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Origin and decay of the *P* element-associated latitudinal cline in Australian *Drosophila melanogaster*

Ian A. Boussy[1], Masanobu Itoh[1,2], David Rand[3] & R.C. Woodruff[4]
[1] *Dept. of Biology, Loyola University of Chicago, 6525 N. Sheridan Rd., Chicago, Illinois 60626, USA;* [2] *Present address: Dept. of Applied Biology, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan;* [3] *Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912, USA;* [4] *Dept. of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403, USA*

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Abstract

The latitudinal cline in *P* transposable element-associated characteristics in eastern Australian populations of *Drosophila melanogaster* has changed between 1986 and 1991–1994. New collections were made in 1991–1994 from localities along the eastern coast of Australia. *P* element-associated properties of 256 isofemale lines from 43 localities were evaluated using gonadal dysgenesis and/or *singed-weak* hypermutability assays. The overall results indicate that both P activity and P susceptibility have declined, with all populations showing a tendency towards a state with little P activity potential but with P repressor function (neutral or 'Q'). P repressor function is strong in all populations except some of the most southerly. P activity potential peaks at about 27 ° SLat, and drops off to the south (as in 1983–1986 collections) and to the north (in contrast to 1983–1986 collections); thus the cline is no longer a simple P-to-Q-to-M pattern from north to south, but is now Q-P-Q-M. A mtDNA RFLP that putatively distinguishes North American and European populations varies in frequency among the populations but the frequency does not vary clinally with latitude, ruling out massive introductions from North America and Europe as causing the cline.

Introduction

P element properties of *Drosophila melanogaster* flies differed dramatically from north to south in collections made in 1983–1986 along the eastern coast of Australia (Boussy, 1987; Boussy & Kidwell, 1987). The *P* element-associated P-M system of 'hybrid dysgenesis' (Kidwell, Kidwell & Sved, 1977) had earlier been shown to be caused by *P* element transposition, and to generate a suite of genetical traits, including non-development of gonads in F1 individuals reared at high temperature (gonadal dysgenesis), F2 egg inviability, chromosomal breaks and rearrangements, chromosome transmission ratio distortion, and genic mutations, which were often unstable (Bingham, Kidwell & Rubin, 1982; Bregliano & Kidwell, 1983; Engels, 1983; O'Hare, 1985). Reference crosses using standard laboratory strains can be used to evaluate the *P* element-associated properties of flies from the wild (Kidwell, Kidwell & Sved, 1977; Engels & Preston, 1980; Kidwell, Frydryk & Novy, 1983). By scoring gonadal dysgenesis (GD) in F1s, reference crosses using Australian lines collected in 1983-1986 revealed that the northeastern coastal populations were 'P' (they had potentially active *P* elements and strong repressor ability; Kidwell, Kidwell & Sved, 1977); southeastern populations were neutral or 'Q' (they did not demonstrate active *P* elements, but could repress such elements introduced in reference crosses; Engels & Preston, 1981); and the southernmost populations were 'M' (demonstrating no *P* element activity potential and relatively weak ability to repress active elements introduced in reference crosses; Engels & Preston, 1980). Thus, in a restricted latitudinal region, a full range of *P* element-associated phenotypes was

encountered, forming a latitudinal cline in P-M hybrid dysgenesis properties (Boussy & Kidwell, 1987).

This pattern was in contrast to the near-uniformity or weak clinal patterns found in other continental areas of the world in the 1980s. North and South American and subsaharan African populations were virtually all P, whereas European, Mediterranean, northern African, and central Asian populations were Q to M, with a weak clinal pattern from weak P in France to Q and to M elsewhere (Engels & Preston, 1980; Anxolabéhère, Nouaud & Periquet, 1982; Kidwell, Frydryk & Novy, 1983; Anxolabéhère et al., 1984; Anxolabéhère et al., 1985; Kidwell & Novy, 1985; Anxolabéhère, Kidwell & Periquet, 1988; Woodruff et al., 1990). Japanese (Kidwell, Frydryk & Novy, 1983; Takada et al., 1983; Yamamoto, Hihara & Watanabe, 1984; Harada, Kusakabe & Mukai, 1991; Matsuura et al., 1993) and South African (Getz & van Schaik, 1988) populations have been described as variable in P-M characteristics, but without the clear clinal pattern seen in Australia. The eastern Australian P-Q-M clinal pattern thus appeared to be unique, and offered a natural experiment in interactions between the different long-term modes to which populations in other areas had developed. A likely origin of the clinal pattern in Australia was the introduction into the north of flies from, for instance, North America, and into the south of flies from, for instance, Europe, with subsequent migration and mixing generating a clinal pattern (Boussy, 1995).

In 1991–1994, we made new collections in eastern Australia to monitor whether changes had occurred in the wild since 1986. We assessed two measures of the *P* element system – gonadal dysgenesis (GD) and *singed-weak* (sn^w) hypermutability – in reference crosses to laboratory strains, and compared the GD data with data from 1983–1986 collections to determine the extent of any changes in the intervening period. We also tested a sample of lines for the frequency of a *Hin*f I site in the mitochondrial DNA that has been shown to differ in its frequency between North American and European populations.

The results of GD comparisons indicate that the clinal pattern has decayed since the 1983–1986 collections, but that there is still a broadly clinal P-Q-M pattern with latitude from about 27 ° SLat to the south. North of about 27 ° SLat, populations now resemble more southerly populations, an unexpected reversal of the clinal pattern. The sn^w tests gave results that correlated with the GD results, indicating that the GD assays were, in fact, measuring *P* element-associated effects. The frequency of the *Hin*f I mtDNA site varied a great deal among the lines, but there was no latitudinal pattern of frequency among the lines tested.

Materials and methods

Isofemale lines and reference strains

Flies were collected either by sweeping over *Drosophila*-attractive sites (e.g., refuse at fruit markets, piles of fermenting fruit waste in orchards, or pomace heaps near wineries) or by attracting them to traps baited with banana mash seeded with live baker's yeast. Within a few hours, collected flies were anesthetized and sorted to separate *D. melanogaster* and *D. simulans* from other species, and the females of the former two were put individually into vials containing food medium. If the male offspring emerging in a vial had genitalia typical of *D. melanogaster*, the population in the vial was kept as an isofemale line (if the genitalia were typical of *D. simulans*, or if no offspring emerged, the vial was discarded).

The localities in which collections were made are shown in Figure 1 and listed in Table 1, along with information about each collection. J.S.F. Barker, J.B Gibson, A. Hoffmann, and N. Jenkins collected many of the lines. Lines were maintained at 20–23° on standard cornmeal-molasses-yeast-agar medium supplemented with live yeast, and with propionic and orthophosphoric acids or with methyl para-hydroxybenzoate (Tegosept®) added to control mold.

Our reference M strain for gonadal dysgenesis assays, Canton-S-brn, has tan eyes due to mutations in both eye-pigment pathways. The stock originated in the laboratory of M.G. Kidwell as a spontaneous mutation in the red pigment pathway in a culture of 'Canton-S-red' that already carried a spontaneous mutation in the brown pigment pathway. The stock has been stable since it was isolated about 1980. Canton-S is a 'wild-type' strain originally collected in Canton, Ohio, USA, in the 1920s or 1930s; Canton-S and its derivatives such as Canton-S-brn are completely devoid of *P* elements. Our reference P strain, Harwich-w, is a substrain of the Harwich strain, started from three females collected in 1967 in Harwich, Massachusetts, USA, by M.L. Tracey, Jr. Harwich-w was isolated as a spontaneous white-eyed mutant in the laboratory of M.G. Kidwell; it is due to a mutation at the *white* locus, and has been stable since its isolation in the

Table 1. The 43 localities from which flies were tested, arranged from north to south.

Locality	° SLat	Collector(s)	Numbers of lines tested for:		
			GD	sn^w	*Hin*f I site
Cairns, Qld	16.9	AAH	2	2	
Bowen, Qld	20.0	AAH	1	1	
Mackay, Qld	21.2	AAH	7	7	8
Nebo, Qld	21.4	JSFB	1	1	
Yeppoon, Qld	23.1	AAH	5	5	7
Westwood , Qld	23.7	JSFB	6	6	
Miriam Vale, Qld	24.3	AAH	5	5	
Hervey Bay, Qld	25.5	AAH	1	1	
Rainbow Beach, Qld	25.9	AAH			7
Bli Bli, Qld	26.7	RCW	8	8	6
Brisbane, Qld	27.5	AAH	2	2	8
Oxenford, Qld	27.9	RCW	4	4	
S. of Brisbane, NSW	28.0	AAH	1	1	
Murwillumbah, NSW	28.2	RCW	10	10	5
Brunswick Heads, NSW	28.6	NJ;AAH	4	4	
Byron Bay, NSW	28.7	RCW	7	7	7
Broadwater, NSW	29.0	RCW	11	11	9
Palmers Island, NSW	29.5	RCW	8	8	5
Coffs Harbour, NSW	30.3	RCW;AAH;NJ	11	11	14
Borah, NSW	30.5	JSFB	9		7
Uralla, NSW	30.6	RCW	1		
Stuarts Point, NSW	30.8	RCW	10	10	10
Coonabarabran, NSW	31.3	RCW	5		4
Port Macquarie, NSW	31.4	RCW	5	5	3
Laurieton, NSW	31.6	RCW	9	9	8
Taree, NSW	31.9	RCW;AAH	7	7	3
Forster, NSW	32.2	RCW;AAH	3	3	
Sydney, NSW	33.8	JS	4	4	
Nowra, NSW	34.9	JBG;RCW	13	13	8
Ulladulla, NSW	35.4	RCW	10	10	
Bateman's Bay, NSW	35.7	RCW	5	5	4
Malua Bay, NSW	35.8	RCW	6	6	4
Moruya, NSW	35.9	RCW	2	2	
Tuross Head, NSW	36.1	RCW	5	5	3
Narooma, NSW	36.2	RCW	1	1	
Bega, NSW	36.7	JBG;RCW	18	18	16
Merimbula, NSW	36.9	RCW	5	5	
Eden, NSW	37.1	RCW	10	10	9
Genoa, Vic	37.5	RCW	7	7	
Cann River, Vic	37.6	RCW	10	10	9
Nicholson River, Vic	37.8	JBG	8	8	8
St. Huberts, Vic	37.9	JBG	8	8	3
Forth, Tas	41.2	AAH	1	1	
Total lines tested:			256	241	175
Localities tested:			42	39	25

Qld=Queensland, NSW=New South Wales, Vic=Victoria, and Tas=Tasmania.
Collectors: A.A. Hoffmann (AAH), J.S.F. Barker (JSFB), R.C. Woodruff (RCW), N. Jenkins (NJ), J. Sved (JS), J.B. Gibson (JBG).
GD=gonadal dysgenesis, sn^w=singed-weak hypermutability, *Hin*f I site=presence of *Hin*f I site in mtDNA (see Materials and methods).

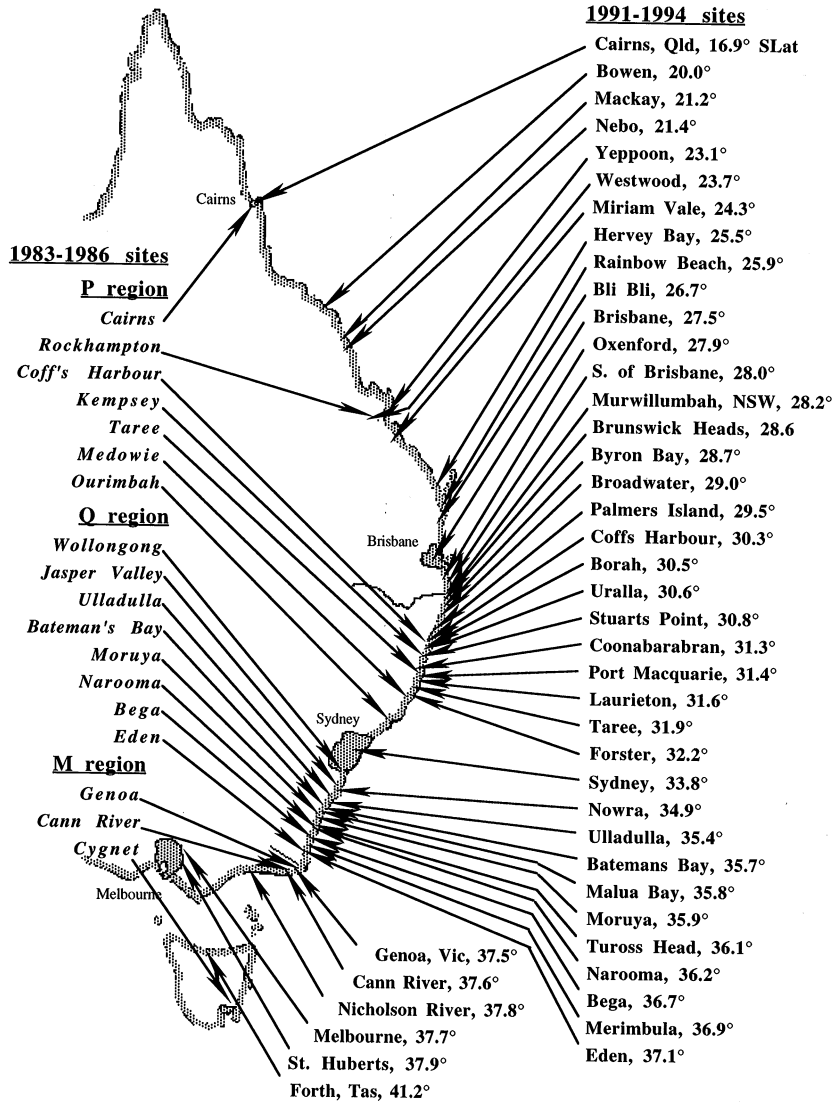

Figure 1. Collection sites along the coast of eastern Australia. Sites sampled in 1983-1986 are shown to the left; 1991-1994 collections are listed to the right, with latitude in degrees south also listed. Qld = Queensland; NSW = New South Wales; Vic = Victoria; Tas = Tasmania.

early 1980s. Like the Harwich strain, Harwich-w is a very strong P strain, carrying at least several full-size *P* elements and deletion-derivatives of various sizes. It completely lacks the deletion-derivatives known as *KP* elements (Black et al., 1987).

Our reference strains for sn^w assays were ysn^w; bw; st and $C(1)DX$, $yf/ywsn^3B$, which carry no *P* elements, and $sn^w(P)$; $\pi 2$, which contains many potentially active *P* elements; these strains were provided by W.R. Engels.

Crosses

Standard crosses to ascertain the P-M characteristics of an isofemale line are diagrammed in Figs 2, 3. Crosses A and A* assess a line's *P* element activity potential and its *P* element repressor potential, respectively (Kidwell, Kidwell & Sved, 1977; Engels & Preston, 1980). We evaluated these characteristics using two hybrid dysgenesis-associated traits, gonadal dysgenesis (GD) and sn^w-hypermutability. Crosses scoring GD were performed using the reference strains Canton-S-brn and Harwich-w, as diagrammed in Figure 2. The crosses were performed at 29 ° C, a

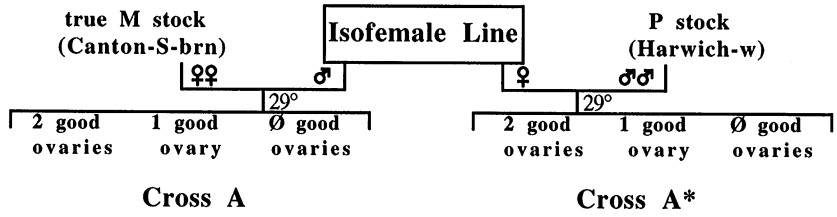

Figure 2. Crosses A and A* to evaluate the P-M characteristics of a line by crossing to reference laboratory stocks and scoring ovarian non-development (gonadal dysgenesis). An ovary was scored as 'good' if it contained one or more full-size eggs in one or more ovarioles.

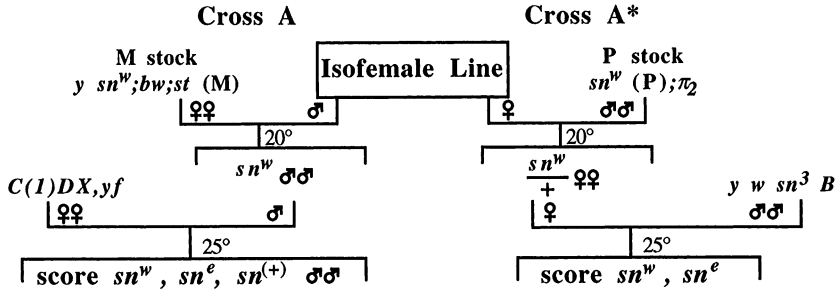

Figure 3. Crosses A and A* scoring sn^w hypermutability in F1 offspring to evaluate P-M characteristics of a line.

temperature at which gonadal dysgenesis occurs in P-M dysgenic crosses (Engels & Preston, 1979; Kidwell & Novy, 1979). For each line, five or more crosses were established with individual males (for cross A) or virgin females (for cross A*), crossed to several individuals of the reference strain. An ovary was scored as 'good' if it contained at least one apparently normal egg in an ovariole. The GD score of a cross was the percent of females with one or both ovaries undeveloped. Appropriate controls were also performed (Canton-S-brn × Harwich-w, Harwich-w × Canton-S-brn, intrastrain, and reciprocal crosses of the lines to the reference strains).

The *singed-weak* (sn^w) allele is due to the head-to-head insertion of two short *P* elements near the 5' end of the *singed* locus (Nitasaka & Yamazaki, 1988; Roiha, Rubin & O'Hare, 1988). In the absence of active *P* element transposase it is stable, but under P-M dysgenic conditions the sn^w allele mutates at high frequency (up to 50%; Engels 1984), to *singed-extreme* (sn^e) or nearly wild-type ($sn^{(+)}$), by excision of one *P* element or the other. Its hypermutability is thus specific to the *P* element and provides a direct assay for *P* element transposase activity. Very weak correlations (or no significant correlations) between the scores for the GD and sn^w assays would have indicated that they were not causally related. Crosses A and A* using sn^w-hypermutability to assess P-M properties are shown in Figure 3. Cross A was performed using the reference M strain $ysn^w; bw; st$, then crossing sn^w offspring males to *C(1)DX, y f* compound-X females and scoring the *singed* phenotypes expressed in male offspring. The cross A sn^w hypermutability score (Engels, 1984) was calculated as:

$$\text{cross A } sn^w \text{ hypermutability score} = \frac{\text{No.}(sn^e\text{males}) + \text{No.}(sn^+\text{males})}{\text{Total males}}$$

Cross A* was performed using the reference P strain $sn^w(P); \pi 2$, then crossing $sn^w/+$ female offspring to sn^3 males; the offspring were then scored for sn^w and sn^e phenotypes. (The expected $sn^{(+)}/sn^+$ offspring cannot be easily differentiated from true wild-type sn^+/sn^+ flies, and therefore cannot be scored.) The cross A* sn^w hypermutability score (Simmons, 1987; Heath & Simmons, 1991) was calculated as:

$$\text{cross A* } sn^w \text{ hypermutability score} = \frac{\text{No.}(sn^e\text{offspring})}{\text{No.}(sn^e\text{offspring}) + \text{No.}(sn^w\text{offspring})}$$

For each line, five or more crosses were established with individual males (for cross A) or virgin females (for cross A*), crossed to several individuals of the reference strain.

Mitochondrial DNA analysis

The mitochondrial genomes of *D. melanogaster* have been shown to be polymorphic in and between populations (Hale & Singh, 1991; Rand, Dorfsman & Kann, 1994). In particular, at position 2603 in the ND5 gene, North American populations appear to be fixed for an A, whereas European and Asian populations have a high frequency of G (Rand, Dorfsman & Kann, 1994; Rand & Kann, 1996). Our designation of nucleotide position 2603 follows Garesse's numbering for the non-coding strand (Garesse, 1988); this site corresponds to nucleotide position 240 on the coding strand in Rand et al. (1994) and Rand and Kann (1996). The occurrence of G at nucleotide position 2603 creates a *Hin*f I site, which can be conveniently assayed using restriction digestion and agarose gel electrophoresis. mtDNA analysis was performed on 175 female lines using methods previously described (Rand, Dorfsman & Kann, 1994; Hutter & Rand, 1995). Briefly, total DNA from a single female from each isofemale line was prepared in a simple 'squish' buffer, and each sample was then amplified individually. We used the following primers, which flank the polymorphic *Hin*f I site:
1600R 5′ AATCCTATTATACCACGGAG 3′ and
3017L 5′ TAGAAGAGGTAAAATTCGAG 3′ (see Table 1 in Rand, 1996).

Using these primers, independent polymerase chain reactions were performed on the DNAs from individual flies to amplify a 1457 bp fragment of the mitochondrial ND5 sequence. The resultant amplification products were then subjected to restriction endonuclease digestion with *Hin*f I, followed by agarose gel electrophoresis which resolved whether they were cut or uncut.

Statistical analyses

Statistical analyses were performed using StatView 4.1 (Abacus Concepts, Inc.) on a Power Macintosh 8100/80 (Apple Computers Inc.).

Results

The results of the gonadal dysgenesis (GD) tests of potential *P* element activity (cross A) and potential repressor function (cross A*) for the 1991-1994 lines are presented as percent gonadal dysgenesis in Table 2, and in Figure 4b they are plotted against the latitude at which the isofemale lines were collected. For comparison, the results from the 1983-1986 collections are presented in Figure 4a. Overall, the results from the 1991–1994 collections are muted compared to those of 1983–1986. There is still a weak P-to-M clinal pattern between about 27 ° SLat and 38 ° SLat, but it is much attentuated from the previous pattern. The southernmost populations are still the ones showing the least repressor function (i.e., the most 'M'), but cross A* scores are somewhat lower than were found in 1983–1986 at similar latitudes (but note that we had only one line from Tasmania, where the most M populations were found in 1983–1986). The very strong P populations found in 1983–1986 in the north (16.9 ° and 23.4 ° SLat) are no longer seen; in fact, the highest P activity is found at about 27 ° SLat, with populations to the north of this region expressing weak P or Q phenotypes like those of populations from the south-central coast.

The results of sn^w tests of crosses A and A* are also presented in Table 2, and are shown graphically in Figure 4c by latitude. The profile of results across latitude is clearly similar for the GD and sn^w tests. The relationships between the GD and sn^w test results of crosses A and A* are graphed in Figure 5a and 5b, respectively. The correlations between gonadal dysgenesis and sn^w scores in cross A and cross A* tests are highly statistically significant (for cross A, Kendall's tie-corrected $\tau = 0.378$, $P < .0001$; for cross A*, Kendall's tie-corrected $\tau = 0.332$, $P < .0001$; see Figure 5 and Table 3). While there was considerable variability in both tests among lines from individual populations, the overall results are sufficient to corroborate our assumption that our GD results are due to *P* element-associated properties of the lines.

The results of evaluating the frequency of haplotypes with the *Hin*f I site in the ND5 gene of mtDNA are also presented in Table 2, and are plotted against latitude in Figure 6. The frequencies of the *Hin*f I site were variable from locality to locality, but did not vary in a systematic way with latitude. The overall mean frequency of the *Hin*f I site haplotype was 0.457 ± 0.038 s.e. (n=175). A linear regression line was fitted using the raw data, so as to take into account the sample sizes at each latitude. As shown in Figure 6, the fitted regression line has a slope that is not significantly different from zero ($P = 0.833$). The results are inconsistent with the hypothesis that large proportions of flies from North America and from Europe were introduced into the north and the south, respectively, of Australia, thereby establishing the clinal pattern.

Table 2. Results of cross A and A* using GD and *sn*^{w} assays, and *Hin*f I frequencies for each locality, arranged by latitude (° SLat). Each mean is presented ± the standard error of the mean (se) if more than one line was tested, followed by the number of lines tested (n).

Locality	° SLat	A GD ± se	n	A* GD±se	n	A sn^{w} ± se	n	A* sn^{w} ± se	n	*Hin*f I site)	n
Cairns	16.9	16.8±5.6	2	0.0±0.0	2	3.60±0.7	2	0.60±0.6	2		
Bowen	20.0	12.4	1	0.0	1	5.00	1	0.00	1		
Mackay	21.2	13.8±1.8	6	0.0±0.0	6	3.90±1.2	6	0.00±0.0	6	0.875	8
Nebo	21.4	0.8	1	0.0	1	0.74	1	0.00	1		
Yeppoon	23.1	10.4±0.7	5	0.4±0.2	5	13.00±2.9	5	0.00±0.0	5	0.429	7
Westwood	23.4	19.1±2.1	6	0.0±0.0	6	8.10±2.0	6	0.00±0.0	6		
Miriam Vale	24.3	12.6±1.5	5	0.2±0.2	5	7.70±1.1	5	0.00±0.0	5		
Hervey Bay	25.5	0.8	1	0.0	1	0.19	1	0.00	1		
Rainbow Beach	25.9									0.714	7
Bli Bli	26.7	36.9±5.4	8	1.9±0.8	8	8.50±2.8	8	0.20±0.1	8	0.000	6
Brisbane	27.5	1.0±0.6	2	0.0±0.0	2	3.60±1.4	2	0.10±0.1	2	0.250	8
Oxenford	27.9	26.6±7.9	4	0.1±0.1	4	16.70±5.1	4	0.00±0.0	4		
S. of Brisbane	28.0	5.2	1	0.8	1	10.30	1	0.18	1		
Murwillumbah	28.2	12.7±1.9	10	1.5±0.5	10	6.40±1.1	10	0.00±0.0	10	0.000	5
Brunswick Head	28.5	3.6±0.6	4	2.5±1.1	4	8.30±3.2	4	0.00±0.0	4		
Byron Bay	28.6	14.8±1.9	7	4.5±1.4	7	8.70±2.4	7	0.00±0.0	7	0.286	7
Broadwater	29.0	3.7±1.5	11	2.3±0.7	11	3.80±1.1	11	0.20±0.1	11	0.333	9
Palmers Island	29.3	2.3±1.3	8	0.3±0.1	8	5.50±1.4	8	0.00±0.0	8	0.600	5
Coffs Harbour	30.3	7.3±1.6	11	0.3±0.1	11	9.00±2.2	11	0.00±0.0	11	0.429	14
Borah	30.5	2.2±0.5	8	4.1±3.2	8					0.143	7
Uralla	30.6	0.8	1	0.0	1						
Stuarts Point	30.8	14.6±3.6	10	0.2±0.1	10	7.80±0.6	10	0.10±0.1	10	0.800	10
Coonabarabran	31.3	2.2±0.5	5	1.4±0.6	5					0.500	4
Port Macquarie	31.3	12.3±4.6	5	0.5±0.2	5	5.80±1.9	5	0.00±0.0	5	0.333	3
Laurieton	31.6	13.3±1.8	9	1.4±0.8	9	6.30±1.2	9	0.00±0.0	9	0.875	8
Taree	31.9	16.3±4.4	7	1.6±0.5	7	12.00±2.2	7	0.20±0.1	7	0.000	3
Forster	32.2	2.1±0.6	3	1.1±0.3	3	7.90±0.7	3	0.10±0.1	3		
Sydney	33.8	11.6±2.3	4	2.4±1.1	4	6.2±3.9	4	0.20±0.1	4		
Nowra	34.9	3.5±0.9	13	1.4±0.7	13	1.10±0.4	13	0.10±0.1	13	0.500	8
Ulladulla	35.4	2.1±0.6	10	0.6±0.4	10	2.40±0.5	10	0.00±0.0	10		
Batemans Bay	35.7	1.7±0.4	5	0.8±0.3	5	2.20±0.6	5	0.40±0.2	5	0.500	4
Malua Bay	35.8	0.8±0.2	6	1.1±0.8	6	2.20±0.8	6	0.00±0.0	6	0.750	4
Moruya	35.9	2.6±0.2	2	10.6±1.0	2	0.70±0.4	2	1.60±1.0	2		
Tuross Head	36.1	0.8±0.4	5	1.7±0.6	5	1.30±0.3	5	0.00±0.0	5	0.000	3
Narooma	36.2	1.2	1	1.2	1	9.40	1	0.00	1		
Bega	36.7	1.5±0.3	18	5.0±2.0	18	1.60±0.5	18	0.30±0.1	18	0.438	16
Merimbula	36.9	2.9±1.2	5	8.2±4.2	5	1.50±0.7	5	0.80±0.8	5		
Eden	37.1	1.6±0.5	10	13.1±3.6	10	1.40±0.5	10	0.40±0.1	10	0.444	9
Genoa	37.5	2.2±0.8	7	8.1±1.6	7	0.70±0.6	7	0.90±0.6	7		
Cann River	37.6	1.2±0.3	10	9.0±3.1	10	0.22±0.1	10	0.54±0.2	10	0.333	9
Nicholson River	37.8	1.6±0.3	8	3.8±1.0	8	0.44±0.2	8	0.79±0.5	8	0.625	8
St. Huberts	37.9	1.7±0.6	8	11.1±2.2	8	0.20±0.1	8	1.80±0.7	8	0.667	3
Forth	41.2	0	1	0.0	1	0.00	1	0.00	1		

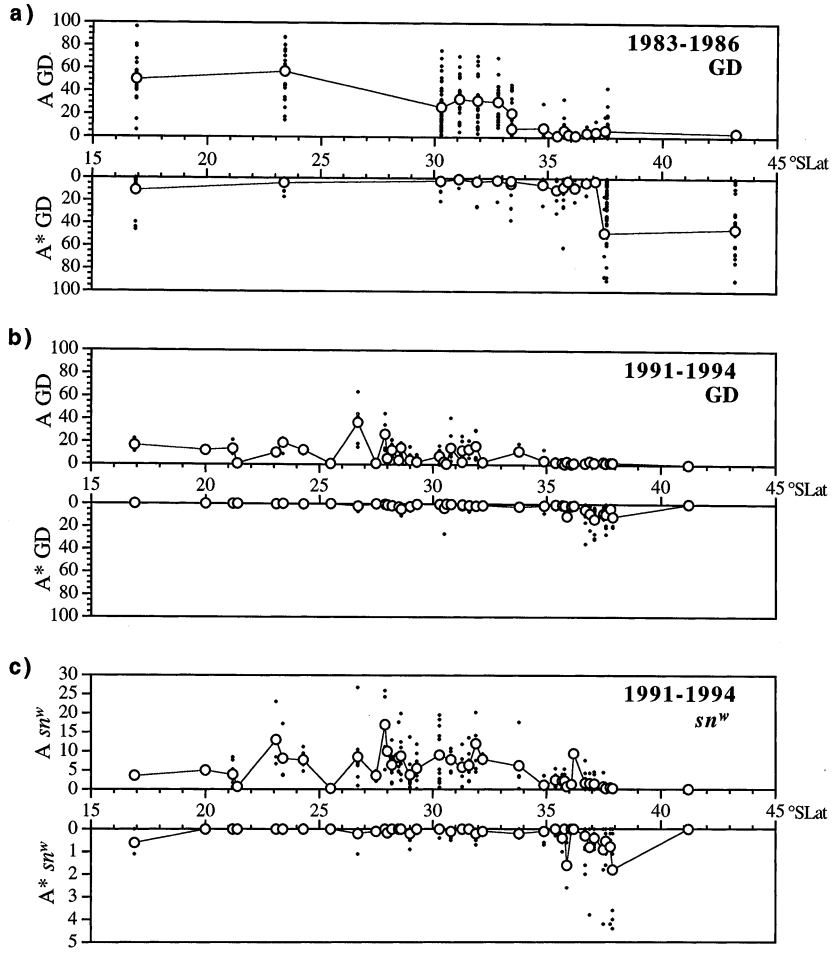

Figure 4. Results of GD and sn^w assays across latitude (° SLat). For each, cross A* results are presented with the Y-axis reversed. The small black dots represent tests of individual lines; the circles connected by lines represent the means for the localities. a) 1983–1986 GD results (data from Boussy (1987), Boussy & Kidwell (1987), and unpublished data (IAB)). b) GD results from 1991-1994 collections. c) sn^w results from 1991-1994 collections.

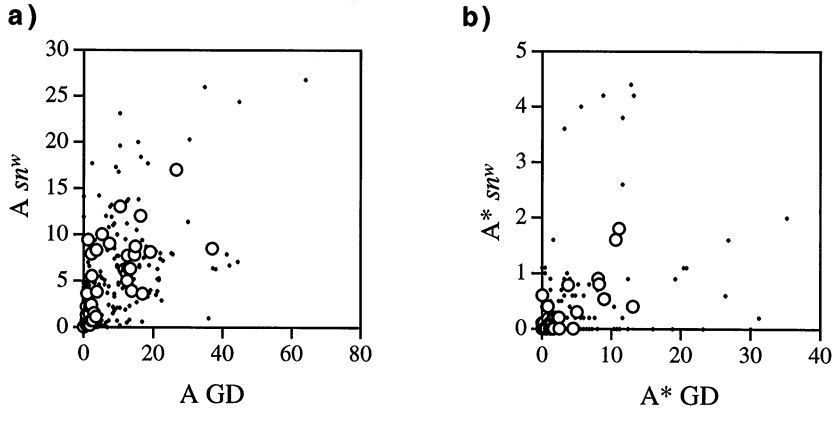

Figure 5. Comparison of GD and sn^w results. Data for each line are presented as dots, with means for populations as open circles. a) Results for cross A tests. b) Results for cross A* tests.

Discussion

P elements are thought to have invaded *D. melanogaster* about 50 years ago (Anxolabéhère, Kidwell & Periquet, 1988), probably by a horizontal transfer from *D. willistoni* (Daniels et al., 1990; Kidwell, 1993), and to have spread thereafter worldwide solely by vertical transmission (for review, see Engels, 1996, or http://www.wisc.edu/genetics/CATG/engels/Pelements/). This remarkable worldwide spread in such a short period must be attributed to the vigorous invasion dynamics of *P* elements in the genome. These dynamics led by the mid-1980s to near-uniformity over continental areas in *P* element-associated properties (see Introduction). This was in strong contrast to the clinal P-Q-M pattern with latitude found in eastern Australia (Boussy, 1987; Boussy & Kidwell, 1987). We saw this remarkable clinal pattern over only a few thousand kilometers as a natural experiment that was worth monitoring, as it was unlikely to be a stable situation and its changes would provide a case study of the long-term population dynamics of a transposable element. The current results for *P* element-associated traits, based on 1991-1994 collections, provide a snapshot of the populations five to ten years after the initial description of the clinal pattern, and probably less than 40 years after the introduction of *P* elements into Australia.

Correlations of hybrid dysgenesis measures

We used sn^w hypermutability as an assay to confirm that the gonadal dysgenesis we measured was in fact due to *P* element activity, rather than activity of *hobo* (Yannopoulos et al., 1987; Blackman & Gelbart, 1988) or some undescribed transposable element system. Very weak correlations (or no significant correlations) between the scores for the two assays would have indicated that they were not causally related. The correlation in cross A tests between gonadal dysgenesis and sn^w scores is highly statistically significant (Kendall's tie-corrected $\tau = 0.378$, $P < .0001$; see Figure 5 and Table 3). This is much lower than the correlation found by Engels for cross A among P strains selected for their high GD scores (Kendall's $\tau = 0.779$; Engels, 1984), but is comparable to that found by Kocur, Drier, and Simmons for isofemale lines collected in Minnesota, Iowa, and Pennsylvania between 1978 and 1983, and tested more than ten generations after capture (using two different strains for GD tests, Kendall's $\tau = 0.32$ and $\tau = 0.41$, both with $P < .01$; Kocur, Drier & Simmons, 1986). The correlation in cross A* tests between gonadal dysgenesis and sn^w scores is also highly statistically significant (Kendall's tie-corrected $\tau = 0.332$, $P < .0001$; see Figure 5 and Table 3). This is lower than that found by Heath and M.J. Simmons for cross A* using a set of inbred M' strains (Kendall's $\tau = 0.92$; Heath & Simmons, 1991), but comparable to the correlation found by G.M. Simmons for cross A* tests of 11 individual X chromosomes isolated from a California population and tested in background free of *P* elements ($\tau = 0.42$, $P < .05$; Simmons, 1987). The studies of Engels and of Heath and Simmons used sets of strains that included several of strong effect, and showed strong correlations, whereas the studies of Kocur and coworkers and of G.M. Simmons used lines of more modest effect and achieved more modest correlations.

Table 3. GD and sn^w correlations. Kendall's τ, with correction for ties, and the probability of type I error (P) were calculated from the raw data; the square of Pearson's correlation coefficient, r^2, was calculated using the transformed data (arcsine-square root transformations).

GD vs sn^w comparison	Kendall's τ, corrected for ties	r^2 of transformed data
Cross A, individual data	0.378 (P<.0001)	0.317
Cross A, population means	0.507 (P<.0001)	0.465
Cross A*, individual data	0.332 (P<.0001)	0.211
Cross A*, population means	0.501 (P<.0001)	0.561

The vast majority of our lines are of weak mean effect in either cross, and with high coefficients of variability for both GD and sn^w scores, thus yielding modest correlations. We therefore have also compared the population means for GD and sn^w scores (Table 3). For these mean comparisons, the correlations are higher (cross A Kendall's tie-corrected $\tau = 0.507$ and cross A* Kendall's tie-corrected $\tau = 0.501$, $P<.0001$ for both). In order to interpret these data, we also calculated a Pearson's r^2 value from the arcsine-square root transformed data. This transformation did not completely remove the dependence of the variance on the mean (data not shown), so we offer the r^2 value only for its interpretation as the proportion of the variance in one variable 'explained' by the other variable. For the mean cross A data, $r^2=0.465$, and for the mean cross A* data, $r^2 = 0.561$. Thus, on the order of 50% of the variability in sn^w scores can be accounted for

by knowing the GD scores for the same populations (or *vice versa*). We conclude that, while the GD and sn^w assays are extremely variable, crosses A and A* both are evaluating *P* element-related phenomena.

Changes in the cline with time

Monitoring the state of the P-M cline in eastern Australia may determine how rapidly such a post-invasion pattern changes in natural populations. One goal of such a study is to determine at what pace wild populations tend toward stable endpoints (with respect to *P* element parameters), and what those endpoints are. Our results show that, while there were substantial changes between 1986 and 1991, differences remain between populations in their *P* element-associated characteristics. There is also considerable variability within populations. Individual lines differ, sometimes a great deal, from other lines from the same locality. More northerly lines, from as far north as 27 ° SLat, are more likely to be P or weak P than lines from further south. Some lines from the south end of the coast (latitudes greater than ca. 36 ° SLat) are M in phenotype. Many lines from all localities are neutral, or Q. The extremes of M and P phenotypes seen among the 1983-1986 collections are not manifest among the 1991–1994 collections.

It seems unlikely that the cross A results would be due to changes in the reference strain. The reference M strains (Canton-S, in the earlier tests, and its derivative Canton-S-brn) are unlikely to have changed significantly in their P susceptibility since the strains completely lack *P* elements. While different strains completely lacking *P* elements can differ in their properties in MxP crosses (Daniels et al., 1987), there is no reason to believe that a highly inbred strain such as Canton-S and its derivatives would change in these properties in the absence of selection. Thus, the relatively low cross A GD scores would seem truly to reflect less P activity potential in the northern populations of Australian flies in 1991–1994 than was there five to nine years previous. Such is certainly the case for the northernmost populations (north of 26 ° SLat), whose mean P activity is now much less than that of their neighbors south of 26 ° SLat. Qualitatively, this means that the clinal pattern in cross A GD has decayed and changed to a weak bidirectional cline, but not disappeared, since 1986. It is seems likely that the situation is still in flux.

The differences between the present results in cross A* GD tests and the results obtained in 1983–1986 may be hypothesized to be due to loss of some of the P activity potential of the reference P strain (Harwich-w). Thus the apparent lack of extreme M strains in the 1991 collections may be due to a diminished P activity of the reference P strain, yielding lower cross A* GD scores. The Canton-S-brn × Harwich-w control crosses resulted in 100% GD, but since Harwich-w was initially an extremely strong P line, a moderate loss in P activity would still produce 100% GD and could not be detected in this control cross. We thus cannot exclude this possibility, but we likewise have no reason to believe that Harwich-w has lost any P activity. Furthermore, cross A* tests of some recently collected lines from South Australia and Tasmania show very high GD scores, suggesting that the Harwich-w reference strain has not lost its power as a P strain (RCW and IAB, unpublished data).

One of the most surprising results is that populations north of about 26 ° SLat no longer manifest the highest P activity potential, as they clearly did in the mid-1980s; indeed, they resemble lines from the southern part of the formerly P region, from 29 ° to 34 ° SLat. The intrinsic genomic dynamics of *P* elements might have led to such a change (Brookfield, 1991; Brookfield, 1996) if repressor-encoding *P* elements increased in frequency and autonomous *P* elements were reduced in number per genome due to selection. Why this would happen more strongly north of 26 ° SLat than between 26 ° and 29 ° SLat is not obvious. It is also possible that new introductions of relatively large numbers of flies from the south (or from another country or region that has flies resembling those from the south) produced the shift from P to weak P or Q phenotype. The northeastern coast of Australia is subtropical in climate, and summer high temperatures of >30 °C may be sufficient to reduce or eliminate local *D. melanogaster* populations, so that new introductions might encounter reduced numbers of 'native' flies at certain times of year and thus be able to strongly influence the resultant population's characteristics.

It is not known when *P* elements were first introduced into Australia, but it was probably after their introduction into other parts of the world. They are hypothesized to have entered *D. melanogaster* from *D. willistoni*, a caribbean and southeastern U.S. species, around 1950 or earlier (Daniels et al., 1990). *P* elements were already present in at least two Australian populations by the mid-1970s (Hunter Valley (near Newcastle, NSW, ca. 33 ° Slat; Kidwell, Kidwell & Sved, 1977; Black et al., 1987) and Para Wirra (near

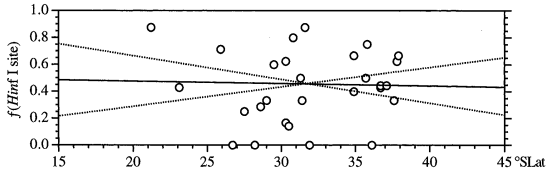

Figure 6. The frequency of the *Hin*f I site in the ND5 gene of mtDNA, tested at 25 localities. The regression line fitted to the raw data has the equation f(*Hin*f I site) = 0.511–0.002* ° SLat; the r^2 value of the fit is 0.0003. The dotted lines show the 95% confidence limits for the slope of the regression.

Adelaide, South Australia, ca. 35 ° Slat; Angus & Colgan, 1978); Angus & Raisbeck, 1979; Black et al., 1987), and might have been introduced a decade or more earlier. Despite a minimum period of at least two decades since their introduction, then, *P* element characteristics have not homogenized within a relatively small geographic area. Within this virtually linear region of only about 3000 km, gene flow should be fairly high, both by natural migration and by human transport of fruit. The degree of change seen between 1983–1986 and 1991–1994 suggests that P-M system uniformity might be achieved within the next decade.

A test of the introductions hypothesis

If the hypothesized initial introductions of flies differing in their *P* element-associated characteristics into the north and the south were large enough, relative to the resident population, the genetic characteristics of the introduced flies (besides *P* element-associated characteristics) should still be reflected in current populations. A mitochondrial DNA polymorphism has been detected in *D. melanogaster* that seems to differ between North American and European populations. European and central Asian populations typically have a high frequency of a *Hin*f I site in the ND5 gene of their mitochondrial DNA that is missing or at low frequency in North American populations (Rand, Dorfsman & Kann, 1994; Rand & Kann, 1996), and these haplotypes are thought to be essentially neutral. Thus, if the hypothesis were true that the P-M cline was established by large introductions of North American and European flies into the north and the south, respectively, there might also be a north-to-south increase in the frequency of mtDNAs with the *Hin*f I site, regardless of the frequencies of the *Hin*f I site in populations prior to the introduction. As shown in Figure 6 and Table 2, the frequency was quite variable among localities across latitude, with an intermediate mean (mean f(*Hin*f I site) = 0.457), but the overall pattern was not different from the null hypothesis of no relationship to latitude (P = 0.833).

Assuming that the RFLP difference cited between North American and European populations (Rand, Dorfsman & Kann, 1994; Rand & Kann, 1996) is representative of all populations from these continents, our result excludes the hypothesis that large introductions of North American and European flies into the north and the south, respectively, established the P-M cline. It does not, however, exclude the hypothesis that the cline was established by relatively small introductions. *D. melanogaster* was first reported from Australia *ca.* 100 years ago (Bock & Parsons, 1981), and was probably widespread by 1923 (Malloch, 1923), so it is likely that the introduced flies that first brought *P* elements encountered many 'native' flies. The infective dynamics of *P* elements in the genome are such that only a few flies would have been sufficient to introduce *P* elements into the *D. melanogaster* populations already present in Australia. Such small introductions would have had negligible impact on the frequencies of mtDNA haplotypes. Thus, our results have only excluded one version of an 'introductions' hypothesis.

Various workers have raised the possibility of using modified transposable elements to transform wild populations of pest or beneficial organisms (e.g., Kidwell & Ribeiro, 1992). In one scenario, a transposable element-derived vector carrying genes sufficient to render their bearer resistant to certain pesticides is used to transform a parasitoid or predatory species used for biological control of a pest insect (Hoy, 1994). Following spread of the vector conferring resistance in the target population, treatment with pesticides will spare the beneficial species, but will lower pest populations to levels that the biological control agent can effectively control. The success of such a plan depends on being able to predict the long-term dynamics of a transposable element vector in a host population. Our monitoring of the evolution of *P* element-associated characteristics in eastern Australian *D. melanogaster* populations should provide data relevant to such considerations.

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