclusively from somatic transcriptional activity of P elements but a part of them could be reflected a P germinal transcriptional activity.

4. Discussion

From a long-established laboratory strain presenting a polymorphism for both P susceptibility and the insertion sites of its P elements, it has been possible to isolate totally non-susceptible lines and lines totally susceptible. The repression of the P-induced hybrid dysgenesis in the non-susceptible lines has been shown to be associated with a reduction of the P-element transcription. Molecular analysis of the P elements in the susceptible and non-susceptible lines has revealed that all have the specific internal deletion of the KP element. Moreover the two kind of lines possess the same mean copy number of the KP elements. Consequently, the non-susceptibility in NS1 and NS2

cannot be due to differences in KP number a mechanism previously proposed by Jackson *et al.* (1988).

The susceptible and non-susceptible lines differ by the chromosomal position of their KP elements, and the non susceptibility has been shown to be correlated with a particular insertion site at 47D1 on the right arm of the second chromosome. The repression of P transcription acts on the P promoter itself reducing P element transcripts. This repression leads us to postulate the existence of a *trans*-activating factor controlling P transcription in the susceptible lines which is absent in the non-susceptible lines.

The results presented in Table 2 show complete recessivity of the resistance to GD sterility. In contrast, the repression seen with the β -galactosidase test occurs in heterozygous adults, thus indicating a dominant mode of action. One possibility is that it acts as a recessive maternal effect gene.

In recent reports several authors (Robertson & Engels, 1989; Simmons *et al.* 1990; Misra & Rio, 1990; Ronsseray *et al.* 1989) have high-lighted the problem of P-element position within stocks. Simmons and collaborators analysed the ability to repress P-element-mediated gonadal dysgenesis in inbred lines derived from a strain called Sexi. Some lines have high or moderate repression potential and others little or none at all. Every line possessed numerous KP elements. The rule of inheritance of repression potential varied among the inbred Sexi lines. In the lines in which the repression ability was not inherited maternally, it appeared to be determined by partially dominant or additive chromosomal factors or to be due to recessive chromosomal factors, as is the case with our non-susceptible lines. Taken together, these results do not support the KP repressor hypothesis, unless, as suggested by Simmons *et al.* (1990), the inbred Sexi lines differ in the position of their KP elements and the production of the KP repressor is affected by chromosomal position. Even this last supposition, with an over-expression of the KP element inserts at the 47D1 chromosomal region, cannot explain the repression of P element activity in NS1 and NS2, since less KP transcript is detected in these lines than in the susceptible line S2.

Among other explanations, one possibility could be that the KP element inserted at 47D1, affects the transcription of genes of this chromosomal region which result in a repression of P transcription in dysgenic hybrids. According to this view, P transcription in our lines would be controlled by a *trans*-activating factor produced by a gene of *Drosophila melanogaster*. A mutation in this gene, as is probably present in NS1 and NS2 lines, must affect not only P-element transcription but also the transcription of other genes. Another possibility is that non-susceptible lines have mutation on the second chromosome which is not caused by the 47D1 insertion. Perhaps the hypothetical mutation inactivates a repressor of a

germline specific transcription unit where the 47D KP element is inserted. The effect would be with 47D, and would be recessive. If this is true, it would be possible to isolate non-susceptible lines devoid of any type of P elements. Other chromosomal regions probably play a role in the control of P-element transposition and some might be responsible for the position effect suspected in recent investigations (Misra and Rio, 1990; Ronsseray *et al.* 1989, 1991).

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