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Evolutionary flux of P element regulation in a *Drosophila melanogaster* hybrid dysgenesis cline

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Summary

Clines of P-induced hybrid dysgenesis provide a means for monitoring the evolution of transposition repression over space and time. We have studied the molecular and phenotypic profiles of flies taken from a 2900 km cline along the eastern coast of Australia, which had previously been characterized over 10 years ago as having P populations in the north, Q populations at central sites and M′ populations in the south. We have found that Q and M′ populations of flies have increased their range within the cline at the expense of P lines. Q populations were found to be in the north of the cline and M′ populations in the south. Some of the northern Q lines transmit repression through both sexes and type I deletion elements have been isolated from them. We suggest that these elements are responsible for Q type repression. The results support our model that populations made up of Q individuals with strong biparentally transmitted repression form an evolutionarily stable strategy for the repression of hybrid dysgenesis in *Drosophila melanogaster*.

1. Introduction

Hybrid dysgenesis is a set of genetic abnormalities brought about by P element transpositions in *Drosophila melanogaster* (Kidwell *et al*., 1977). Hence, the P element is a destructive transposable element against which mechanisms have evolved to control transposition (Engels, 1989). P element mobilization causes increased mutation rate, chromosome rearrangements, male recombination and temperature-dependent sterility (gonadal dysgenesis). P strains of flies contain transposase-producing (autonomous) P elements and can induce transpositions if they transmit these elements to strains lacking a strong repression system. The P elements themselves have been found to be repressors of P element transposition. Therefore, M strains, which are devoid of all P elements, have little potential to prevent transposition and hybrid dysgenesis if invaded by autonomous P elements. Several mechanisms of repression have been discovered and strains of flies can be classified by the type of repression system that they exhibit (P, Q or M′). Complete P elements contain four exons (open reading frames (ORFs) 0 to 3), which together encode a single 87 kDa polypeptide that is the functional transposase. P elements with deletions cannot produce their own transposase but can be mobilized if the enzyme is supplied in *trans*. P elements have been classified into two structural types (Gloor *et al*., 1993). Type I includes the complete 2907 bp P element and elements with specifically located small deletions at the 3′ end, while type II elements (for example, the KP element: Black *et al*., 1987) have much larger deletions.

Experimentally, strains are classified by their potential to induce transposition/hybrid dysgenesis when crossed to M strain females (A cross) and their ability to repress transposition when crossed to P strain males (A* cross). The strengths of induction and repression are measured by the number of dysgenic ovaries observed in the progeny of the crosses (Kidwell *et al*., 1977; Engels & Preston, 1980). Fig. 1 shows how M, P, M′ and Q strains are classified in the gonadal dysgenesis assays. Two characteristics are assayed for each line classified.

P strains of flies display a strong maternally inherited repression system called P cytotype (Engels

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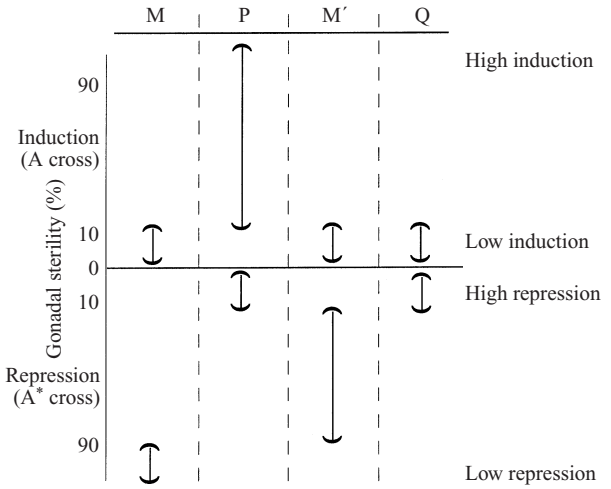

Fig. 1. Classification of repression systems by gonadal dysgenesis (GD) assays. GD scores for the A cross (measuring induction potential) and the A* cross (measuring repression potential) are above and below the horizontal axis respectively. The GD scores represent ideal M, P, M′ and Q strains of *Drosophila melanogaster* (Kidwell *et al*., 1977).

et al., 1987). P cytotype is mediated by a 66 kDa protein produced by differential splicing of the complete element's primary transcript (Laski *et al*., 1986; Misra & Rio, 1990). When P strain females are crossed to a strong P reference line with autonomous P elements in the A* cross, less than 10% of the ovaries examined in the progeny are dysgenic. Therefore, P strains are strong repressors of hybrid dysgenesis. If males from a P strain are crossed to females of a reference M strain in the A cross, more than 90% of ovaries are dysgenic in the progeny. Therefore, P strains are strong inducers of transposition.

M′ strains contain deletion derivative repressor elements, in particular the abundant and widespread type II repressor called KP, which we isolated from European and Russian populations (Black *et al*., 1987). KP has been shown to be a repressor of P element transposition (Black *et al*., 1987; Jackson *et al*., 1988; Andrews & Gloor, 1995). M′ strains display intermediate levels of repression of dysgenesis when crossed to P strain males and induce less than 10% gonadal dysgenesis sterility when crossed to M strain females. Both males and females from M′ strains are capable of passing a repressing factor to their progeny (Lemaitre *et al*., 1993), differentiating them from the uniparental inheritance characteristic of P cytotype.

Q strains possess strong repression of transposition, comparable to the levels seen in P cytotype, in that crosses with strong P strains produce less than 10% dysgenic gonads. Q strains also display a low induction of transposition in the A cross, as seen in M′ lines, with a GD score of less than 10%. Some Q strains have maternal inheritance of repression whilst others have biparental inheritance (Jackson *et al*., 1988). It is not known how repression in Q strains is mediated, but we proposed that type I deletion elements possessing small deletions, close to the ORF2–3 boundary, are involved in the repression mechanism, mediated by the three P element leucine zipper motifs (Corish *et al*., 1996). We have isolated one particular type I deletion element, the SR element, which has a 309 bp deletion at the 3′ end of the element and is a good candidate for the strong biparental repression observed in a Q line, collected from the North Wootten UK population (Corish *et al*., 1996). The SR repressor cannot produce functional transposase but can potentially produce the 66 kDa repressor and a novel 75 kDa protein, both of which are candidates for Q type repression.

What are the evolutionary dynamics of repression in natural populations? On the discovery of the KP (type II) element (Black *et al*., 1987), we proposed that different repression systems arose by chance in different parts of the world and that local selection would promote a repression system on a first-come-first-served-basis, in order to alleviate the debilitating hybrid dysgenesis effects of P transposition. On the discovery of the SR (type I) element in a Q population (Corish *et al*., 1996) and after the classification of Q individuals into Q_{mat} (strong repression inherited through the maternal side only) and Q_{bip} (strong repression inherited biparentally) (Jackson *et al*., 1988), we proposed an evolutionary model in which Q_{bip} would be the most stable evolutionary end state, superior to P cytotype, M′ and Q_{mat} (for arguments see Corish *et al*., 1996).

In order to test this model we have carried out a phenotypic and molecular analysis of an extensive P–M cline along the eastern coast of Australia, approximately 10 years after it was first sampled and analysed (Boussy, 1987; Boussy & Kidwell, 1987; Boussy *et al*., 1988). According to our model, populations of flies with different repression systems will inevitably interact and clinical patterns of repression may change over time as they evolve to the most stable state. The results of our phenotypic analysis confirm one of our conjectures that Q_{bip} is the stable end point to which other population phenotypes (in particular P cytotype populations) will evolve. Additionally, deletion mapping of cloned elements from northern Q_{bip} populations shows us that some elements have the structures of type I repressor elements.

2. Materials and methods

(i) *Fly strains used*

The Australian cline consists of flies collected from the east coast by Linda Partridge in 1993 (James *et al*., 1995), with locations similar to those collected for the 1983 study (Boussy, 1987; Boussy & Kidwell, 1987;

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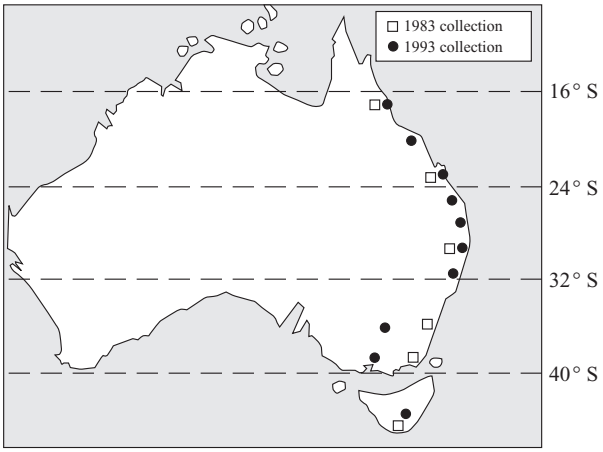

Fig. 2. Geographical locations of collection points on the east Australian cline. Collection points from north to south are: Cairns, Bowen, Yeppoon, Hervey Bay, South Brisbane, Coff's Harbour, Taree, Cobram, Melbourne and Tasmania. Each collection point has two sites, approximately 16 km apart, from which flies were sampled in 1993. The collection points have locations similar to those sampled in 1983 (Boussy, 1987).

Boussy *et al*., 1988). The collection spans 26 degrees of latitude, from Cairns in the north to Tasmania in the south (Fig. 2). Each location has two collection sites that are approximately 16 km apart. The collection points are as follows: Cairns (16·9 °S), Bowen (20·0 °S), Yeppoon (23·1 °S), Hervey Bay (25·3 °S), South Brisbane (27·9 °S), Coff's Harbour (30·3 °S), Taree (31·9 °S), Cobram (35·8 °S), Melbourne (38·2 °S) and Tasmania (42·9 °S). The Australian collections consist of several isofemale lines that have been kept at 18–25 °C on standard sugar food. An individual isofemale line is described in the form: Collection point, site number, isofemale line number. For example, Cairns 1.4 is isofemale line number four from site 1 of Cairns. The reference lines used to measure induction potential and repression potential were Canton-S (a true M strain) and Harwich (a strong P strain), respectively (Bingham *et al*., 1982; Kidwell, 1985).

(ii) *Gonadal dysgenesis assays*

Determination of repression system (P, Q or M′) was carried out with the standard tests for measuring gonadal sterility potential (Kidwell *et al*., 1977; Engels & Preston, 1980). The following crosses were set up for each isofemale line and incubated at 29 °C: A cross (15 Canton-S virgin females × 10 males from the isofemale line) and A* cross (15 virgin females of the isofemale line × 10 Harwich males). Parent flies were removed from the vials after 4–5 days of egg laying. After eclosion and 2–3 days of maturation, at least 50 female progeny were dissected and classified into: (i) both ovaries normal, (ii) one ovary normal and one dysgenic, (iii) both ovaries dysgenic. From this a gonadal dysgenesis (GD) score can be generated: [% gonadal sterility = (no. of dysgenic ovaries/total no. of ovaries scored) × 100]. The GD score in the A cross gives a measure of the induction potential (or P activity); a high score means a high level of unrepressed transposition in the progeny of the cross. The score in the A* cross gives a measure of the repression potential (or P susceptibility); the lower the percentage dysgenesis, the stronger the repression. Fig. 1 shows how each system is classified from the two criteria.

(iii) *Biparental analysis*

Virgin females of the line to be assayed were crossed to Canton-S males (maternal cross) and Canton-S virgin females were crossed to test line males (paternal cross); both crosses were kept at 25 °C. F1 virgin females from both crosses were mated separately to Harwich males and incubated at 29 °C. F2 progeny were scored for dysgenic ovaries and χ^2 analysis was performed on 2×2 contingency tables, to determine whether the test line exhibits strong biparental repression.

(iv) *Southern blot analysis*

Standard techniques were used for DNA extraction, gel electrophoresis, blotting and hybridization (Sambrook *et al*., 1989). Approximately 5 μg of genomic DNA was digested with *Dde*I in accordance with the manufacturer's instructions (GIBCO BRL). Digestions were carried out in large volumes at 37 °C overnight; DNA was precipitated before electrophoresis in 1 % TAE agarose gels followed by transfer to Hybond N^+ (Amersham) and hybridization in Church buffer (0·5 M Na_2HPO_4, 1 % BSA, 1 mM EDTA, 7 % SDS; Church & Gilbert, 1984). Filters were probed with a ^{32}P-labelled internal 900 bp *Pvu*II fragment isolated from a complete P element, and were washed at high stringency (0·5 × SSC, 0·1 % SDS) at 65 °C before drying and exposure to film for 1–2 days.

(v) *Polymerase chain reaction*

Standard polymerase chain reaction (PCR) (Saiki *et al*., 1988; Erlich, 1989) was used to amplify the array of P elements in each test line. A single primer was used in the reactions, homologous to the 31 bp terminal inverted repeat of P elements (Haring *et al*., 1995). The sequence of the p31 oligonucleotide is: 5′(CATAAGGTGGTCCCGTCG)3′. Genomic DNA for the template was extracted from 50 adult flies by the method of Mian & Dover (1990). PCR reaction volumes were 30 μl containing approximately 100 ng of genomic DNA. P elements were amplified using 20 cycles comprising denaturation (94 °C for 30 s), primer

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annealing (55 °C for 1 min) and extension of products (72 °C for 4 min). The low cycle number improved the detection of the larger P elements by limiting the preferential amplification of smaller deletion derivatives such as KP.

(vi) *Isolation of potential type I repressors*

Candidate repressor DNA was excised from PCR gels, purified and blunt cloned into phosphatased *Eco*RV cut pBluescript KS$^+$ (Stratagene) (Sambrook *et al*., 1989). Colonies were picked and screened by PCR using the same p31 primer.

(vii) *Analysing repressor structure*

Once isolated, the elements' structures were analysed by PCR, generating a deletion map. Primer 0 is homologous to 5′ P element sequence at position 90 and was used in all reactions. The other primers used have homology to the opposite strand at various points along the P element. The positions of the other primers relative to the complete element are as follows: (1) 476, (2) 881, (3) 1081, (4) 1328, (5) 1565, (6) 1834, (7) 2009, (8) 2204, (9) 2419, (10) 2764 and (11) 2873. It was possible to locate approximately where in an element a deletion resides by which PCR reactions do not amplify (see Fig. 7). After deletion mapping, the presence or absence of the leucine zippers was assayed by PCR, using primers homologous to the leucine zipper sequences, and individual elements were sequenced to identify the exact position of the deletion breakpoint.

3. Results

(i) *Gonadal dysgenesis in Australian populations collected in 1993*

The latitudinal cline in eastern Australia was studied (Boussy, 1987; Boussy & Kidwell, 1987; Boussy *et al*., 1988) to assay gonadal dysgenesis characteristics. In 1983 collections, P populations were found exclusively in the northern locations, Q populations at central locations and M′ populations in the south (Fig. 4*b*, *c* show the GD scores of these populations). There was no apparent shift in the cline between 1983 and 1986 (Boussy & Kidwell, 1987).

Table 1 shows the results of the A and A* crosses, performed on flies collected from the Australian cline in 1993. The table includes the results for individual sites within each location and the pooled data for induction and repression potential. Multiple isofemale lines were studied for each site and the standard errors for the GD scores are included. Despite the 16 km separation between the two sites at any collection point, in most cases the GD scores for induction and repression are similar for the two sites. At the most northern locations (Cairns, Bowen, Yeppoon and site 1 of Hervey Bay), the induction potential is very low and the repression potential is high, characteristic of Q populations. Moving south through the cline, the induction potential increases 2- to 3-fold and the repression potential decreases significantly to levels not seen in Q populations. The lowest levels of repression are observed in the most southern locations (Melbourne and Tasmania). The data reveal that the populations are Q in the north and M′ in the south. Fig. 3 shows the data set in a graphic form, including the results of the individual isofemale lines tested. The scatter plot shows much less variation in induction and repression potentials in the northern populations compared with the southern populations. The high variation seen in repression potential GD scores in the south is to be expected of M′ populations as these have been described as having variable and intermediate repression strengths (Kidwell, 1983). However, the induction potential is higher and more variable than would be expected of M′ populations

Table 1. *Unweighted means of gonadal dysgenesis scores for the A and A* crosses carried out on Australian isofemale lines collected in 1993, including data for individual sites and pooled data for each collection point*

1993 Populations	(°S)	No. of lines	Site 1: mean ± SE (%)		Site 2: mean ± SE (%)		Pooled data	
			A cross	A* cross	A cross	A* cross	A cross	A* cross
Cairns	16·9	12	10·9 ± 2·2	12·7 ± 4·4	9·2 ± 2·9	6·8 ± 1·9	10·2 ± 1·7	10·2 ± 2·8
Bowen	20·0	5	9·8 ± 2·1	6·2 ± 2·3	—	—	9·8 ± 2·1	6·2 ± 2·3
Yeppoon	23·1	4	4·5 ± 0·5	7·0 ± 3·0	4·8 ± 2·8	18·5 ± 7·5	4·7 ± 1·2	12·8 ± 4·7
Hervey Bay	25·3	6	9·7 ± 2·0	5·7 ± 1·9	22·3 ± 9·9	18·0 ± 3·1	16·0 ± 5·4	11·8 ± 3·2
South Brisbane	27·9	7	19·5 ± 6·5	19·3 ± 7·7	14·0 ± 4·0	17·0 ± 5·0	17·1 ± 4·0	18·3 ± 4·6
Coff's Harbour	30·3	8	17·8 ± 5·7	21·9 ± 12·8	23·0 ± 2·7	26·0 ± 10·2	19·8 ± 3·6	23·5 ± 8·4
Taree	31·9	9	20·0 ± 4·1	27·2 ± 6·5	7·0 ± 0·6	25·0 ± 6·1	15·7 ± 3·5	26·4 ± 4·5
Cobram	35·8	6	18·0 ± 5·1	8·3 ± 4·4	15·0 ± 3·8	33·0 ± 18·2	16·5 ± 2·9	20·7 ± 10·0
Melbourne	38·2	9	24·0 ± 6·3	41·2 ± 8·7	5·0 ± 1·7	37·3 ± 5·7	17·7 ± 5·2	39·9 ± 5·9
Tasmania	42·9	6	20·0 ± 3·8	33·5 ± 10·7	18·3 ± 6·0	57·0 ± 9·6	19·2 ± 3·2	45·2 ± 8·3

Standard errors for each data set are included as a measure of variation within a site.

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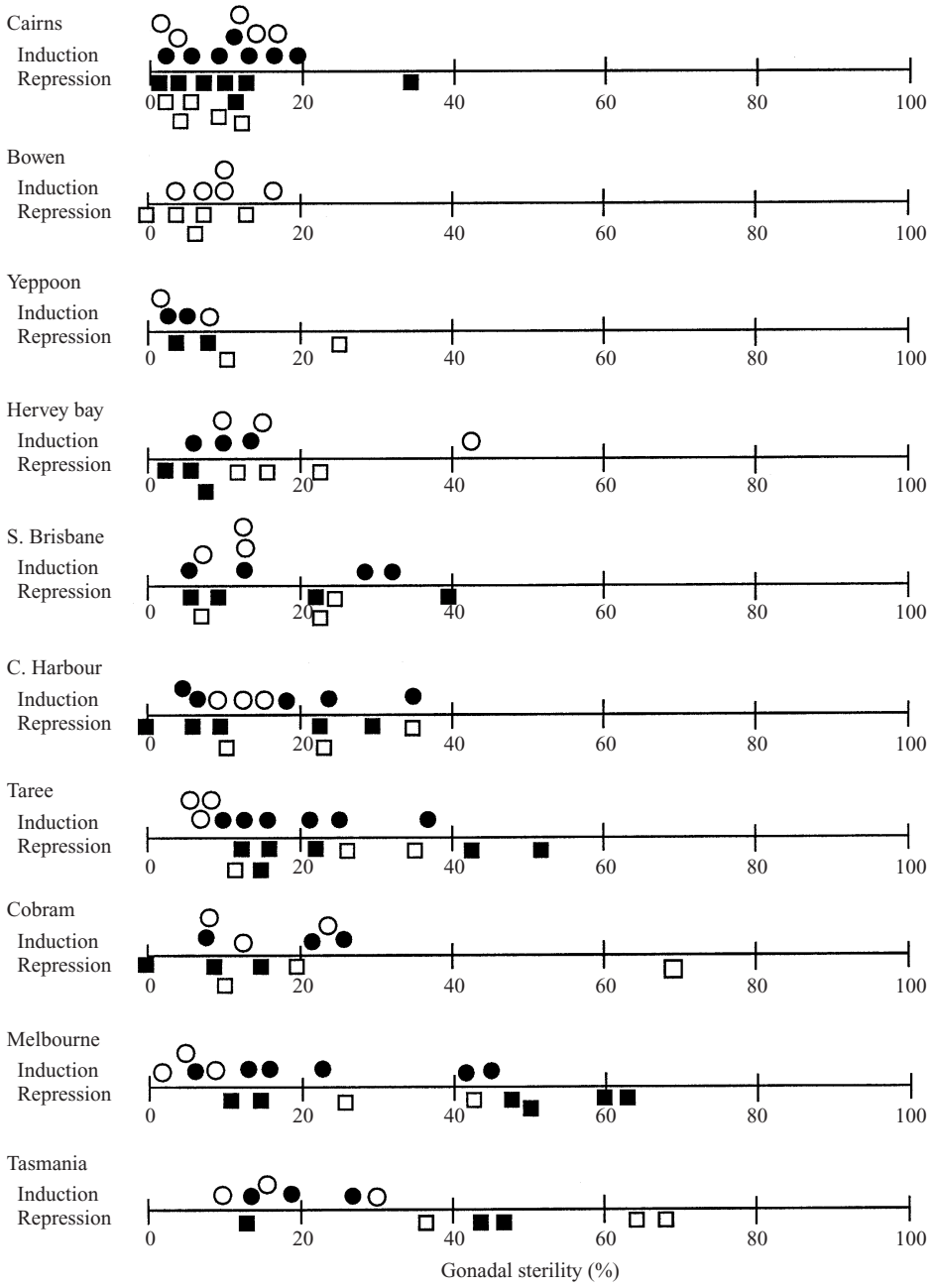

Fig. 3. A scatter plot showing the whole data set for A and A* crosses. The induction potentials are above the horizontal line and repression potentials are below, showing the GD scores for each individual isofemale line assayed. The collection points run north (top) to south (bottom). Induction potential is represented by circles; the greater the percentage sterility, the higher the induction of transposition. Repression potential is represented by squares; the higher the percentage sterility, the lower the repression strength. Site 1 data are shown with filled shapes and site 2 data with open shapes.

and suggests a mixture of repression systems in these regions. Fig. 4*a* shows the percentage gonadal sterility observed in the A and A* crosses moving from north to south. The induction potential is lowest in the north, while a marginal increase in P activity is observed in the southern lines. On average, therefore, the induction potential is low, as would be expected of Q and M′ populations and in contrast to the strong induction of P strains. Along this same cline, however, repression ability decreases dramatically from north to south with levels typical of Q populations in the north. In the south, reduced, highly variable levels of repression are observed, consistent with the M′ phenotype.

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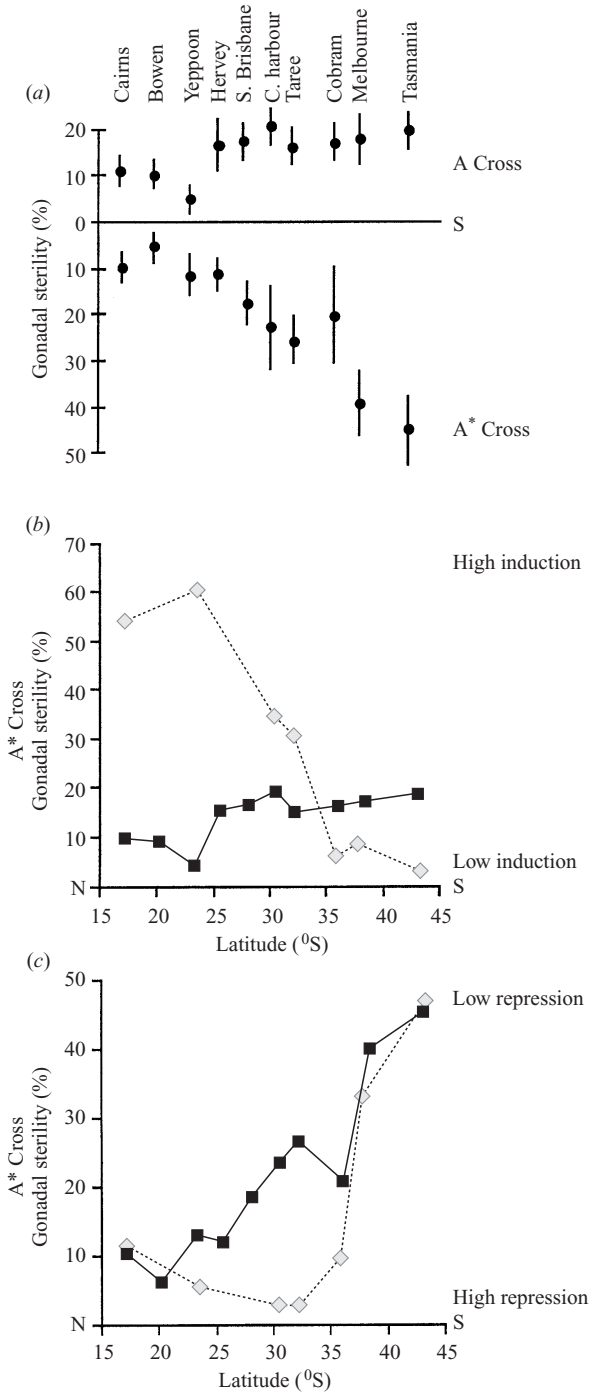

Fig. 4. Comparison of the 1983 and 1993 collections. (*a*) Induction potentials (above the *x*-axis) and repression potentials (below the *x*-axis) for isofemale lines from 1993 are plotted against latitude, northern populations at the left and southern populations at the right. Multiple lines were studied at each collection point and standard errors are included. (*b*) Induction potentials vary through the cline. The 1993 Australian collections are shown with black squares, whilst data from 1983 collections (Boussy, 1987) and data from flies collected at Taree in 1985 (Boussy & Kidwell, 1987) are shown with grey diamonds. (*c*) Repression potentials vary through the cline. The 1993 Australian collections are shown with black squares and data from 1983 and 1985 collections (Boussy, 1987; Boussy & Kidwell, 1987) are shown with grey diamonds.

(ii) *Natural alterations in the dysgenesis cline*

Flies collected in 1983 were classified as P in the north, Q in central sites and M′ in the south (Boussy, 1987). Fig. 4*b*, *c* show the results of the A and A* crosses that were carried out on these 1983 populations. Data for flies collected from Taree in 1985 have also been included in the graphs (Boussy & Kidwell, 1987). There is a dramatic difference between the induction potentials observed in the 1983 and 1993 collections (Fig. 4*b*). The older collection revealed high induction potentials throughout the north, characteristic of P strains. This decreased moving south through the cline, to levels characteristic of Q and M′ populations. The more recent collections show significantly lower levels of induction at the northern sites with only a slight increase in induction at southern sites, where the results are not significantly different from the 1983 collections. The induction potential observed in southern populations for both 1983 and 1993 collections is out of the range that defines the P phenotype. However, some of the 1993 isofemale lines do have induction potentials that are higher than Q and M′ flies should possess, but are reduced compared with a typical P strain. These lines displaying induction potentials greater than typical Q and M′ phenotypes were predominantly found at the boundary between northern Q and southern M′ populations. For example, the isofemale line Brisbane 1.3 displays 6% and 32% gonadal dysgenesis in the A* and A tests respectively. This is the largest induction potential observed in a 1993 isofemale line possessing less than 10% dysgenesis in the A* test. Brisbane 1.3 and two other isofemale lines collected from central sites may be classified as P. The induction potentials are out of the range that defines the Q phenotype, but are lower than potentials observed in the 1983 northern P populations.

The repression potential is approximately identical in the most northern and the most southern sites when comparing the two collections. However, the decrease in the strength of repression, moving north to south, occurs across a broader geographical range (Fig. 4*c*). Consequently, populations at central sites are no longer Q in the 1993 samples, in contrast to the well-defined Q region from 1983.

(iii) *Inheritance of repression*

Lines classified as M′ are presumed to display biparental inheritance of repression potential (Black *et al.*, 1987), but Q lines can exhibit either maternal (Q_{mat}) or biparental (Q_{bip}) inheritance patterns (Jackson *et al.*, 1988). Biparental test crosses were performed on ten northern Q strains, four of which (Cairns 1.4, Cairns 2.4, Cairns 2.5 and Bowen 1.1)

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Table 2. *Patterns of inheritance of repression ability in lines classified as Q strains, using the A and A* gonadal dysgenesis crosses*

Isofemale line	A cross	A* cross	Maternal % GD	Paternal % GD
Cairns 1.4	6	3	12	19
Cairns 1.5	2	6·4	19	41***
Cairns 2.2	3	2	11	28·6***
Cairns 2.4	12	4	37	63*
Cairns 2.5	2	10	1	4
Bowen 1.1	8	4	17	10
Bowen 1.4	10	8	6	61***
Yeppoon 2.2	2	11	18	56***
Hervey Bay 1.1	6	7	27	76***
Hervey Bay 1.3	10	2	14	68***

Isofemale lines are named in the form: Collection point, site number, isofemale line number. Gonadal dysgenesis (GD) scores for maternal and paternal components of repression are included, showing the repression potential of inherited P elements. Maternal and paternal components of repression were compared with a χ^2 analysis performed on 2×2 contingency tables ($*P < 0{\cdot}05$, $***P < 0{\cdot}005$; all other comparisons were non-significant).

were found to have statistically significant biparental inheritance of repression (Table 2; the remaining lines were classified as Q_{mat}. However, some Q_{mat} lines (Cairns 1.5 and Cairns 2.2), whilst displaying a significant difference between maternal and paternal inheritance of repression, show a paternal component of repression (41 % and 28·6 %, respectively) that is higher than that seen in many biparental M′ strains.

(iv) *Molecular analysis of isofemale lines*

The distribution of P elements in the 1993 collections was examined with Southern blots of genomic DNA probed with an internal P element fragment. Fig. 5 shows the results of the Southern blots carried out on a subset of lines, representing the full Australian cline, from Cairns in the north (top left) to Tasmania in the south (bottom right). Some lanes, for example Taree 1.6, are devoid of bands because of a failure in the Southern blot and not because the line has no P elements. Molecular analysis on these lines has been repeated with PCR (data not shown) and they have been shown to contain many P elements. For example, the Southern blot of Cairns 2.2 failed to reveal P elements (Fig. 6*a*) whilst the PCR technique revealed many elements from the same sample (Fig. 6*b*). The Canton-S control lane is totally devoid of P element bands, whilst the Harwich lane possesses multiple bands including a very intense band of 2·2 kb. The 2·2 kb and 0·42 kb bands, possessed by most isofemale lines, correlate with the expected sizes of *Dde*I-digested 2·9 kb complete element and KP element, respectively. Although there are differences between isofemale lines, there is no apparent clinal change in the abundances of the complete element or KP element in the 1993 collections, in contrast to the clines of abundances observed in the 1983 populations (Boussy *et al*., 1988).

Further molecular analysis was performed on a subset of seven of the Q isofemale lines, four of which have biparental inheritance of repression (Table 2). Fig. 6 shows a comparison of Southern blot analysis with PCR amplification assays, carried out on these seven Q isofemale lines. The PCR strategy amplifies the array of P elements in the genomes of isofemale lines with a single primer homologous to the 31 bp terminal inverted repeats (Haring *et al*., 1995). Complete P element results in a 2·2 kb or 2·9 kb band in the Southern blot (Fig. 6*a*) and PCR assay (Fig. 6*b*), respectively, whilst the KP element produces 0·42 kb and 1·1 kb bands in the same tests. PCR gels were probed with ^{32}P-labelled P element fragment (data not shown) to ensure that all PCR fragments were indeed P elements. Furthermore, the 2·9 kb and 1·1 kb PCR fragments, from Bowen 1.1, were cloned and deletion mapped with PCR (Fig. 7*a*) to confirm that they were complete and KP elements respectively (see next section).

Although no comparison between band intensities in Southern blots and PCRs can be made, the patterns of element size distribution are similar in their key features. All lanes, with the exception of Canton-S (lane 8) and Bowen 1.2 (lane 5), possess a strong complete P element band in both the Southern blot and PCR assay, and Q isofemale lines (lanes 2–7) possess KP-sized bands in both assays. Additionally, bands other than the full-sized P element and the KP repressor can be seen to be shared between Southern blots and PCRs. The two large bands, lying just below the complete P element, in Cairns 2.5 (lane 3) are

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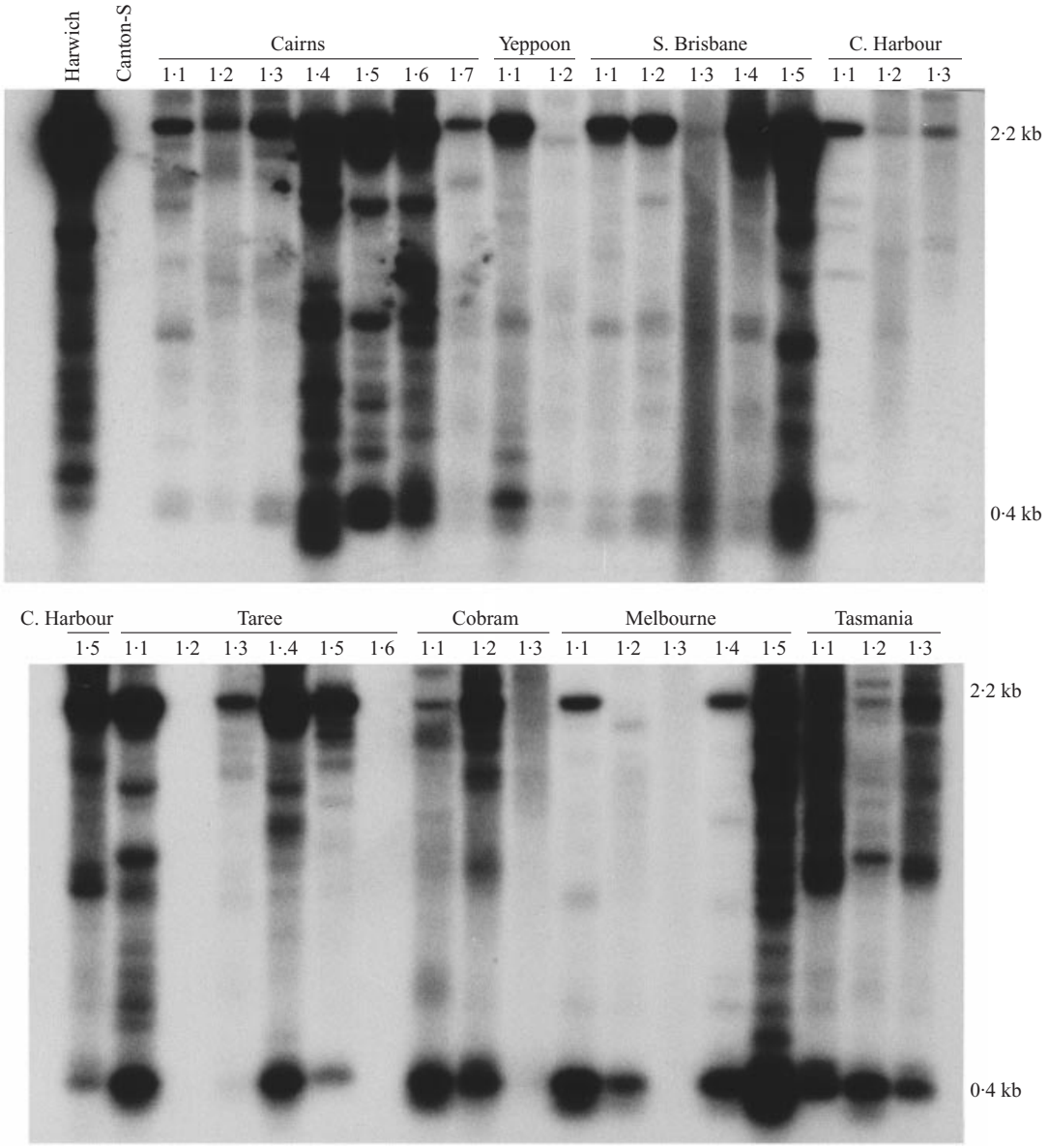

Fig. 5. P element molecular profiles, obtained by Southern blots, of a subset of isofemale lines screened with A and A* crosses. Collection points run from the north (top left) to the south (bottom right) with positive and negative controls, Harwich and Canton-S respectively. Bands corresponding to complete P element (2·2 kb) and KP element (0·42 kb) are indicated.

present in the Southern blot (~ 2 kb) and PCR (~ 2·8 kb), and two prominent bands of approximately 1·6 kb present in the Cairns 1.4 (lane 7) Southern blot lane are also present as bands of approximately 2·3 kb in the PCR. From these comparisons it would appear that PCR is a viable alternative to Southern blots for the qualitative screening of populations for P elements.

A significant difference in the P element molecular profiles of Q_{mat} and Q_{bip} isofemale lines is not evident in Figs 5 and 6. Excepting lane 5, the Southern blots of Q lines do not show any major differences in full-sized P element and KP element intensities; and pooled maternal and pooled biparental lines possess approximately equal numbers of P element deletion derivatives.

(vi) '*SR-like*' *elements in Q strains*

Some of the large elements present in Q isofemale lines were excised from PCR gels and cloned into pBluescript. Four potential 'SR-like' repressors have been isolated from Q strain flies: two elements (described in the previous section) from Cairns 1.4 (Q_{bip}), one from Cairns 1.5 (Q_{mat}) and one from Bowen 1.1 (Q_{bip}). These were selected by virtue of the similarity of their size to previously described type I elements (Gloor *et al.*, 1993; Corish *et al.*, 1996). All four elements are between 2·3 and 2·4 kb in length and contain all three leucine zippers. Fig. 7*b* shows the structure of one of these elements (the smaller of the two from Cairns 1.4) determined by PCR deletion

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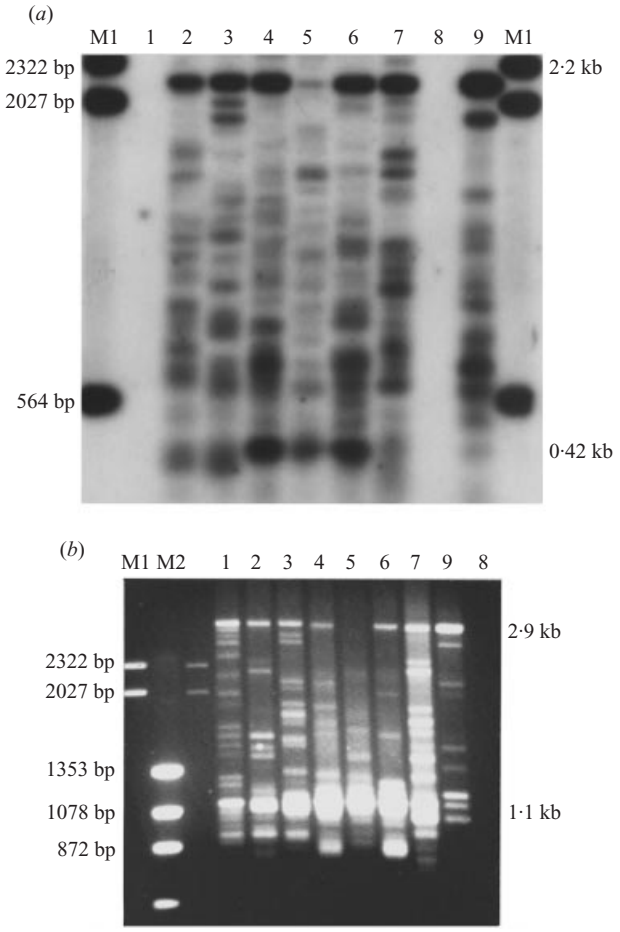

Fig. 6. A qualitative comparison of Southern blot and polymerase chain reaction techniques to study P element molecular profiles in isofemale lines. No attempt is made to compare band intensities in the two assays. (*a*) Southern blots of seven Q strains: (1) Cairns 2.2, (2) Cairns 2.4, (3) Cairns 2.5, (4) Bowen 1.1, (5) Bowen 1.2, (6) Bowen 1.4 and (7) Cairns 1.4. 1, 5 and 6 and 2, 3, 4 and 7 were classified as Q_{mat} and Q_{bip} respectively. Canton-S (8) and Harwich (9) controls are included. M1 lanes are the marker λ*Hin*dIII. The complete P element results in a 2·2 kb band and KP produces a 0·42 kb band. (*b*) PCR analysis performed on the same isofemale lines. Amplification of P elements was done using a single primer homologous to the 31 bp terminal inverted repeats. The complete P element gives a band of 2·9 kb and the KP elements yields a band of 1·1 kb.

mapping and sequencing. This element, with a deletion between positions 2023 and 2625, conforms to the defined structure of type I repressors (Gloor *et al*., 1993). In addition, two other elements, from Bowen 1.1 and Cairns 1.5 (deleted between 2012 and 2567), conform to the structural requirements of a type I repressor, as they too possess the entire sequence of exons 0, 1 and 2 and have the first 9 bp of the 2–3 intron (data not shown). The fourth element, the larger fragment from Cairns 1.4, has a deletion between positions 1211 and 1686 and does not conform to the type I definition. This is structurally similar to an element isolated from a Japanese Q strain by Sakoyama *et al*. (1985), which was deleted between positions 1194 and 1953.

4. Discussion

(i) *Changes in repression and induction over time*

The clinal pattern of P element repression has changed since 1983. The cline P to Q to M′ (Boussy, 1987) has become Q in the north and has remained M′ in the south. We proposed (see Corish *et al*., 1996) that Q_{bip} populations are the stable end state for P, M′ and Q_{mat} populations, in that the Q_{bip} phenotype can transmit strong repression through both sexes, accompanied by little induction of GD. This study provides evidence in support of the proposition. The range of the Q phenotype has increased into the northern part of the cline at the expense of P populations.

The southern boundary of Q has also moved northwards, possibly due to competition with M′ populations. A similar shift from Q_{mat} to M′ has been observed in Europe, where we have studied a P–M hybrid dysgenesis cline in France and Spain (data not shown). Flies were collected in 1995 at locations similar to those performed for a previous study (Fleuriet *et al*., 1992). The cline was classified as Q in the north and M′ in the south and we found that the cline had not changed dramatically since 1991. However, there was an influx of M′ characteristics into the most northern Q location and the Q populations in this region are Q_{mat}, as is the case with the Australian populations where M′ invades Q_{mat}. Such examples of local superiority of M′ over Q could be due to the fact that the southern Q populations have maternal inheritance of repression (see Table 2), whilst the KP repression system is biparentally transmitted and hence, potentially, more effective. However, the French/Spanish study of Fleuriet *et al*. (1992) showed, in one particular location, a reverse trend from M′ to Q over a few years. It could be, therefore, that Q_{mat} and M′ populations can co-exist but the balance of power can shift in favour of either repression system.

Two further observations deserve comment. First, the induction potential has decreased dramatically in the 10 years between collections and there has been a significant decrease in the repression potential at central sites. It could be that the acquisition of low induction potential has been at the expense of loss of repression strength (Fig. 4*b*, *c*). It may be that, in the long term, low induction is more important than strong repression, especially if this is accompanied by biparental inheritance of KP replacing the uniparental inheritance of Q_{mat}. Secondly, southern populations display more variation in induction and repression than northern lines (Fig. 3). M′ populations are expected to have variable strength of repression, possibly due to differences in KP copy number and

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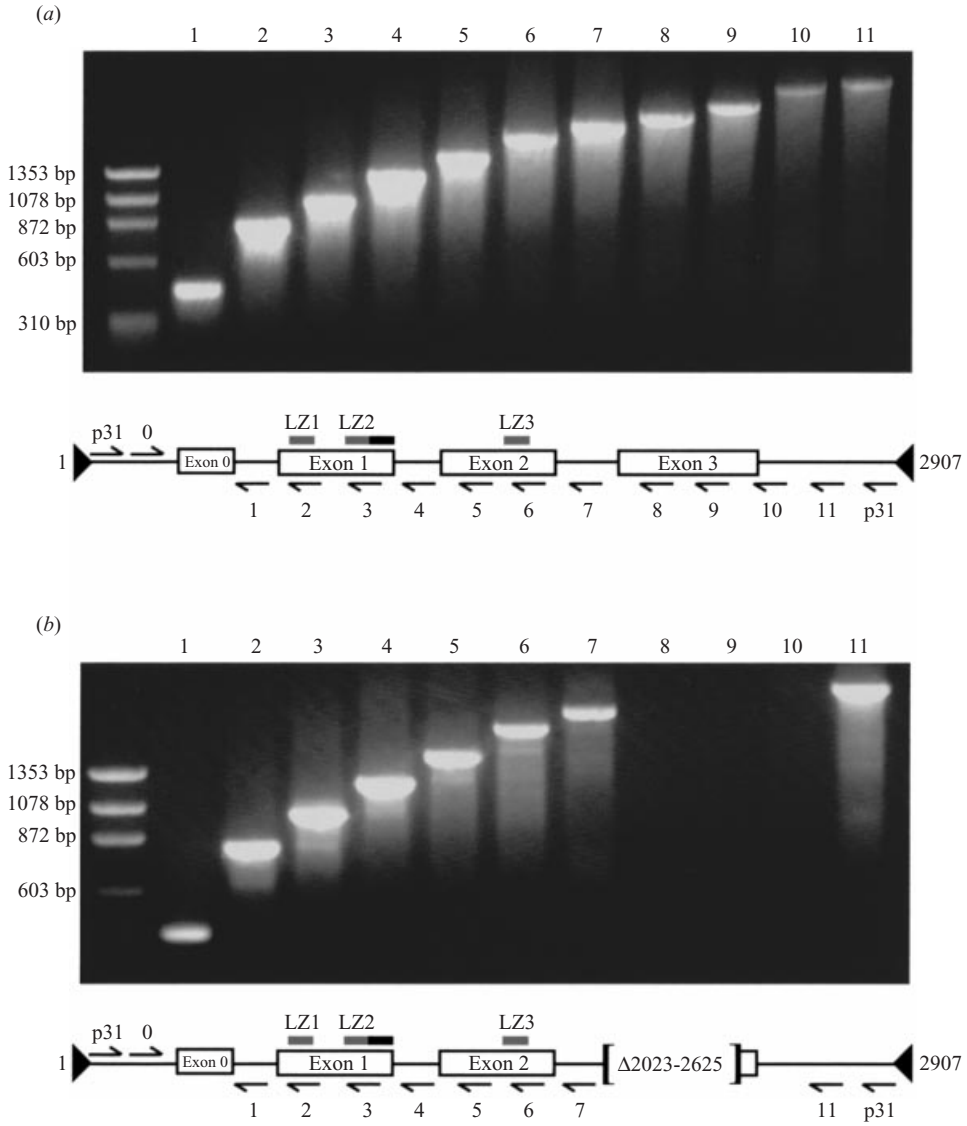

Fig. 7. PCR analysis, showing the structure of cloned elements from Q strains. Primer 0 is homologous to P element DNA at position 90 relative to the complete element and was used in all reactions. The other primers anneal at various sites along the P element sequence on the opposite strand. Lane 1 on the gels represents the reaction containing primers 0 and 1, lane 2 has primers 0 and 2, etc. The approximate locations of primers were indicated below the gel pictures. (*a*) The 2·9 kb PCR band from Bowen 1.1 was closed and analysed. All lanes contain amplified product of expected size, confirming that the 2·9 kb band is indeed the full-sized P element. (*b*) A 2·3 kb PCR fragment from Cairns 1.4 was cloned and analysed. Lanes 8, 9 and 10 are devoid of PCR product, indicating a deletion in the 3′ end of the element. The deletion element was fully sequenced and the exact breakpoints are included.

transcriptional effects from genomic positioning of KP inserts (Misra *et al*., 1993). However, the large variation in induction potential seen in the southern populations is not expected of stable M′ lines and could be due to the population still being in flux, in that populations with highly variant levels of induction have not yet reached a stable state.

(ii) *The molecular basis of Q repression*

We have cloned and analysed the structure of four deletion derivatives from the northern Australian Q populations. These are smaller than SR, and three of these elements fulfil the structural requirements to be classified as type I and all four possess all three leucine zippers (Gloor *et al*., 1993). These elements may be responsible for the Q characteristics observed, producing a strong repressor that prevents transposition by protein–protein or protein–DNA interactions mediated by the leucine zippers (Landshultz *et al*., 1988; Rio, 1991; Lee *et al*., 1996, 1998). The three elements that fulfil the type I structural requirements have the capacity to produce the 66 kDa repressor protein. They may also be able to produce a protein similar to the 75 kDa protein predicted for SR by Corish *et al*. (1996), as well as the 66 kDa repressor.

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The P element molecular profiles of Q_{mat} and Q_{bip} isofemale lines, in general, do not appear to be significantly different and elements with type I structure have been isolated from Q_{mat} and Q_{bip} lines. The differences in repression characteristics observed could be explained by variation in copy number and insertion sites of these putative repressors. The differences in genomic position may result in transcriptional effects directly (Misra *et al*., 1993) or may promote the formation of certain chromatin structures that have roles in P element regulation (Roche & Rio, 1998; Ronsseray *et al*., 1998).

In conclusion, the P element cline in eastern Australia has changed since last studied and a trend towards Q_{bip} has been observed. P cytotype and M′ mechanisms of repression may have been early, emergency responses to the deleterious consequences of transposition. Surviving populations have subsequently evolved relatively superior mechanisms to improve fitness by the acquisition of biparental Q repression, possibly mediated by *de novo* generated type I repressor elements, as predicted in the model (Corish *et al*., 1996).

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