Molecular Analysis of the P-M Gonadal Dysgenesis Cline in Eastern Australian *Drosophila melanogaster*

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ABSTRACT

The latitudinal cline in P-M gonadal dysgenesis potential in eastern Australia has been shown to comprise three regions which are, from north to south respectively, P, Q, and M, with the P-to-Q and Q-to-M transitions occurring over relatively short distances. The *P* element complements of 30 lines from different regions of the cline were determined by molecular techniques. The total amount of *P* element-hybridizing DNA was high in all lines, and it did not correlate in any obvious way with the P-M phenotypes of individual lines. The number of potentially full-sized *P* elements per genome was high in lines from the P regions, but variable or low among lines from the Q and M regions, and thus declined overall from north to south. A particular *P* element deletion-derivative, the *KP* element, occurred in all the tested lines. The number of *KP* elements was low in lines from the P region, much higher in lines from the Q region, and highest among lines from the M region, thus forming a cline reciprocal to that of the full-sized *P* elements. Another transposable element, *hobo*, which has been described as causing dysgenic traits similar to those of P-M hybrid dysgenesis, was shown to be present in all lines and to vary among them in number, but not in any latitudinal pattern. The P-M cline in gonadal dysgenesis potential can be inferred to be based on underlying clinal patterns of genomic *P* element complements. P activity of a line was positively correlated with the number of full-sized *P* elements in the line, and negatively correlated with the number of *KP* elements. Among Q and M lines, regulatory ability was not correlated with numbers of *KP* elements.

THE P-M hybrid dysgenesis system in *Drosophila melanogaster*, initially described as a complex set of observations of unknown etiology (KIDWELL, KIDWELL and SVED 1977), was given a causal basis with the discovery of the *P* element and its role in P-M hybrid dysgenesis phenomenology (BINGHAM, KIDWELL and RUBIN 1982). The initially described pattern of *P* element complements among strains, in which P strains carried many *P* elements and M strains completely lacked them (with rare exceptions), has proven naive. We now know that the molecular basis of the P-M system is quite complex. Most current phenotypically M strains bear many *P* elements, including full-sized ones, and degenerative processes form deletion-derivative *P* elements, some of which may participate in regulation (O'HARE 1985; KIDWELL 1986; BLACK *et al.* 1987). The picture is further complicated by the recent description of another possible system of hybrid dysgenesis, based on the *hobo* transposable element, that may mimic the P-M system in its phenotypic effects (YANNOPOULOS *et al.* 1987; BLACKMAN *et al.* 1987). The finding of dramatic variability in gonadal dysgenesis potential among popu-

lations along the east coast of Australia (BOUSSY 1987; BOUSSY and KIDWELL 1987) provides the opportunity to examine the molecular basis of hybrid dysgenesis characteristics in wild populations. Two hypotheses can thus be framed about the molecular basis of the clinal pattern: the first is that the gonadal dysgenesis cline is caused by a clinal pattern of the *P* element complements carried by the flies; the second is that the phenotypic cline is caused by a clinal pattern of *hobo* element complements. Since different size classes of P elements exist and seem to have different properties, these hypotheses predict that genomic complements would vary in a way correlated with the gonadal dysgenesis cline. This paper reports the results of investigations designed to test these hypotheses by asking whether such correlations exist.

The P-M hybrid dysgenesis system: The characteristic phenomena of P-M hybrid dysgenesis are manifested in the germ line of F₁ offspring from crosses between females of strains lacking regulatory ability (M strains) and males carrying active *P* elements (P strains), but usually not at all or only at very low rates in the reciprocal crosses, within strains, or in crosses between strains within the P or M types (KIDWELL, KIDWELL and SVED 1977; BINGHAM, KIDWELL and RUBIN 1982). Q strains are neutral, showing little or

no dysgenesis in crosses to M or P strains (KIDWELL 1979). The reference crosses used to classify strains are cross A [males of the strain are crossed to females of a strong M strain to measure P activity (KIDWELL, KIDWELL and SVED 1977)] and cross A* [females of the strain are crossed to males of a strong P strain to measure susceptibility to P activity (ENGELS and PRESTON 1980)]. The traits most usually scored in offspring of the reference crosses are F_1 gonadal dysgenesis at high developmental temperature, male recombination, and mutation rate of hypermutable alleles, but other traits are also characteristic of P-M hybrid dysgenesis, including embryonic lethality of F_2 eggs, chromosomal transmission ratio distortion, and chromosomal breaks and rearrangements [for reviews, see BREGLIANO and KIDWELL (1983) and ENGELS (1983)] Although cross A and cross A* would seem to measure independent parameters, results of extensive testing of lines suggest that they do not, since lines generally manifest hybrid dysgenic traits in one or the other cross (or neither) but not both. Almost all lines therefore can be classified along a phenotypic continuum from strong P (high cross A dysgenesis, no cross A* dysgenesis) to weak P to Q (no dysgenesis in either cross) to weak M to strong M (no cross A dysgenesis, high cross A* dysgenesis) (YAMAMOTO, HIHARA and WATANABE 1984; KIDWELL, FRYDRYK and NOVY 1983; KIDWELL 1986; BOUSSY and KIDWELL 1987).

The various traits of P-M hybrid dysgenesis are now known (*e.g.*, insertional mutations, chromosomal rearrangements, hypermutable alleles) or inferred (*e.g.*, gonadal dysgenesis, transmission ratio distortion) to be the result of activity of the P element (BINGHAM, KIDWELL and RUBIN 1982; ENGELS 1983), although it should be noted that certain of these traits do not always correlate well together (GREEN 1984; ENGELS 1984; SIMMONS 1987). Full-sized P elements are 2907 bp in length, but smaller P elements are common, and they are usually internally deleted derivatives of full-sized elements (O'HARE and RUBIN 1983; SAKOYAMA *et al.* 1985; BLACK *et al.* 1987). The formation of these degenerate P elements may be directly linked to the transposition process, as it can occur at high frequency during P element mobilization (VOELKER *et al.* 1984; DANIELS *et al.* 1985; DANIELS, STRAUSBAUGH and ARMSTRONG 1985; TSUBOTA and SCHEDL 1986; DANIELS *et al.* 1987). Phenotypically M strains completely lacking P elements are known as true M strains (KIDWELL 1985), to differentiate them from pseudo M or M' strains (ENGELS 1984) which contain P elements. The latter seem to predominately carry degenerate P elements (TODD *et al.* 1984; ANXOLABÉHERE *et al.* 1985; BLACK *et al.* 1987) and at least some pseudo M lines seem to have as few as seven detectable P elements (S. RONSSERAY, personal communication). Cur-

rently, the only strains that are known to completely lack P elements are old laboratory strains. The most recently collected true M strains from the wild are from 1966 in North America, 1969 in France, and 1974 in the USSR (ANXOLABÉHERE, KIDWELL and PERIQUET 1988). In all regions of the world, all M strains collected and tested since 1975 have been shown to bear P elements (TODD *et al.* 1984; ANXOLABÉHERE *et al.* 1985; BLACK *et al.* 1987; ANXOLABÉHERE, KIDWELL and PERIQUET 1988; S. RONSSERAY, personal communication; I. A. BOUSSY, unpublished data).

The putative *hobo* dysgenesis system: The *hobo* element (McGINNIS, SHERMOEN and BECKENDORF 1983; STRECK, MACGAFFEY and BECKENDORF 1986) is structurally similar to the P element in having short inverted terminal repeats. It is present in many copies per genome in some strains of *D. melanogaster*, but in few or none in others. As for the P element, internally deleted *hobo* elements of different sizes are very common. The *hobo* element has recently been claimed to generate hybrid dysgenesis traits, independently of the P-M system, in crosses between males of strains with many and females of strains with few or no *hobo* elements (YANNOPOULOS, STAMATIS and EEKEN 1986; YANNOPOULOS *et al.* 1987; BLACKMAN *et al.* 1987). As in the P-M system, the reciprocal cross is nondysgenic. The traits described include insertional mutations, male recombination, chromosomal deletions and rearrangements, and, most importantly for this study, gonadal dysgenesis.

The Australian gonadal dysgenesis cline: There is a strong latitudinal cline in gonadal dysgenesis potential, as determined in reference crosses, among eastern Australian populations of *D. melanogaster* (BOUSSY 1987). The clinal pattern comprises three regions of which each is qualitatively uniform: a northern region, in which populations are P (with single lines or individuals varying from strong P to Q), a middle region of Q populations (with little variability among lines or individuals), and a southern region of M populations (with lines and individuals varying from Q to strong M) (BOUSSY and KIDWELL 1987). The experiments reported here were designed to evaluate the P and *hobo* element complements of 30 lines from the eastern Australian gonadal dysgenesis cline, in order to test hypotheses concerning its causality.

MATERIALS AND METHODS

Reference lines: The following laboratory stocks were used as standards in reference crosses and for molecular analyses:

Harwich-w: A subline of the standard strong P line Harwich (KIDWELL, KIDWELL and SVED 1977) that bears a mutant allele at the *white* (*w*) locus that arose spontaneously and was isolated in the laboratory of one of us (M.G.K.);

Canton-S-red: A subline of the standard true M line

Canton-S that bears a recessive red eye-color mutation on the second chromosome that arose spontaneously and was isolated in the laboratory of M.G.K. (locus not identified; phenotype of homozygotes similar to rosy);

Canton-S-brn: A subline of Canton-S-red that bears, in addition to the red eye color mutant, a tan-to-brown eye color mutation on the third chromosome that arose spontaneously in the Canton-S-red line and was isolated in the laboratory of M.G.K. (locus not identified);

Sexi: A pseudo M strain collected in Spain in the 1970s, probably near Almuñecar (Almuñecar was a Phoenician trading post founded about 1000 B.C. and called at that time Sexi).

Isofemale lines: The 40 Australian isofemale lines used in the experiments were collected in 1983, 1985 and 1986 [see Figure 1 for names of localities; see BOUSSY (1987) and BOUSSY and KIDWELL (1987) for details]. The abbreviations used in the text and the figures refer to the locality names and the numbers of the isofemale lines. The 1983 collections used here are from six localities distributed along 2900 km of the eastern coast of Australia, from Cairns (16.9°SLat) in the north to Cygnet (43.2°SLat) in the south. The 1985 and 1986 collections used here are from eight localities along the southeast coast, from Coff's Harbour (30.3°SLat) in the north to Cann River (37.6°SLat) in the south. Collections from Coff's Harbour, Bateman's Bay (35.7°SLat) and Cann River are from the same sites as the 1983 collections. They have been kept in the laboratory in vials (population census size 20–50) on standard sugar-yeast-corn meal medium since their isolation.

Gonadal dysgenesis (GD) in reference crosses: The GD in reference crosses of the lines was retested (in 1987) for this study. Ten males of each line were mass mated at 29° to Canton-S-red virgins (cross A) and ten virgins of the line were mass mated at 29° to Harwich-w males (cross A*); egg laying was allowed for 2–4 days, until about 100 eggs had been laid. After eclosion and 2–3 days of maturation with fresh food, female offspring were dissected and the proportions of females with one or both ovaries undeveloped was determined as the GD score (BOUSSY 1987; BOUSSY and KIDWELL 1987). For the cross A tests, the mean number of females dissected per line ($\pm$SE) was 58.3 ± 2.6; for the cross A* tests, it was 68.0 ± 2.9. As controls, intrastrain sterilities at 29° were also evaluated. The mean number of females dissected per line was 54.4 ± 3.0, and the mean intrastrain GD was $4.4 \pm 1.0\%$ (mean $\pm$ SE) among all strains.

DNA preparation, restriction enzyme digestion and Southern blot analysis: The DNA tested for P elements was extracted within the same month (April 1987) in which the GD reference crosses were established for the 12 1983 lines and 18 1985–1986 lines. The DNA tested for hobo was extracted from the 30 1983 lines 5 months earlier (October 1986). DNA was extracted from 100 females of each line essentially as described by DANIELS and STRAUSBAUGH (1986). Restriction enzyme digestion of about 1 μg of DNA was performed according to the manufacturer's instructions (Bethesda Research Laboratories, Inc.). Digestion was stopped after 2–4 hr by addition of 1/10 volume of stop-dye [20% (w/v) Ficoll (type 400), 0.25% bromphenol blue (sodium salt), 0.1 M ethylenediaminetetraacetate (EDTA), 1.5% sodium dodecyl sulfate (SDS), 1 mM Tris (pH 7.4), 0.5% sodium azide].

Agarose-gel electrophoresis, gel blotting to nitrocellulose filters (Schleicher and Schuell), and preparation of DNA probes were performed essentially as described by RUSH-LOW, BENDER and CHOVNICK (1984). Before blotting, gels were soaked for 30 min in 0.5 μg/ml ethidium bromide in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA), then de-

stained for 30 min in 1× TAE. A photograph was taken of the stained gel illuminated with ultraviolet light on a transilluminator (UVP, Inc.). Filters were prehybridized at 42° for 2–4 hr with "prehyb" mixture. The mixture is 50% formamide (Fluka), 25% 20× SSPE (3.0 M NaCl, 0.2 M NaHPO$_4$, 0.04 M EDTA, pH 7.0), 1% SDS, 2.5% 100× Denhardt's solution [2% (w/v) Ficoll (type 400), 2% polyvinylpyrrolidone, 2% bovine serum albumin (Pentax fraction V; Sigma)], to which salmon testis DNA (type III, sodium salt; Sigma) was added to 250 μ/ml after denaturation at 90–95° for 10 min. Hybridization was carried out overnight at 42° in a similar mixture, but which contained 500 μg/ml of salmon testis DNA and ^{32}P-labeled probe DNA added, the DNA denatured by heating (90–95° for 10 min). Two posthybridization washes were of 10 min each in 2× SSPE, 1% SDS at room temperature, followed by two washes of 30 min each in 0.1× SSPE, 0.5% SDS at 42°. Excess liquid was then removed with blotting paper, and the damp filters were wrapped in plastic film and exposed to X-ray film (XAR-5; Eastman Kodak Co.).

The P probe used was pπ25.1 (O'HARE and RUBIN 1983), which contains a complete P element flanked by genomic DNA from the 17C region of the X chromosome of the D. melanogaster strain π2, inserted in the BamHI site of the plasmid pBR322. The genomic DNA of the probe corresponds to a unique fragment of the genome, and hybridization to the genomic DNA on the filter potentially offers the advantage of an internal control on each autoradiogram for the relative amount of DNA loaded in each lane. The hobo probe consisted of pRG2.6X (BLACKMAN et al. 1987), which contains the internal 2620-bp XhoI fragment from a 3.0-kb hobo element, inserted in the SalI site of the plasmid pUC8. Partial restriction maps of P and hobo elements, inferred from published sequences (O'HARE and RUBIN 1983; STRECK, MACGAFFEY and BECKENDORF 1986), are shown in Figure 2.

Scanning densitometry and statistical analyses: Each lane of the autoradiograms of the Southern blots was scanned with a scanning densitometer (E-C Corporation) to measure densities of individual bands, and a photograph of the UV-fluorescence of the ethidium bromide-stained original gel was scanned across the lanes at an intermediate position (avoiding RNA at the bottom of the lanes) to assess relative loading of DNA. The height of each peak corresponding to a band of interest on the Southern blot was measured on the printout, then divided by either the relative density of the ethidium bromide staining of the lane in the picture of the original gel (for Figures 3, 4 and 9), or the height of the peak corresponding to the 17C band on the autoradiogram (for Figure 7), in order to adjust for variation in amount of DNA loaded per lane. These adjusted heights were used as data representing the relative amount of DNA in a band.

Spearman's rank correlation coefficients (r_s, corrected for ties) and the probability of getting an absolute value of r_s equal to or exceeding that found given no correlation of rank (P) were calculated using a statistical program (Statview 512+, Brainpower, Inc.) on a microcomputer (Apple Macintosh Plus).

RESULTS

Gonadal dysgenesis (GD) in lines over time: Figure 1a shows the results of retesting in 1985 and 1987 the lines originally collected from the full length of the coast of eastern Australia, about 2900 km, and tested in 1983 (BOUSSY 1987). The lines shown were
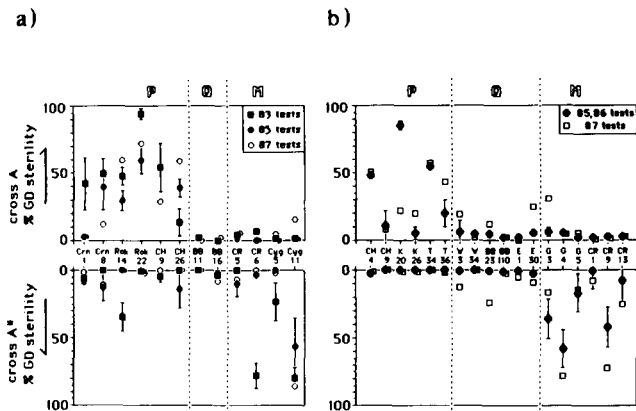
a)   b)

FIGURE 1.—Gonadal dysgenesis scores of cross A (above the horizontal axis) and cross A* (below the horizontal axis, reading down) for lines for which Southern blots are shown in Figures 3, 4 and 7. a) Data for the 1983 lines shown in Figure 3. Data for 1983 (represented by solid squares) and 1985 tests (solid diamonds) are means, with standard error brackets shown; data for 1987 tests (open diamonds) are from single mass tests. Lines are arranged from north to south [see BOUSSY (1987) for details of collections]. Abbreviations are as follows: Crn: Cairns; Rok: Rockhampton; CH: Coff's Harbour; BB: Bateman's Bay; CR: Cann River; Cyg: Cygnet. b) Similar data for the 1985–1986 lines shown in Figures 4 and 7. Data for 1985 and 1986 testing (represented by solid diamonds) are means, with standard error brackets shown; data for 1987 tests (open squares) are from single mass tests. Lines are arranged from north to south [see BOUSSY and KIDWELL (1987) for details of collections]. Abbreviations are as follows: CH, Coff's Harbour; K, Kempsey; T, Taree; W, Wollongong; BB, Bateman's Bay; E, Eden; G, Genoa; CR, Cann River.

originally tested by crossing individuals from them to reference strains, and averaging the GD scores of the F_1s; most of the lines were retested in this way in 1985. In 1987, mass crosses were made; thus the 1987 data do not have standard errors, whereas the earlier data do. Some lines show apparent changes in phenotype. For instance, Crn 1 dropped significantly in P activity between 1983 and later years, and CR 6 lost its high susceptibility to P activity. However, differences between the results of the tests done in different years cannot be meaningfully interpreted, since the numbers of individuals originally tested were small for each line, and the reference strains were different in different years. Most other lines did not change as much as did Crn 1 and CR 6, and no major qualitative changes are manifest overall. Lines from Coff's Harbour and north are P to weak P or Q, lines from Bateman's Bay are Q, and lines from Cann River and Cygnet are Q to M.

Figure 1b shows similar data for retests of lines originally collected and tested in 1985 and 1986 from coastal southeastern Australia, a region of about 900 km (BOUSSY and KIDWELL 1987). The 1985 and 1986 testing was done with individual crosses, the 1987 tests by mass crosses. One P region line, K 20, apparently lost considerable P activity between tests; the GD scores of other lines changed relatively little. In

these tests the same reference strains were used, although the 1985 tests were done in a different laboratory from the 1986 and 1987 tests. There were no qualitative changes in pattern between subsequent tests of the lines; lines from the northern region are still P to weak P, lines from the central region are still Q, and lines from the south are still Q to M.

Full-sized and deleted P elements in ACCI digests: *Acc*I cuts a full-sized P element near its ends, generating a single 2.4-kb internal fragment, as shown in Figure 2a. Figure 3 shows *Acc*I digests of genomic DNA from the lines for which GD scores are shown in Figure 1a, hybridized with the P element probe. All the lines tested show high levels of hybridization to P element DNA, as indicated by the total numbers and densities of bands in Figure 3. The Drosophila genomic DNA carried by the pπ25.1 probe appears to hybridize to two bands of very high molecular weight that, unfortunately, do not transfer well to the nitrocellulose filter, and can thus only barely be seen even on the original autoradiogram. However, ethidium bromide staining of the original gels showed that comparable amounts of DNA had been loaded in each lane.

The lines included in Figure 3 represent the full span of the P-to-Q-to-M cline of about 2900 km. The lines from the P region (lanes 3–8) all have a moderate number of potentially full-sized P elements, judging by the relative density of the 2.4-kb band. Their number is clearly less than in Harwich-w, a strong P strain (lane 1); this is expected since none of the lines had very strong P activity in cross A (Figure 1a) relative to that of Harwich-w (100%). The two Q lines from Bateman's Bay (lanes 9 and 10) appear to carry some potentially full-sized P elements, but apparently fewer than do any of the P lines. The four M region lines (lanes 11–14) have even fewer potentially full-sized P elements per genome; indeed, Cyg 11 appears to lack any DNA banding at 2.4 kb.

Figure 4 shows *Acc*I digests of genomic DNA from the lines from the southeastern coast for which GD scores are shown in Figure 1b, hybridized with the P element probe. The results are similar to those for Figure 3. In all lines, many P elements are present. Among P region flies (lanes 3–8) the number of potentially full-sized P elements is relatively constant (as shown by the intensity of the 2.4-kb band). The number of potentially full-sized P elements per genome is variable among the Q and M region lines, but in general this number is less than among the P region lines.

Figure 5 presents analyses of the relative densities of the 2.4-kb bands shown in Figures 3 and 4, as measured by scanning densitometry and corrected for DNA loading. The relative densities of the 2.4-kb bands decline with increasing latitude among the lines
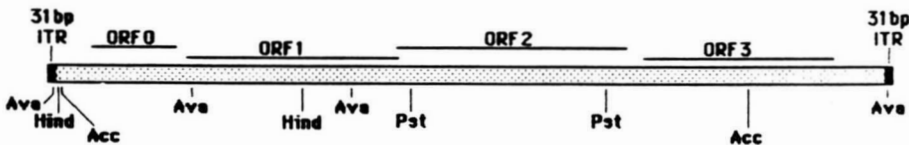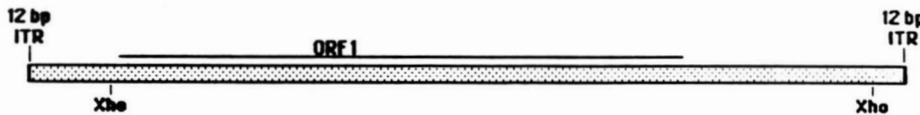
a) *P* element (2907 bp)

b) *hobo* element (3016 bp)

FIGURE 2.—Partial restriction maps of: a) the 2.9-kb *P* element, and b) the 3.0-kb *hobo* element. The restriction sites shown are based on published sequences of the *P* element-bearing plasmid pπ25.1 (O'HARE and RUBIN 1983) and the *hobo* element-bearing plasmid pH108 (STRECK, MACGAFFEY and BECKENDORF 1986). ITR means "inverted terminal repeat"; ORF means "open reading frame." The abbreviations for the enzymes are as follows; Acc = *Acc*I; Ava = *Ava*II; Hind = *Hind*III; Pst = *Pst*I; Xho = *Xho*I.
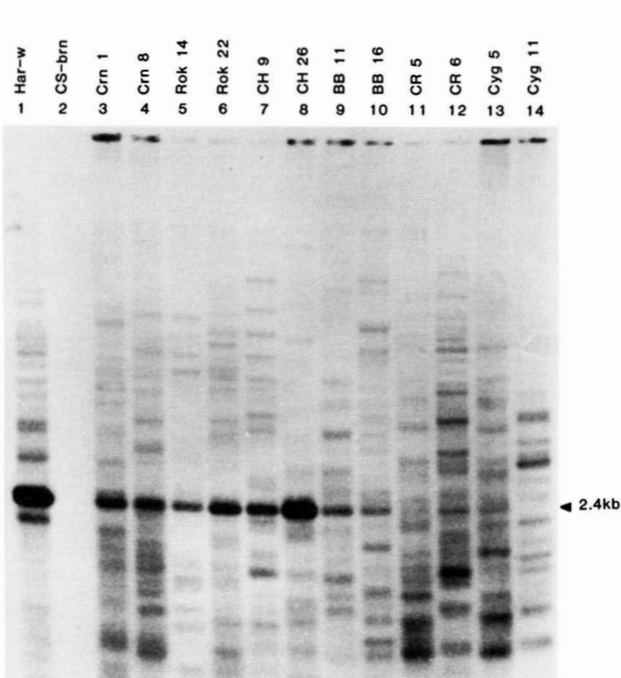

FIGURE 3.—Autoradiogram of *Acc*I digests of *ca.* 1 μg of genomic DNA from lines from the 1983 collections, probed with the *P* element-containing plasmid pπ25.1. Lanes 1 and 2 are control lanes containing DNA from Harwich-w and Canton-S-brn, respectively. The remaining lanes contain DNA from lines arranged as in Figure 1a, in order from north to south: 3) Crn 1; 4) Crn 8; 5) Rok 14; 6) Rok 22; 7) CH 9; 8) CH 26; 9) BB 11; 10) BB 16; 11) CR 5; 12) CR 6; 13) Cyg 5; 14) Cyg 11. Abbreviations are as for Figure 1a. The 2.4-kb *Acc*I fragment expected from a complete *P* element is indicated.

FIGURE 4.—Autoradiogram of *Acc*I digests of *ca.* 1 μg of genomic DNA from lines from the 1985 and 1986 collections from southeastern Australia, probed with the plasmid pπ25.1. Lanes 1 and 2 are control lanes containing DNA from Harwich-w and Canton-S-brn, respectively. The remaining lanes are arranged as in Figure 1b, in order from north to south: 3) CH 4; 4) CH 9; 5) K 20; 6) K 26; 7) T 34; 8) T 36; 9) W 3; 10) W 34; 11) BB 23; 12) BB 110; 13) E 1; 14) E 30; 15) G 3; 16) G 4; 17) G 5; 18) CR 1; 19) CR 9; 20) CR 13. Abbreviations are as for Figure 1b. The 2.4-kb *Acc*I fragment expected from a complete *P* element is indicated, as is a band of approximately 0.75 kb.

shown in Figure 3 (Figure 5a, $r_s = -0.82$, $P < 0.01$) and Figure 4 (Figure 5d, $r_s = -0.77$, $P < 0.002$). Figure 5, b and e, compares the cross A GD scores of the lines to their *P* element complements. Cross A GD correlates with the density of the 2.4-kb band in the two data sets ($r_s = 0.49$, $P ≈ 0.11$ for Figure 3 data, $r_s = 0.55$, $P ≈ 0.02$ for Figure 4 data). Examination of Figure 5, b and e, suggests that cross A GD is low unless the number of full-sized *P* elements exceeds a
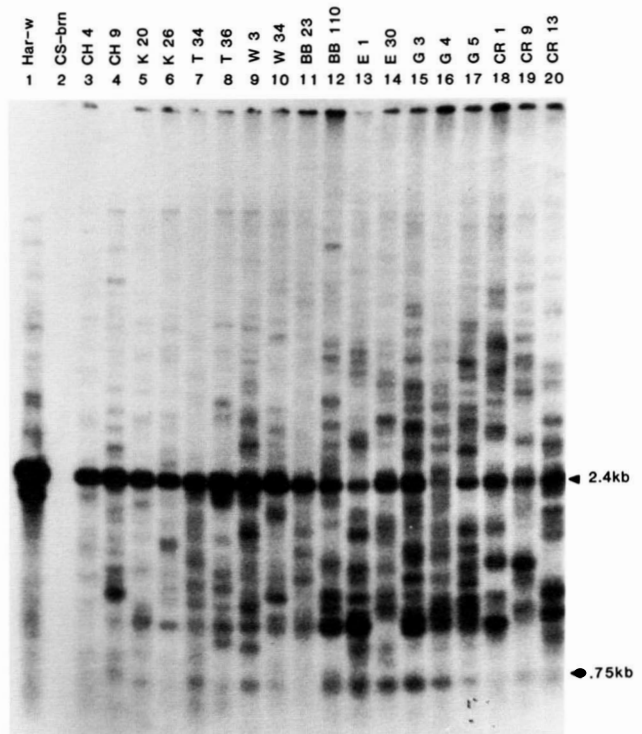
certain value (but that a high number of such elements can occur with low cross A GD). Similarly, as shown in Figure 5, c and f, cross A* GD correlates with the relative density of the 2.4-kb band ($r_s = -0.47$, $P ≈ 0.12$ for Figure 3 data, $r_s = -0.71$, $P ≈ 0.004$ for Figure 4 data). It appears that, for cross A* sterility to be high, it is necessary that the number of full-sized
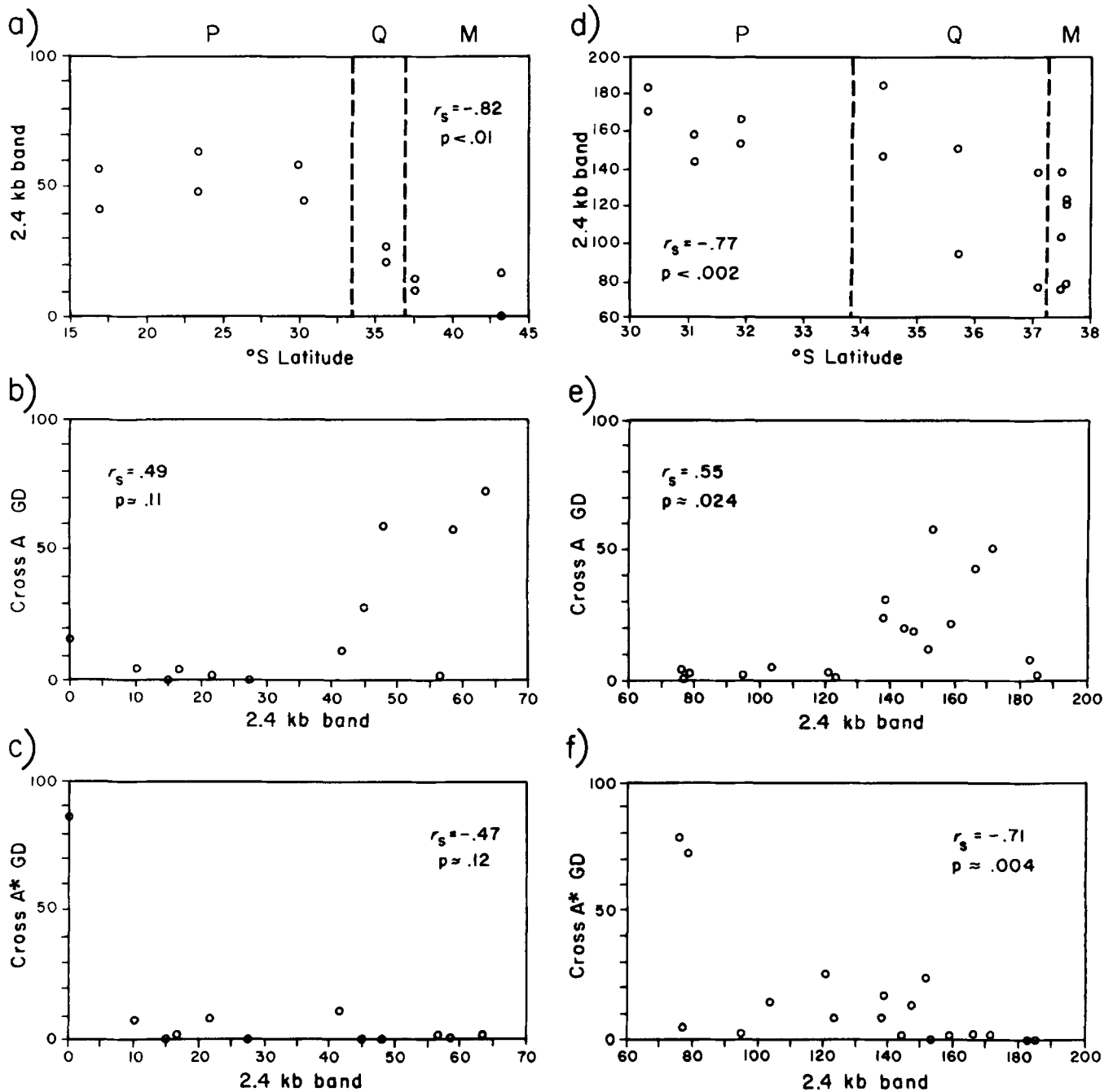
FIGURE 5.—Relative densities of the 2.4-kb bands shown in Figures 3 and 4, normalized with respect to the relative densities of ethidium bromide staining of the lanes in the original gel, and compared to other data for each line. Spearman's rank correlation coefficient (r_s) is shown with each graph, along with the probability (P) of exceeding that absolute value if no correlation exists. a) Relative densities from Figure 3 plotted against degrees south latitude of the collection localities of the lines. b) Cross A GD scores of each line plotted against relative densities of the 2.4-kb bands from Figure 3. c) Cross A* GD scores of each line plotted against relative densities of the 2.4-kb bands from Figure 3. d) Relative densities from Figure 4 plotted against degrees south latitude of the collection localities of the lines. e) Cross A GD scores of each line plotted against relative densities of the 2.4-kb bands from Figure 4. f) Cross A* GD scores of each line plotted against relative densities of the 2.4-kb bands from Figure 4.

P elements be quite low, but it may not be sufficient or causal (among the Q and M region lines only of Figures 3 and 4, cross A* GD and the relative density of the 2.4-kb band do not correlate well; $r_s = -0.52$, $P \approx 0.24$, and $r_s = -0.29$, $P \approx 0.33$, respectively). There seem to be thresholds for these two effects, rather than linear relationships with the number of full-sized P elements.

Classes of P elements revealed in AvaII digests: AvaII cuts a full-sized P element at four sites, to generate three internal fragments of 478, 544 and 1838 bp in length; the KP element (a particular deletion-derivative P element found at high frequency in European populations; BLACK et al. 1987) is cut at three sites, generating internal fragments of 478 and 628 bp in length (see Figure 6a). Figure 7 shows AvaII

a)

P element
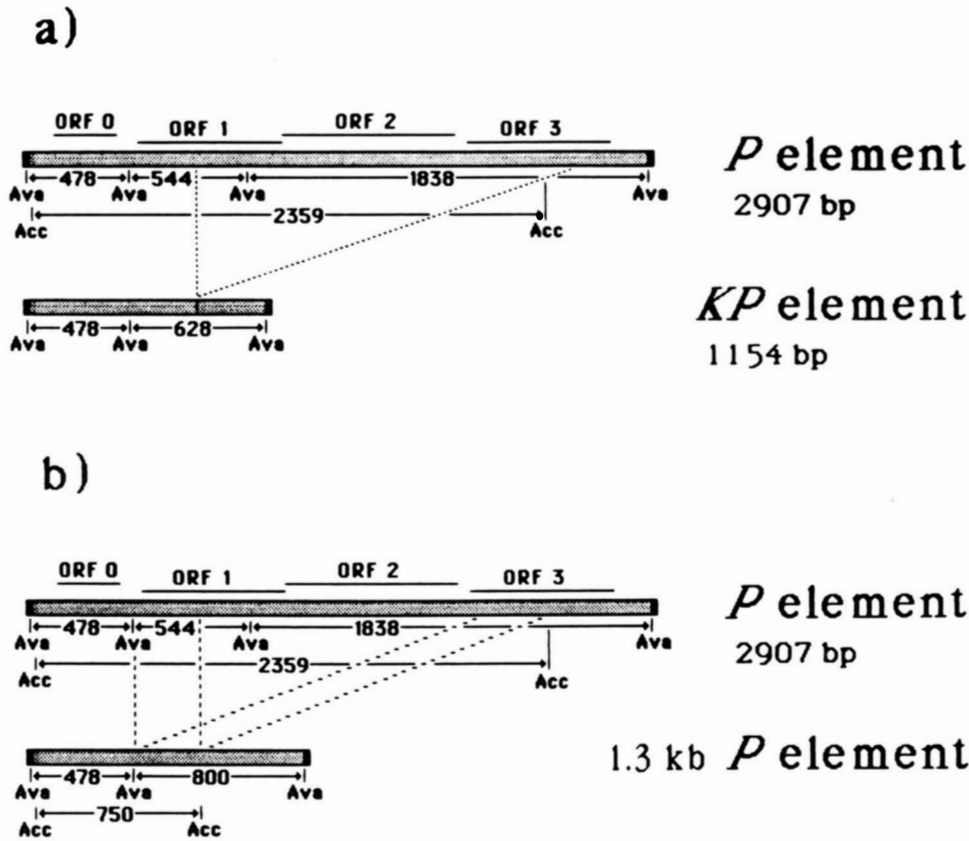2907 bp

KP element
1154 bp

FIGURE 6.—Structures of *P* elements. a) Diagram of the complete *P* element, showing the open reading frames (ORF), the *Acc*I and *Ava*II sites, and the sizes of the restriction fragments generated by them (based on O'HARE and RUBIN 1983), and the *KP* element, with its *Ava*II sites and the restriction fragments generated by them (based on BLACK *et al.* 1987). Dotted lines indicate the 1753 bp internal deletion that derives a *KP* element from a complete *P* element. b) A similar diagram, showing the hypothesized structure of a 1.3-kb *P* element, derived from the complete *P* element by a single internal deletion of 2.6 kb. The *Acc*I and *Ava*II sites are indicated, as well as the fragments generated by them. The dotted lines show the probable limits for the break sites of the deletion.
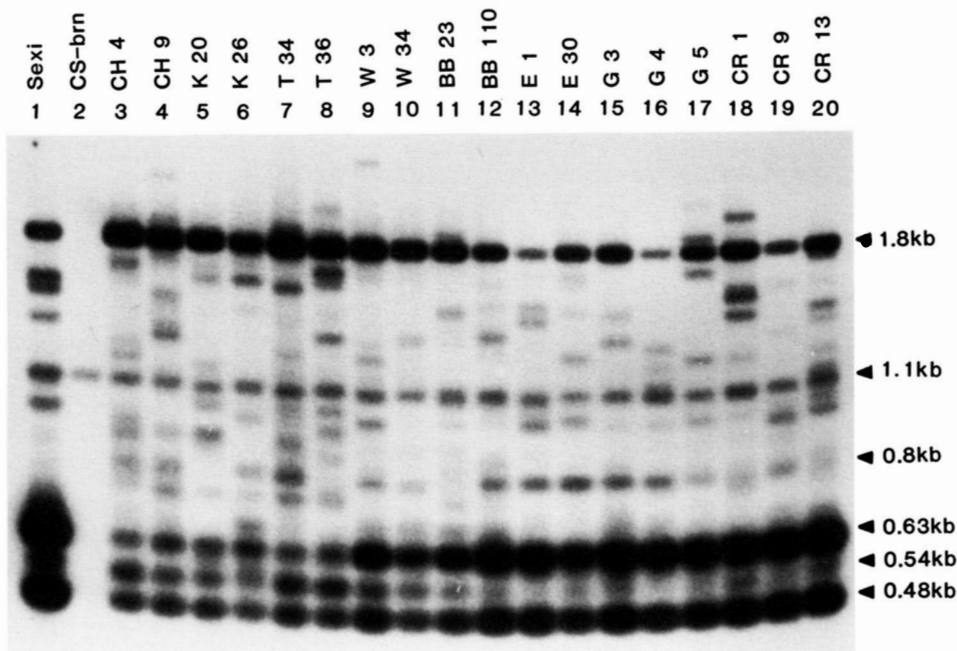
b)

P element
2907 bp

1.3 kb *P* element

FIGURE 7.—Autoradiogram of *Ava*II digests of ca. 1 µg of genomic DNA from the same lines as in Figure 4, probed with the plasmid pπ25.1. Lanes 1 and 2 contain DNA from Sexi and Canton-S-brn, respectively. The remaining lanes are arranged as in Figure 4. The 1.8-, 0.54- and 0.48-kb fragments expected from a complete *P* element are indicated, as are the 0.63 and 0.48 kb fragments expected from a *KP* element. Also indicated are the band of approximately 1.1 kb corresponding to the genomic DNA of the pπ25.1 plasmid, and a band of approximately 0.8 kb.

digested genomic DNA from the same lines as in Figure 4, hybridized to the *P* element probe. The *Drosophila* genomic DNA carried by the pπ25.1 plasmid is seen to hybridize to a band of about 1.1 kb; comparison between lanes of the density of this band allows an estimate of the relative amount of DNA loaded in each lane.

The densities of the 1.8- and 0.54-kb bands, representing full-sized *P* elements plus any other elements without deletions in the 1.8 and 0.54 regions, decline

with increasing latitude. The 0.63-kb band, representing the *KP* element (BLACK *et al.* 1987), is present in all the Australian lines tested and its relative density rises dramatically with increasing latitude. (The 0.48-kb band represents both the full-sized *P* elements and *KP* elements and any others not deleted in the 22–500 region.) Lane 1 on Figure 7 contains genomic DNA from Sexi, a strain from Spain that has been shown to bear many *KP* elements (BLACK *et al.* 1987); the prominent 0.63-kb band matches in position those

seen in the Australian lines. Comparison of Figures 4
and 7 reveals that, although Figure 4 shows a "ladder"
of *P* element-hybridizing DNA in each lane, indicating
many *P* elements besides the potentially full-sized *P*
elements banding at 2.4 kb, Figure 7 has only a few
bands besides those representing potentially full-sized
P elements (at 0.48, 0.54 and 1.8 kb) and *KP* elements
(at 0.48 and 0.63 kb). The relative lack of bands at
positions other than 1.8, 0.63, 0.54 and 0.48 kb in
Figure 7 indicates that the large majority of *P* ele-
ments in each Australian line comprises only full-sized
P elements and *KP* elements.

A comparison of Figures 4 and 7 also indicates that
a band at about 0.75 kb on Figure 4 and a band at
about 0.8 kb on Figure 7 have an identical pattern of
occurrence and density among the lines tested. The
band is present in lanes 7, 9, 10 and 12 through 20
on both figures, and is strongest in lanes 12 through
16. Figure 6b shows that a deletion of about 1.6 kb
from a full-sized *P* element, somewhere between the
*Ava*II site at position 500 and the *Acc*I site at 2410,
would generate a 1.3-kb *P* element that would mani-
fest bands of the sizes seen.

Figure 8 presents analyses of relative densities of
bands from Figure 7. The densities of the 1.8-kb band,
representing potentially full-sized *P* elements, decline
with increasing latitude ($r_s = -0.67$, $P \approx 0.006$; as do
the 0.54-kb bands, $r_s = -0.74$, $P \approx 0.002$, data not
shown), corroborating the results from the *Acc*I analy-
sis above. The relative densities of the 0.63-kb band,
representing *KP* elements (BLACK *et al.* 1987), rise
dramatically with increasing latitude ($r_s = 0.83$, $P \approx 0.001$).

The relative densities of the 1.8-kb band from Fig-
ure 7 correlate well with the relative densities of the
2.4-kb band for each line seen in Figure 4 ($r_s = 0.93$,
$P < 0.001$; as do the 0.54-kb band densities, $r_s = 0.88$,
$P < 0.001$, data not shown), indicating that these
*Ava*II fragments of the *P* element measure the fre-
quency of potentially full-sized *P* elements nearly as
well as does the 2.4-kb *Acc*I fragment. Accordingly,
the relationships of cross A and A* GD to the relative
densities of the 1.8-kb fragments are essentially as
described above for the 2.4-kb fragment of Figure 4;
cross A GD appears to be manifested above a certain
frequency of full-sized *P* elements (Figure 8b), and
cross A* GD appears to be manifested below a certain
frequency (Figure 8c).

The frequency of *KP* elements correlates with the
phenotype, but the correlation seems to involve
thresholds. High cross A GD scores occur only among
lines with a very low frequency of *KP* elements (Figure
8e), and cross A* GD only occurs among lines with
KP elements above a certain number (Figure 8f).
Among lines from the Q and M regions alone, the

relationship of *KP* frequency to cross A* GD appears
random ($r_s = 0.14$, $P \approx 0.65$).

The *hobo* element in Australian lines: Figure 9
shows the results of *Xho*I digests of genomic DNA
probed with *hobo* DNA. The lines are from 1983
collections spanning the GD cline (including the lines
shown in Figures 1a and 3), as well as the reference
strains Harwich-w and Canton-S-red. Our reference
M strain, Canton-S-red, is shown to completely lack
full-sized or smaller *hobo* elements (Figure 9, lanes 2).
Since Canton-S-red is the female parent in cross A
tests, *hobo* dysgenesis could potentially contribute to
the gonadal dysgenesis of cross A tests if active *hobo*
elements exist in the Australian lines (YANNAPOULOS
et al. 1987). Our reference P strain, Harwich-w, seems
likewise to lack full-sized *hobo* elements, although it
has some smaller *hobo* sequences (Figure 9, lanes 1).
Since Harwich-w is the male parent in cross A*, *hobo*
dysgenesis should not be contributing to cross A*
gonadal dysgenesis.

While all of the Australian lines contain potentially
full-sized (as shown by the 2.6-kb bands of Figure 9)
and smaller *hobo* elements, and certain smaller *hobo*
elements seem to be common in some localities, there
is no latitudinal pattern to the frequencies of smaller
or of full-sized *hobo* elements (for full-sized *hobo* ele-
ments, as measured by the density of the 2.6-kb bands
in Figure 9, the correlation with latitude is $r_s = -0.20$,
$P \approx 0.27$). Since the *hobo* system seems to be inde-
pendent of the *P* element system (YANNOPOULOS, STA-
MATIS and EEKEN 1986; YANNOPOULOS *et al.* 1987),
and since the *hobo* complements of the Australian lines
do not vary in a clinal pattern, it seems unlikely that
hobo could contribute a clinally varying effect in ref-
erence crosses.

DISCUSSION

The 30 Australian lines examined here range from
P to Q to M in phenotype, and derived from 18
localities along the eastern coast. The information
provided by the Southern blot analysis resolves the
issues originally raised, namely, whether the gonadal
dysgenesis clinal pattern correlated with genomic
complements of *P* or *hobo* elements. The genomic
complements of potentially full-sized *P* elements and
of *KP* elements both show clinal patterns with latitude,
and correlate with the GD phenotypes observed. In
contrast, the genomic complements of *hobo* elements
do not show a latitudinal pattern. Therefore one can
infer that the GD cline is, in fact, due to underlying
clinal patterns of *P* elements and not due to *hobo*
elements. The data are also relevant to broader ques-
tions of *P* element function and phenotype.

The Southern blots show that the overall amount
of *P* element-hybridizing DNA in all the lines tested
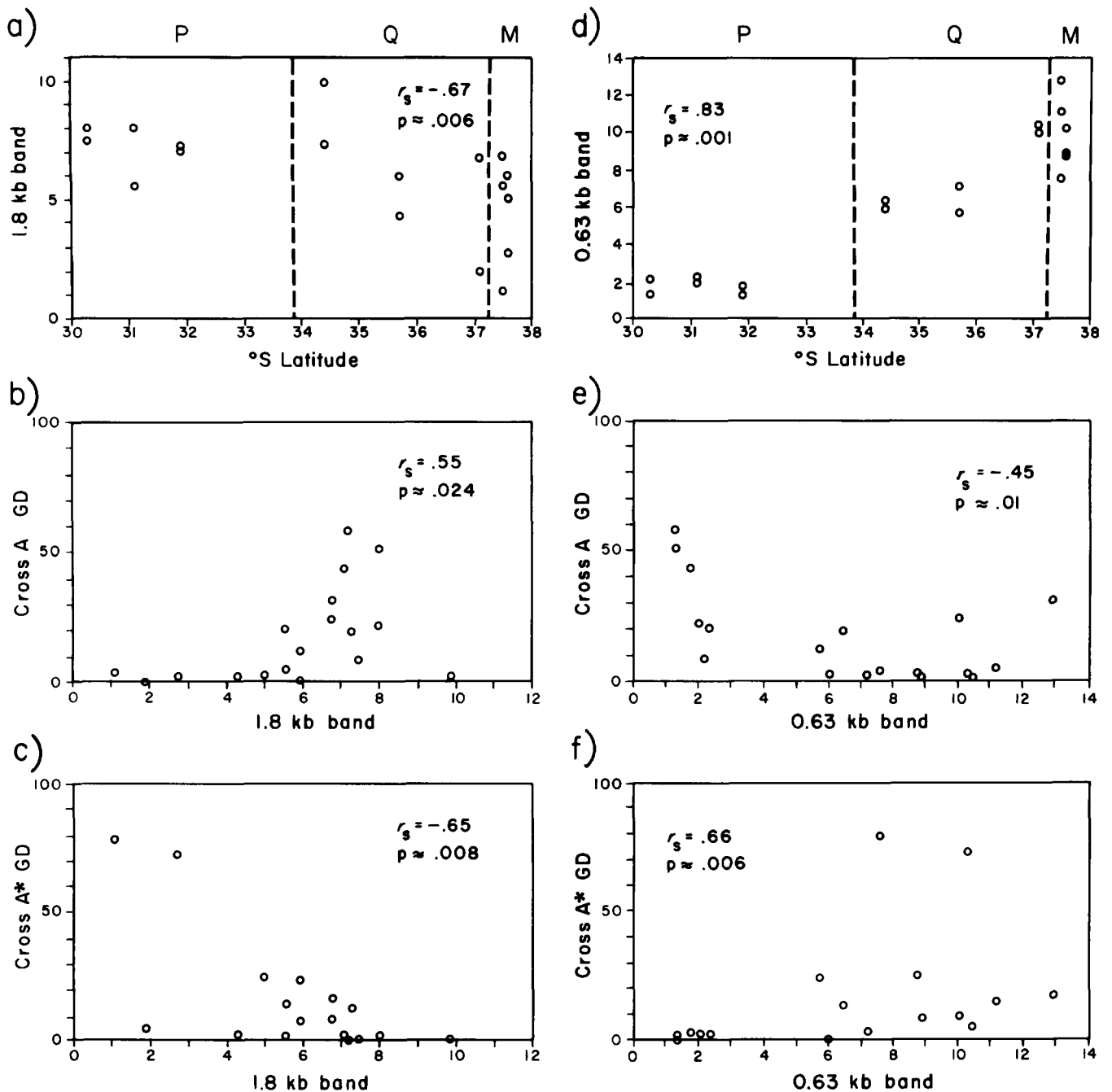is similar to the amount in the strong P control (Har-

FIGURE 8.—Relative densities of the 1.8- and 0.63-kb bands shown in Figure 7, normalized with respect to the densities of the 1.1-kb bands (representing single copy genomic DNA), and compared to other data for each line. Spearman's rank correlation coefficient (r_s) is shown with each graph, along with the probability (P) of exceeding that absolute value of r_s if no correlation exists. a) The relative densities of the 1.8-kb bands plotted against degrees south latitude of the collection localities of the lines. b) Cross A GD scores of each line plotted against the relative densities of the 1.8-kb bands. c) Cross A* GD scores of each line plotted against the relative densities of the 1.8-kb bands. d) The relative densities of the 0.63-kb bands plotted against degrees south latitude. e) Cross A GD scores of each line plotted against the relative densities of the 0.63-kb bands. f) Cross A* GD scores of each line plotted against the relative densities of the 0.63-kb bands.

wich-w). Furthermore, there is no obvious pattern with latitude or phenotype of variability among the lines tested here. A simple hypothesis that P lines have many *P* elements and M lines few, with Q lines intermediate, is thus excluded in this case.

Such an hypothesis seems naive, since it has been shown that different proportions of complete and

incomplete elements exist in strains with different P-M phenotypes (BINGHAM, KIDWELL and RUBIN 1982; TODD *et al.* 1984; SAKOYAMA *et al.* 1985) and that certain incomplete elements may play a direct role in regulation (NITASAKA, MUKAI and YAMAZAKI 1987; BLACK *et al.* 1987; H. M. ROBERTSON and W. R. ENGELS, personal communication), and yet a correla-
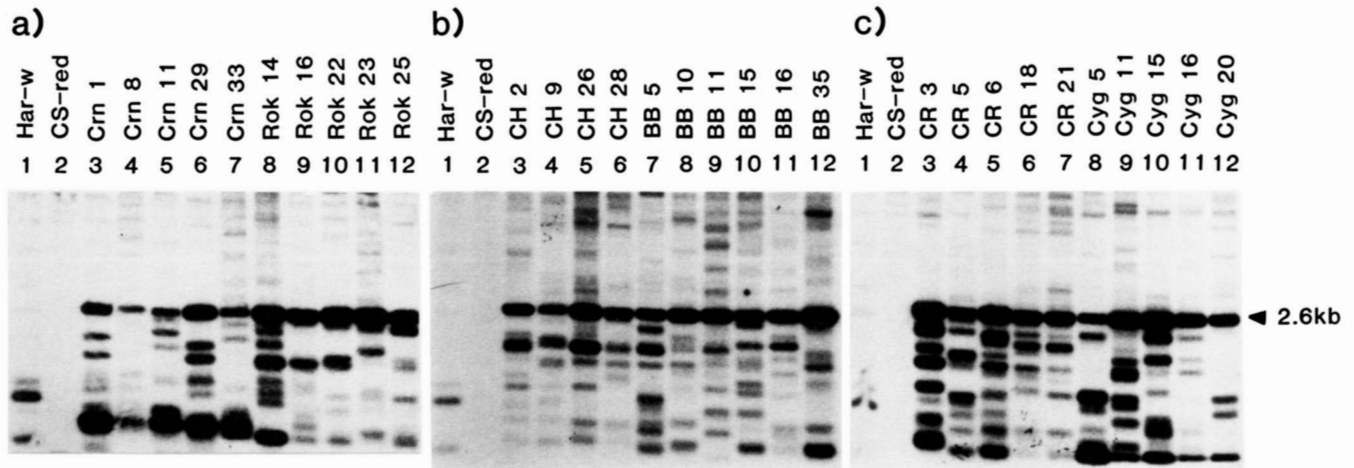
FIGURE 9.—Hybridizations of a *hobo* element probe (pRG2.6X) to *Xho*I-digested genomic DNA from a representative set of 1983 lines, shown as autoradiograms. On each autoradiogram, lanes 1 and 2 contain DNA from Harwich-w and Canton-S-red, respectively. The 4–6 lines shown from each of the six localities include the 12 lines for which data are shown in Figures 1a and 3. The localities are arranged in order from north to south. The lanes are as follows: a) 3) Crn 1; 4) Crn 8; 5) Crn 11; 6) Crn 29; 7) Crn 33; 8) Rok 14; 9) Rok 16; 10) Rok 22; 11) Rok 23; 12) Rok 25; b) 3) CH 2; 4) CH 9; 5) CH 26; 6) CH 28; 7) BB 5; 8) BB 10; 9) BB 11; 10) BB 15; 11) BB 16; 12) BB 35; c) 3) CR 3; 4) CR 5; 5) CR 6; 6) CR 18; 7) CR 21; 8) Cyg 5; 9) Cyg 11; 10) Cyg 15; 11) Cyg 16; 12) Cyg 20. Abbreviations are as in Figure 1a. The 2.6-kb fragment expected from *Xho*I digestion of a 3.0-kb *hobo* element is indicated.

tion has been found in Eurasian lines between phenotype and *P* element numbers. ANXOLABÉHERE *et al.* (1985) examined the genomic DNA of individual flies and found that, among the weak P, Q and M strains they tested from Eurasia, the mean degree of hybridization to *P* element probes correlated with the P-M phenotype of the flies. For example, the correlation with cross A* GD was $r_s = -0.83$ ($P \approx 0.002$), thereby showing a cline from west to east, with most hybridization among weak P and Q lines from France and least hybridization among M lines from central Asia. Their "P1" probe was the 0.84-kb *Hind*III-*Hind*III fragment of the *P* element, which is situated at the left end of the element (see Figure 2a). This P1 probe should have detected nearly all *P* elements in the genome, since most *P* element deletion-derivatives have been found to have more or less central deletions and are therefore conserved near their ends. Examining the same set of lines by *in situ* hybridization of *P* element probes to larval salivary gland chromosomes, S. RONSSERAY (personal communication) has estimated the total number of *P* elements per haploid genome to be about 30 in lines from western Europe (including France), declining with longitude to only about 12 in flies from Chimkent (USSR; 70°ELong), and seven in flies from Alma Ata (USSR; 77°ELong). These correlations suggest a role in regulation for the total number of elements per genome, and seem to bear on the "titration" model of regulation proposed by SIMMONS and BUCHOLZ (1985), in which episomal and chromosomal *P* elements compete for limited transposase. However it seems more likely that the correlation is not causal, and that a model based on the kinds of elements present in each genome (as well

as their numbers) would yield better predictions.

ENGELS (1984; his Figure 2) presented data on North American P lines that are in contrast to the data from Eurasian Q to M lines. He reports on gonadal dysgenesis and *sn^w* hypermutability for a set of inbred, wild-derived P lines. These two measures of P activity are well-correlated among the lines tested, but they do not correlate well with the total number of autosomal *P* elements in each line (as estimated by *in situ* hybridization to larval salivary gland chromosomes). The data from North America for P lines and from Eurasia for Q to M lines thus seem to differ. The Australian lines examined here have phenotypes that span the P-to-M continuum, and among them there is no correlation of P-M phenotype and total number of *P* elements; they therefore seem to extend the results of ENGELS (1984) but contradict the results of ANXOLABÉHERE *et al.* (1985) and S. RONSSERAY (personal communication). The discrepancy between Australian and Asian M lines implies that there are different ways to achieve an M phenotype (besides the true M and pseudo M types already known). It may well be that the histories of introduction of *P* elements into the populations in question differ.

An alternative hypothesis of P-M phenotype based on types of *P* elements is suggested by the data of TODO *et al.* (1984; their Table 1) from a set of wild-derived lines. Their data show a strong correlation between the P-M phenotype, assayed by gonadal dysgenesis tests, and the number of elements per genome. The latter number is estimated by the number of bands hybridizing to an internal, central fragment of the *P* element (the *Pst*I-*Pst*I fragment; see Figure 2) on Southern blots of genomic DNA. This central-

fragment criterion can be interpreted as an upper estimate of the actual number of full-sized P elements. Their results showed that the cross A* GD of a line was high unless the line had a certain threshold number of the internal fragments per genome (the correlation of cross A* GD with the estimate of the internal fragment copy number is $r_s = -0.59$, $P \approx 0.02$). Likewise, if a line had more than a certain number of the internal fragments (a higher number than the A* threshold) it was likely to manifest cross A GD (overall, the cross A GD correlation with internal fragment numbers is $r_s = 0.89$, $P \approx 0.002$). All their lines had comparable amounts of total P element-hybridizing DNA when probed with longer probes. Assuming that the amount of DNA loaded per lane was similar, the correlations found in their data suggest the hypothesis that the degree of P activity measured in phenotypic tests of P-M hybrid dysgenesis correlates with the number of full-sized P elements (as opposed to the total number of P elements of all sizes) per genome.

The present results would seem to corroborate this hypothesis. The number of full-sized P elements correlates with both the P activity (cross A GD) and susceptibility to P activity (cross A* GD) among the Australian lines tested. Furthermore, there seems to be a threshold in number below which susceptibility to P activity is possible, and another higher threshold above which P activity is possible, just as found by TODO et al. (1984). A role of full-sized P elements in P activity is reasonable, but the lack of a significant correlation between cross A* GD and full-sized P element number among the Q and M lines alone suggests that full-sized P elements do not have a direct role in regulation of P activity. This latter idea is consistent with certain experimental data. For instance, in lines into which P elements have been introduced by crossing of lines or by chromosomal contamination (A. G. GOOD, H. BROCK, T. A. GRIGLIATTI and D. A. HICKEY, unpublished data; KIDWELL, KIMURA and BLACK 1988) or by transformation (W. R. ENGELS and C. R. PRESTON, personal communication; DANIELS et al. 1987), regulation often lags well behind the build-up of large numbers of full-sized P elements or of P activity. Indeed, DANIELS et al. (1987) reported one transformed line that had not developed complete regulatory ability even after 34 months (cross A* GD of 60%), although it had a large number of full-sized and deleted P elements per genome, and generated 100% GD in cross A. It seems likely that either critical numbers of elements or, more likely, a particular class of elements is necessary for regulation.

Other evidence suggests that certain incomplete or mutated P elements may play a direct role in regulation. SAKOYAMA et al. (1985) showed that a Japanese Q strain with strong regulation (no cross A* GD) completely lacked full-sized P elements, implying that incomplete elements must be involved in the regulatory ability of this strain. Regulatory ability has been directly demonstrated by H. M. ROBERTSON and W. R. ENGELS (personal communication) for certain P elements that carry mutations in the 2–3 intron splice region or mutations that put open reading frame (ORF) 3 out of frame (LASKI, RIO and RUBIN 1986). NITASAKA, MUKAI and YAMAZAKI (1987) have isolated a deleted P element they claim to be associated with regulation in a Japanese Q strain (although their initial screening to isolate it may have ignored other regulatory elements). The putative regulating element they isolated has a deletion that removes intron 2–3 and most of ORF 3, and thus is structurally similar to the mutant elements tested by H. ROBERTSON and W. R. ENGELS (personal communication). These may represent a class of regulatory P elements. The 1.2-kb KP element (BLACK et al. 1987) has been implicated in regulation of transpositional activity, and would seem to represent another class of potentially regulatory P elements, since it is quite different structurally from the ORF 3 mutated elements (see Figure 6a).

It must be noted that P-M regulation is probably not a single phenomenon, since the type of regulation called P cytotype has been shown to be at least partly maternally inherited (ENGELS and PRESTON 1979), but another sort of regulation, apparently associated with KP elements, seems to be strictly chromosomally inherited (KIDWELL 1985; BLACK et al. 1987). The regulation associated with elements mutated in ORF 3, cited above, may correspond to P cytotype, but this has not yet been demonstrated. Maternally and chromosomally inherited types of regulation, of possibly divers sorts, could of course be present within any one strain. It is also possible that differences in background genotypes of strains could affect P element activity or regulation (DANIELS et al. 1988). The biochemical basis of any type of regulation is not currently known, and the inheritance (whether maternal or chromosomal) of susceptibility to P activity has not been examined in the Australian lines.

It is immediately apparent from Figure 7 that the Australian lines tested all carry KP elements, and that the large majority of all elements present in most lines comprises only full-sized P elements and KP elements. In some lines, 60–80% of the total hybridization is accounted for by these two classes. This is consistent with the results of BLACK et al. (1987) in European and Asian lines. The clear preponderance of only these two types strongly suggests that the KP element has special properties, whether regulatory [as argued by BLACK et al. (1987)] or transpositional (a special aptitude for replicative transposition).

The number of KP elements per genome in lines from the P region is much less than in lines from the Q and M regions (Figure 7, lanes 3–8; this result is

corroborated by results from other lines, not shown). The overall pattern is a reciprocal latitudinal cline in KP elements compared with that of full-sized P elements. The finding of KP elements in P lines, albeit at low levels, is in contradistinction to the findings of BLACK *et al.* (1987). Among the lines they examined from North America, Europe and Asia, no P lines were found that had any KP elements. They have hypothesized that, upon introduction of P elements to a true M population, the population responds by developing one of two types of regulation, either cytotype (largely maternally inherited) or that due to KP elements (chromosomally inherited). If so, then the Australian lines may reflect introductions from two or more sources that differed in their mode of regulation, as do European and North American populations. The presence of a few KP elements per genome in the P lines then might be the result of diffusion into the already stabilized P populations from the likewise stabilized Q or M populations containing many KP elements. A prediction of this theory is that the P populations of the north coast should slowly become more and more Q- or M-like, as the mean frequency of KP elements increases in them by diffusion from the south. Testing this prediction will require more collections from Australia. This theory also predicts that the inheritance of regulatory ability should differ between lines from the P and from the Q and M regions, the former (P) having a preponderance of cytotype regulation and the latter (Q and M) a preponderance of KP element regulation. This prediction has not yet been tested.

As noted above, an increased number of KP elements per genome correlates with a reduced level of P activity (cross A GD) among the lines examined. This is consistent with a simple regulatory or repressor role for KP elements, in which a product of the element competes with, blocks, or in some way impedes the activity of the transposase of the complete P elements (KP elements retain in frame the end of ORF 3 and therefore could make a protein product (BLACK *et al.* 1987)). However in going from the Q region lines to the M region lines, one finds more KP elements (Figure 8d), not fewer as would be expected if the KP elements provided a repressor product. Furthermore, among the Q and M region lines, higher numbers of KP elements per genome do not correlate with higher regulatory ability (Figure 8f). One interpretation of this observation is that KP element regulation may operate on potentially active P elements in the same genome with the KP elements (as in cross A), but not on active P elements introduced in another genome (as in cross A*).

The 750-bp band in the AccI digests of Figure 4 and the 800-bp band in the AvaII digests of Figure 7 occur with a similar pattern among lines in the two autoradiograms, suggesting that they are due to the same set of deleted P elements, present in some lines at moderate copy number. Analysis of the sequence of the full-sized P element shows that a 1.6-kb deletion, somewhere between the AvaII site at 500 and the AccI site at 2410, could generate the bands seen in the two digests. Figure 6b shows the hypothesized structure of such an element, with dotted lines indicating the probable limits of the deletion. The presence of this element in many copies in several lines from a region of several hundred kilometers could indicate a special role for this element (transpositional or regulatory) once it had arisen, but could also simply indicate that the lines shared a common ancestor in which this element was at moderate frequency.

The difference observed between the M lines of southeastern Australia, in which even the most extreme carry many P elements, and the M lines of Eurasia, in which more extreme M lines carry fewer P elements, suggests that the same phenotype may be produced by different underlying genomic components in the P-M system. The possible interactions of full-sized P elements, KP elements, and other P element deletion-derivatives must be better understood than they are now before the population and genomic dynamics of P elements can be satisfactorily modelled. The only population dynamics model of a transposable element that takes into account more than one class of element is that of KAPLAN, DARDEN and LANGLEY (1985), and it treats degenerate elements as a single category of nonautonomous elements. The assumptions and simplifications used in this model make its application to the P element system dubious, especially since the complexities of P element biology are so little understood. DANIELS *et al.* (1988) showed that females from different true M lines could give very different GD results in cross A to males of a single P strain. Thus the genotype of the fly, possibly including its complement of other transposable elements, could affect the dynamics of the P-M system, which adds another degree of complexity to a realistic analysis. The correlations reported here between the P-M phenotypic cline and the full-sized P element and KP element reciprocal clines suggest causal relationships, but the actual dynamics of the origin and maintenance of the cline await a better understanding of P element biology.

The lack of *hobo* elements in the reference strain Canton-S-red makes it possible that *hobo* dysgenesis contributes to the moderate level of cross A GD seen in such strains as Cyg 11 (Figure 1a) and G 3 (Figure 1b), which seem otherwise to be M (moderate to high cross A* GD scores). The strain G 3 contains many full-sized P elements, and a weak or variable P-M regulation system in that strain could possibly allow some cross A GD, so that a *hobo* (or another element)

hypothesis may not be necessary to account for the 30.8% GD. However, as noted above, Cyg 11 seems to lack any full-sized *P* elements at all, so unless certain mutated *P* elements are capable of causing GD sterility in the absence of full-sized *P* elements, the 16.2% GD observed in the 1987 cross A test for this strain must be ascribed to another cause, such as *hobo* dysgenesis. Cyg 11 carries many copies per genome of full-sized *hobo* elements (Figure 9c, lane 9), so such an effect is plausible. On the other hand, the other Q and M region strains shown in Figure 1b also carry many full-sized *hobo* elements (e.g., Figure 9b, lanes 9 and 11; Figure 9c, lanes 4, 5 and 8), yet manifest only insignificant levels of GD in cross A. Since nothing is known about the molecular basis for possible regulation in the *hobo* system, a real effect of *hobo* in the cross A GD tests cannot be confirmed or denied with these data.

Another, unknown transposable element system, with similar effects to those of the P-M system, can still be postulated as contributing to the clinal pattern. The hypothesized system would have to vary clinally in Australia as does the *P* element system, and also have correct complements in the reference strains, in order to produce GD sterility in patterns that could be interpreted as due to the P-M system. Parsimony argues against it.

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