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Mutability, sterility and suppression in P–M hybrid dysgenesis: the influence of *P* subline, cross, chromosome, sex and P-element structure

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Summary

Three Harwich P sublines with different *P*-element activity potential were used to investigate the influence of P-derived chromosomes on sn^w mutability and *vg* suppression and to relate the induction of these dysgenic traits to the number and structure of *P* elements. Destabilization of the sn^w allele, a measure of *P* transposase activity, was differentially influenced by the major autosomes. Chromosome 2 of the standard Harwich subline, H^w, induced only 60% of the level of mutability relative to chromosome 3, whereas chromosome 3 of the weakest Harwich subline, H^f, induced only 50% of the mutability relative to chromosome 2. In somatic suppression of the vg^{21-3} allele, chromosome 3 of the H^f subline produced a lower level of complete suppression as compared to chromosome 3 of the H^w or the H^s subline (the high hybrid-dysgenesis-inducing subline). The level of these dysgenic traits and GD sterility, was not correlated with the number of *P* elements per individual (67–68) or per chromosome arm which was very similar among the sublines. The number of complete *P* elements per genome, based on Southern blot analysis of the X and major autosomes, ranged from 15 to 19. Destabilization of the sn^w allele and *vg* suppression by chromosome 3 was correlated with a greater number of complete *P* elements. Two novel unexpected observations emerged from these studies: both sn^w mutability and *vg* suppression data demonstrated high *P*-element activity in hybrids derived from non-dysgenic crosses irrespective of Harwich subline, indicating a lack of P-cytotype regulation. Mutability in non-dysgenic males ranged from 40 to 60% of the level found in dysgenic males. The high sn^w mutability and low GD sterility in non-dysgenic hybrids suggests that these traits may arise by a different mechanism.

1. Introduction

P–M hybrid dysgenesis is characterized by a number of germ line traits that typically occur in hybrid offspring, particularly in those derived from a cross of an M-strain female, devoid of *P* elements, with a P-strain male, bearing *P* elements (Kidwell, Kidwell & Sved, 1977; Bingham, Kidwell & Rubin, 1982). The syndrome includes high mutability, chromosome breaks and rearrangements, transmission ratio distortion, and temperature-dependent sterility (reviewed in Engels, 1989; Rio, 1990). These aberrant traits in hybrids are associated with the mobilization of the *P* family of transposable elements (Bingham, Kidwell & Rubin, 1982) whose members are heterogenous in size, many of which are deletion derivatives of the 2·9 kb complete P element (O'Hare & Rubin, 1983). Typically, between $\frac{2}{3}$ and $\frac{3}{4}$ of the *P* elements in P strains are the smaller deleted non-autonomous members (Rio, 1990). Only the complete *P* elements encode *P* transposase, an enzyme essential for *P* mobility, whose synthesis is restricted to the germ line and controlled at the level of RNA splicing (Karess & Rubin, 1984; Engels, 1984; Laski, Rio & Rubin, 1986; Rio, Laski & Rubin, 1986). Germ line mobility of *P* elements is regulated by cytotype, a maternally transmitted state which is determined by *P* elements themselves (Engels, 1979*b*), the molecular basis of which is still not completely understood. The repression of *P*-element transposition in the *P* cytotype can be controlled by *P*-encoded repressor elements (Nitasaki *et al.* 1987; Engels, 1989), such as the 66 kDa protein (Misra & Rio, 1990) which is a truncated transposase. In addition, KP deletion derivatives of *P* elements, commonly found in M′ strains, have also been implicated in the repression of

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hybrid dysgenesis (Black *et al.* 1987; Jackson, Black & Dover, 1988; Simmons *et al.* 1990; Margulies & Griffith, 1991) by a mechanism which can be inherited through either sex and which may involve protein–protein interaction (reviewed by Rio, 1990).

Suppression of a *P*-element insertion allele at the *vg* locus (Williams, Pappu & Bell, 1988*a*) has been shown to be dependent on P elements and postulated to be due to somatic P element repressor activity. On the other hand, the sn^w *P*-element insertion allele (Roiha, Rubin & O'Hare, 1988) which is highly mutable in dysgenic crosses has been used as a sensitive test for *P* transposase and *P* activity (reviewed in Engels, 1989; Rio, 1990). sn^w mutability has been generally correlated with other dysgenic traits, namely GD sterility, although rare exceptions, with a lack of correlation, have been reported in studies of natural populations (Kokur *et al.* 1986; Simmons, 1987). The molecular basis of gonadal dysgenic sterility (GD) (Engels, 1979*a*; Engels & Preston, 1979; Kidwell & Novy, 1979) has not been elucidated. The proposal that GD sterility is due to dominant lethal mutations caused by chromosome breakage (Engels, 1989) has been supported by the findings that X rays and DNA repair deficiencies act synergistically in conjunction with *P* activity (Margulies *et al.* 1989, Margulies & Griffith, 1991).

The hybrid dysgenesis syndrome has been invariably defined as a *P*-element destabilizing state resulting exclusively from the one-way cross of M-cytotype females to P-strain males (reviewed by Engels, 1989; Rio, 1990). We have reported (Margulies & Griffith, 1991) that the sn^w allele can be markedly destabilized in reciprocal cross hybrids when P-cytotype females derived from the high hybrid dysgenesis-inducing Harwich subline (Margulies, 1990) were used for the non-dysgenic cross. In this study we report that destabilization of the sn^w mutation in non-dysgenic cross hybrids also occurs at high frequencies in hybrids derived from other Harwich sublines. Moreover, this phenomenon is not correlated with the very low GD sterility and is sex-dependent. In addition, the studies reported here have focused on investigating the influence of different chromosomes on sn^w mutability and suppression of the vg^{21-3} allele (Williams, Pappu & Bell, 1988*b*) in hybrids derived from Harwich P-strain sublines with different *P*-activity-inducing potential. The correlation of these genetic data and the molecular analysis of *P* elements by Southern blot and *in situ* hybridization is presented.

2. Materials and methods

(i) *Cultures and strains*

A cornmeal–Brewer's yeast medium was employed in all cultures. The developmental temperature was 18 or 21 °C, as specified in each experiment.

P strains

1–3. The three sublines of the strong wild-type P strain, Harwich (Kidwell, Kidwell & Sved, 1977) were obtained through the courtesy of M. G. Kidwell between 1983 and 1989 and characterized by us. The designation shown below as high (H^s), standard (H^w), and weak (H^r) dysgenesis-inducing sublines is based mainly on differences in the GD sterility pattern at 21 *vs.* 29 °C (Margulies, 1990; Margulies & Griffith, 1991; and see Table 3 here) and on partial X/Y chromosome loss frequencies (Margulies *et al.* 1989; Balter, Griffith & Margulies, 1992).

1. H^w. Harwich white, a standard Harwich reference subline, marked with the spontaneous X-linked mutation, *w* (white eyes).

2. H^s. The unusual properties of this subline include virtually 100% GD sterility of dysgenic cross hybrids reared at 21 °C, high sensitivity to DNA repair deficiencies in induction of GD sterility, and a five-fold higher X/Y chromosome loss than in hybrids derived from H^w or H^r.

3. H^r. f stands for relatively greater fertility of dysgenic hybrids reared at 29 °C (see table 3). This P subline is unusual in that it carries a few KP elements (Margulies & Griffith, 1991). The different temperature-specific patterns of induction of GD sterility by the H^s and H^r sublines, compared to the 'typical' H^w pattern could be caused by variation in abilities of their constituent *P* elements to induce and/or repress *P*-element mobility.

4–6. (4) *C(1)DX y f*/Y-H^w; (5) *C(1)DX y f*/Y-H^s; (6) *C(1)DX y f*/Y-H^r. Attached-X strains in which the free X and Y and the autosomes were derived from the respective inbred Harwich subline by backcrossing attached-X females (strain 8 below) to inbred Harwich subline males for 12 generations in the case of the H^s-derived stock, 18 generations for the H^w-derived stock and 10 generations for the H^r stock. The stocks were subsequently maintained in mass matings at 18 °C for approximately 45, 15, and 12 generations, respectively.

7. *C(1)DX y f*/*y*-H^s. This is a stock derived from a yellow body colour mutant; the X-linked mutation arose in a dysgenic cross early in the evolution of the H^s strain in our laboratory (Margulies, 1990). The stock was started by crossing the *y* H^s male to attached-X P-cytotype females derived from the H^s subline (stock 5) and inbreeding for many generations (4 years, by the time of these experiments). Males of this stock induce almost 100% sterility in dysgenic cross hybrids reared at 21 °C, just as the H^s strain males do. Unlike H^s males, however, which by the time of these experiments induced virtually complete sterility of dysgenic cross hybrids reared at 18 °C, *y*-H^s males allow much more fertility of hybrids reared at 18 °C. *y*-H^s is thus a weaker P strain than H^s based on GD sterility as well as sn^w mutability data, as shown below.

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M strains

8. *C(1)DX, y f*/Y. The attached-X females of this stock are marked with *y* (yellow body) and *f* (forked bristles). Their sons have a patroclinous origin of the X chromosome.

9. *J188*: *Cy*/*Pm*; *Sb*/*D*, a stock carrying four dominant markers: *Cy* (curly wings), In(2L)*Cy sp*^{2}, and *Pm* (Plum eyes) associated with the inversion, In(2LR)*bw*^{V1}, are on the second chromosome; *D* (dichaete wings), In(3LR) DCXF *ru h D*, and *Sb* (stubble bristles) are on the third chromosome.

10. J188-*Basc*. This stock was derived in this laboratory by crosses of the J188 stock with the *Basc* stock and thus also has the X chromosome balancer (*B*, *Bar* eye, *w*^{a}, apricot eye colour, *sc*, inversion). For further details about genetic markers, see Lindsley & Zimm, 1992.

M-cytotype stocks with P-element insertion mutations

11. *y sn*^{w}; *bw st*/*y*$^+$ Y. *P*-element insertion at the X-linked *singed* bristle locus (Engels, 1984) which causes a weak *singed* phenotype. Two defective *P* elements were found at *singed* and one other defective *P* element is closely linked; the genome is otherwise devoid of *P* elements (Roiha, Rubin & O'Hare, 1988). The autosomes carry the recessive eye colour markers, *bw* (brown eyes) on the second chromosome and *st* (scarlet) on the third chromosome. The stock behaves as an M strain. The *singed weak* allele is destabilized in P–M hybrid dysgenesis, providing a mutability assay. Excision of one defective *P* element leads to a more extreme singed bristle phenotype, whereas excision of the other results in a wild-type bristle phenotype (Roiha *et al.* 1988).

12. *vg*$^{21-3}$. A 2·6 kb *P*-element insertion at the vestigial locus on chromosome 2, causing a vestigial phenotype in an M strain background, which is suppressed in a P-strain background (Williams, Pappu & Bell, 1988*a*, *b*). The stock is also homozygous for the brown (*bw*) and cinnabar (*cn*) eye colour mutations on chromosome 2 which cause a white eye phenotype.

13 and 14. *Sb* Δ2–3/*TM6*; and *mei332*; Δ2–3/*TM6*. The Δ2–3 [P(*ry*$^+$ Δ2–3)(99B)] refers to an insertion at 99B of a modified *P*-element construct with a 189 bp deletion (Laski, Rio & Rubin (1986; Robertson *et al.* 1988) and includes two elements inserted in a head-to-head arrangement. The other markers, including the *TM6* third chromosome balancer are described in Lindsley & Zimm (1992). Strains 8, 9 and the *Basc* were obtained from the Bowling Green University Stock Center. The *sn*^{w} strain was a gift from M. J. Simmons, the *vg*$^{21-3}$ strain was kindly provided by J. A. Williams, and the Δ2–3 stocks were a gift of A. Chovnick.

(ii) *Crosses and mating schemes*

(*a*) *sn*^{w} *mutability studies*. Fig. 1 presents the mating scheme for testing the effect of the major autosomes of each Harwich P strain on *sn*^{w} mutability utilizing the dominant markers of the J188 M strain. The P_1 crosses were made with about 20–25 pairs in multiple half-pint milk bottles allowing mating for 2 to 3-day periods. F_2 hybrid males were aged for 4 days to ensure uniform age for P_3 crosses. For studying the effect of sex on *sn*^{w} mutability (Table 2), first generation dysgenic and non-dysgenic crosses were mass matings as above which were made over a period of 1–2 weeks. The parental males or females of each Harwich subline were obtained from at least two cultures set up weekly in bottles and reciprocal crosses were made using the *sn*^{w} M-cytotype stock (stock 11 above). F_1 hybrid males and females were collected daily from the multiple cultures and aged at 21 °C to maintain uniformity of age. Hybrid males were aged for 4 days and mated singly for a period of 4 days to three attached-X M-strain females (stock 8) 3–6 days of age. Hybrid females were mated immediately in mass matings, 30 per bottle, to stock 8 males. After 1 day of mass mating, the females were placed singly in a vial with 3 males and allowed to deposit eggs for 3 days. All P_2 cultures were kept for a week at 21 °C. *sn*^{w} mutability was scored usually between 20–22 days after the cultures were established.

(*b*) *Suppression of vg*$^{21-3}$. Fig. 2 shows the mating scheme for determining the influence of specific P-strain chromosomes of each Harwich subline on suppression of the vestigial phenotype. The derivation of *B*/Y; *cn vg bw*/*cn vg bw*; M/*Sb* males used for the P_1 crosses is not shown in Fig. 1. These males were constructed by crossing the *vg*$^{21-3}$ stock females with males of the multiple balancer strain J188-*Basc* (stock 10) to derive the stock, *Basc*; *cn vg bw*; M/*Sb* which was maintained by selection.

(iii) *Statistical analyses*

The significance of differences in *sn*^{w} mutability was analysed by the non-parametric Wilcoxon rank sum test, using the mutation rate computed for each male tested (Engels, 1979*c*). The non-parametric multi-dimensional contingency analysis (MDCA) (Rockwell, Findlay & Cooke, 1987) was used to examine differences in *vg* suppression in hybrids derived from P_2 cross 1 or 2, and testing the progeny derived from different Harwich sublines simultaneously. If significant differences were obtained from the MDCA analysis, 2 × 2 contingency analysis was performed to test the effect P chromosomes in pairwise combinations of subline data, namely H^w *vs.* H^s, H^w *vs.* H^t and H^s *vs.* H^t.

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(iv) *Gonadal dysgenesis sterility (GD)*

F_1 females derived from M × P (dysgenic) or P × M (non-dysgenic) crosses were reared at 29 °C and aged at 21 °C on yeasted food vials for 3–4 days to mature the gonads. GD sterility was scored by the absence of eggs in dissected gonads.

(v) *Molecular analysis of P elements on Southern blots*

Genomic DNA was isolated by homogenization of 50 male flies by a modification of the method of Marcus (1985). The isolation of plasmid pπ25.1 DNA (O'Hare & Rubin, 1983), restriction endonuclease digestion, agarose gel electrophoresis and elution from low-melting agarose were essentially as described by Maniatis, Fritsch & Sambrook (1982). All gels used for Southern blots were 0·7% agarose. Southern blot analysis was performed as follows. Genomic DNA from 50 male flies was digested to completion and purified by phenol and chloroform treatment. DNA concentration of each preparation was determined by the sensitive fluorometric assay using the Hoechst fluorochrome (Hoefer Scientific Instruments, San Francisco, CA) and the Farrand optical fluorometer. In order to estimate the number of intact *P* elements originating from a *P*-derived chromosome or per genome of a strain, genomic DNA was digested with *Acc* I or *Dde* I which cut twice within the *P* sequence as shown in Fig. 4. Different amounts of the restricted DNA of each Harwich strain were loaded per lane and run on an agarose gel together with the genomic DNA of the standard reference strain, Δ2–3 (strains 13 or 14) which contains two *P* elements. The gel was blotted to a framed nylon filter in a blotting apparatus (Bios Corporation, New Haven, Connecticut, CT) for 8 h. Probes were prepared by random prime labeling (Amersham Co., Arlington Heights, Illinois, IL). Filter hybridization was performed at 65 °C in a cassette by a modification of the described procedure (Bios Corporation). In order to perform molecular analysis of *P* elements on each of the major chromosomes of the H^w, H^s and H^r strains, we utilized the mating scheme shown in Fig. 3. First generation non-dysgenic crosses of P-strain females to M-strain males minimized *P*-element mobility, and thus contamination of M-strain chromosomes with *P* elements (Kidwell, 1983). The dominant autosomal markers of the M strain permitted the selection of second generation males with only one chromosome, the X chromosome, 2 or chromosome 3, derived from the denoted P strain. Genomic DNA was isolated from 50 second-generation males of each type and Southern analysis was performed as described above.

(vi) In situ *hybridization*

Polytene chromosome spreads from salivary glands of third instar larvae were prepared for hybridization similarly to the described procedure (Bingham, Levis & Rubin, 1981) and treated with a nick-translated biotinylated DNA probe (Gibco/BRL) of pπ25.1.

3. Results

(i) sn^w *mutability*

The mating scheme to test the influence of the major autosomes on sn^w mutability is shown in Fig. 1. The data presented in Table 1 show that destabilization of this *P*-insertion allele in F_2 dysgenic hybrids is dependent on both the subline and the *P*-derived chromosome. Reversion of sn^w to either the extreme or wild-type allele was highest in hybrids of the H^s subline, with each chromosome exerting a similar effect. In contrast, chromosome 2 of the standard reference H^w subline and chromosome 3 of the weaker H^r subline had a much smaller influence than the respective chromosomes of the H^s subline. The results with each chromosome tested individually confirmed previous characterization (Margulies & Griffith, 1991) that *P*-transposase activity is highest in hybrids of the H^s subline.

Since our previous studies showed an unexpectedly high destabilization of the sn^w insertion in hybrids of a non-dysgenic cross (P female × M male) of the high dysgenesis-inducing subline, H^s, it was of considerable interest to examine if this phenomenon is uniquely associated with the high dysgenesis syndrome or whether it is a more common trait induced in hybrids derived from other Harwich P strains. In addition, we wanted to determine if sn^w mutability also occurs in appreciable frequencies in females derived from non-dysgenic crosses of each subline. To compare the effect of subline, cross and sex, reciprocal crosses were made using the sn^w stock as the M strain. To test for destabilization of the sn^w allele in P × M cross hybrid males, attached-X females were derived with the P-genetic background of each subline as described in Materials and methods. The results of these experiments are shown in Table 2. It should be noted that the H^s stock used for the M × P crosses and for the P × M crosses with attached-X;H^s P females is a weaker P strain than the original H^s strain from which it was derived (see Materials and methods). The use of this weaker strain was necessary at the time of these experiments, since M × P crosses of the high hybrid dysgenesis-inducing subline (Margulies, 1990) resulted in almost complete GD sterility of male and female hybrids even when reared at 16 °C. As can be seen in Table 2, sn^w mutability in females derived from the dysgenic M × P crosses of the standard H^w subline, as well as those of the H^s subline, amounted to about $\frac{1}{2}$ of the frequency found in males derived from the same crosses. Since the mutation rate in females was based solely on the reversion of sn^w to the sn^e allele, the mutability was very similar in dysgenic cross male and female hybrids, regardless of the subline used. Mutation rate was not determined here in H^r subline

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P_1 cross: $\frac{p^+}{p^+}$ $\frac{p^+}{p^+}$ $\frac{(H^s)\ (H^f)\ (H^w)}{}$ ♀♀ × $\frac{Pm}{Cy}$ $\frac{D}{Sb}$ (M) ♂♂

Mass mating at 21 °C

P_2 cross: $\frac{sn^w}{sn^w}$ $\frac{bw}{bw}$ $\frac{st}{st}$ (M) ♀♀ × $\frac{p^+}{\Rightarrow}$ $\frac{p^+}{Cy}$ $\frac{p^+}{D}$ ♂♂

Mass mating at 18 °C

P_3 cross: XXY (M) 3 ♀♀ × $\frac{sn^w}{\Rightarrow}$ $\frac{bw}{Cy}$ $\frac{st}{p^+}$ dysgenic ♂

or × $\frac{sn^w}{\Rightarrow}$ $\frac{bw}{p^+}$ $\frac{st}{D}$ dysgenic ♂

Single male at 18 °C

Score sn^w mutability to sn^e or to sn^+ in sons with patroclinous X origin

Fig. 1. The mating scheme to test sn^w mutability (Table 1) in F_2 dysgenic males with either chromosome 2 alone or chromosome 3 alone derived from the H^w, H^s, or H^f Harwich P subline. *Pm*/*Cy* and *D*/*Sb* are the dominant-autosomal markers from the M strain. p^+ refers to the wild-type homologues originating from the Harwich P strain, and M to wild-type M-strain-derived homologues. XXY attached-X females were used in P_3 crosses to obtain sons with patroclinous origin of the X chromosome.

Table 1. *The effect of each major P-strain autosome on* sn^w *mutability in dysgenic male hybrids derived from different Harwich sublines*

P strain subline	P autosome tested	Progeny scored			Mutation rate	S.E.
		sn^w	sn^e	sn^+		
H^w	2	533	28	19	0·092	0·0188
H^w	3	1526	145	109	0·148**	0·0123
H^s	2	1460	139	138	0·150*	0·0102
H^s	3	2745	243	263	0·158**	0·0072
H^f	2	1497	122	162	0·161*	0·0126
H^f	3	2716	128	136	0·086	0·0087

H^w is the standard Harwich subline, H^s is a high level hybrid dysgenesis inducer subline, and H^f is a weak GD-inducing subline, which contains a few KP elements. The mating scheme to test the effect of different autosomes is shown in Fig. 1. Mutation rate was computed using the unweighted method (Engels, 1979*b*). S.E. is the standard error. The progeny data shown represent pooled data of all individually tested males. (See Materials and methods for statistical analysis.) * $P < 0{\cdot}005$, in comparing the H^s and H^f subline with H^w. ** $P < 0{\cdot}001$, comparing hybrids of the H^w and H^s subline with those derived from H^f.

M × P cross hybrids, but in previous studies (Margulies & Griffith, 1991) the rate was very similar to that in H^w hybrids. In sharp contrast, sn^w mutability in non-dysgenic cross hybrids differed greatly in the sexes. Regardless of the subline used, the mutation rate in non-dysgenic females was very low by comparison to dysgenic females, indicating the typical maternal effect governed by cytotype. The mutation rate in non-dysgenic males, however, was very high compared to females and amounted to 62 and 40% of the rate found in dysgenic male hybrids of the H^w and H^s subline, respectively.

(ii) *GD sterility*

In view of these findings, it was important to ascertain the cytotype of the attached-X females derived from each *P* subline which were used for the study of sn^w mutability in P × M cross male hybrids. To do so we examined the frequency of GD sterility, the trait normally associated with hybrid dysgenesis. Table 3 shows that the frequency of GD sterility (Schaeffer, Kidwell & Faysto-Sterling, 1979) among F_1 females reared at the nonpermissive temperature of 29 °C was very low when the P-strain-derived attached-X females were used for the P × M cross hybrids. Indeed there was no significant difference in GD sterility when compared to the incidence found when H^w or the H^s

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Table 2. *Effect of sex on* sn^w *mutability in hybrids derived from dysgenic and non-dysgenic crosses of different Harwich P-strain sublines*

Cross ♀		♂	Sex of F_1	Number tested	F_2 progeny scored sn^w	sn^e	sn^+	Mutation rate	S.E.
					H^w subline				
M	×	P	Male	83	1419	423	242	0·299	0·0166
M	×	P	Female	138	1713	315	—	0·150	0·0010
P	×	M	Male	50	1929	201	197	0·186	0·0027
P	×	M	Female	115	1903	3	—	0·001	0·00006
					H^s subline				
M	×	P	Male	138	3469	304	432	0·179	0·0092
M	×	P	Female	166	501	41	—	0·081	0·0013
P	×	M	Male	104	4381	162	164	0·072	0·0058
P	×	M	Female	139	2065	5	—	0·002	0·0001
					H^r subline				
P	×	M	Male	65	3115	53	109	0·053	0·0010
P	×	M	Female	128	2860	3	—	0·001	0·0008

M × P is the dysgenic cross, P × M is the non-dysgenic cross. The M strain is the sn^w stock in an M-strain background. The P strain is the specific Harwich subline, H^w, H^s or H^r. Attached-X females with P autosomes derived from each respective P subline were used to test sn^w mutability in non-dysgenic males. The progeny data shown represent pooled data of individually tested males and females. Mutation rate was calculated by family size of all individuals tested, using the unweighted method (Engels, 1979*b*). S.E. is the standard error. The class of sn^+ revertants could not be scored among offspring of heterozygous females tested for sn^w mutability.

Table 3. *GD sterility of reciprocal cross hybrids derived from different Harwich sublines*

Harwich subline	Type of cross ♀				♂	Number females tested	Number sterile	Per cent sterile
H^w	M	×	P	sn^w	× H^w	95	95	100
	P	×	M	H^w	× sn^w	66	1	1·5
	P	×	M	H^wXXY	× sn^w	47	3	6·4
H^s	M	×	P	sn^w	× H^s	100	100	100
	P	×	M	H^s	× sn^w	70	3	4·3
	P	×	M	H^sXXY	× sn^w	59	4	6·8
H^r	M	×	P	sn^w	× H^r	86	60	69·8
	P	×	M	H^r	× sn^w	78	11	14·1
	P	×	M	H^rXXY	× sn^w	50	2	4·0

M × P are dysgenic crosses, P × M are non-dysgenic crosses. GD sterility of hybrid females reared at 29 °C was scored by dissection of gonads. XXY are the attached-X P-cytotype females used to study sn^w mutability in non-dysgenic males, whereas the Harwich stock females of each representative subline were used to determine sn^w mutability in non-dysgenic females (see Table 2).

parental subline females were used for the P × M crosses. In H^r subline hybrids, GD sterility was lower in attached-X P × M cross offspring. We also tested male hybrids from these crosses in other experiments (results not shown) which also exhibited very low frequencies of sterility typical of P-cytotype maternal control. These results confirmed the P cytotype of the attached-X P-strain-derived females and showed that GD sterility was not correlated with sn^w mutability in P × M cross male hybrids.

(iii) *Suppression of the vestigial phenotype due to the* vg^{21-3} *allele*

Since it was demonstrated that suppression of the vestigial phenotype manifested by the *P*-element

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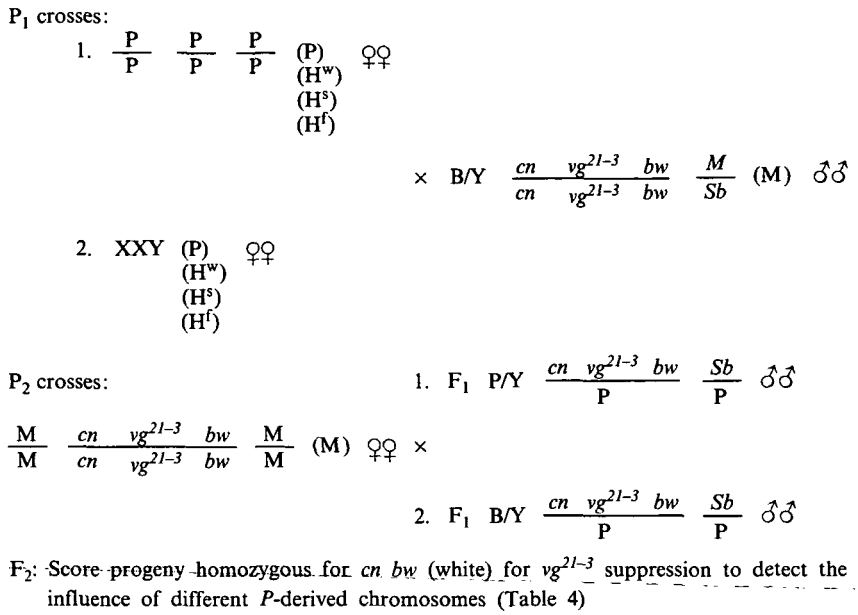

Fig. 2. Mating scheme to test suppression of vg^{21-3} phenotype in hybrids derived from Harwich sublines with different *P*-activity-inducing ability. P-strain chromosomes are designated by P. The derivation of males used for P_1 crosses and genetic markers are described in Materials and methods. XXY (P) are attached-X P-cytotype females with P autosomes derived from the respective Harwich subline.

insertion allele was occurring only when *P* chromosomes were present and was thus dependent on *P* elements (Williams *et al.* 1988*a*), a mating scheme was designed (Fig. 2) to test the influence of different *P*-strain-derived chromosomes on the somatic suppression of this mutant phenotype. The scheme permitted us to test the effect of chromosome 3 alone, the X chromosome alone, chromosome 3 and the X combined, and the absence of any *P*-derived chromosome on the expression of the *vg* phenotype. It should be noted that the P_1 P × M non-dysgenic crosses were designed to minimize *P*-element mobility and thus contamination of M-strain chromosomes with *P* elements. The results presented in Table 4 show that both chromosome 3 and the X were effective in suppressing the vestigial phenotype, the suppression being for the most part complete. There was a lesser ability, however, by the X to affect complete suppression in comparison to chromosome 3 in the case of H^w-derived and H^s-derived hybrids ($P < 0{\cdot}001$ and $< 0{\cdot}01$, respectively). In contrast, chromosome 3 of hybrids of the weaker subline, H^f, was significantly less effective in suppression as noted by the higher number of partial suppression events. This finding was reproducible in three different genotypic classes of F_2 progeny. Thus the lesser effect of chromosome 3 in *vg* suppression correlated with its lower induction of *sn^w* mutability in H^f subline hybrids relative to hybrids derived from the standard and high dysgenesis inducing sublines, where chromosome 3 was more effective in both *sn^w* mutability and *vg* suppression.

Table 4 also shows that hybrids which did not inherit any *P* chromosomes manifested some partial and complete *vg* suppression. Moreover, the degree of this suppression differed significantly among hybrids derived from different sublines. In the case of one genotypic class, suppression was most effective in H^f subline hybrids, whereas in the other two classes, the *vg* phenotype was most effectively suppressed in hybrids of the standard H^w subline, and was least suppressed in H^s subline hybrids. The most likely explanation for suppressibility of the *vg* phenotype in the absence of *P*-strain derived chromosomes is that some mobility of *P* elements occurred in the male parents derived from the non-dysgenic P × M crosses and that this *P* activity resulted in contamination of one or more M chromosomes with *P* elements.

(iv) *Molecular characterization of P elements in Harwich sublines*

This approach was designed to assess if the relative degree of *sn^w* mutability and *vg* suppression manifested in hybrids of each subline, could be related to the number of *P* elements as determined by *in situ* hybridization and, more specifically, to the number of full-size *P* elements based on Southern blot analysis.

(*a*) *In situ hybridization patterns.* The number of *P* elements that was detected in different chromosome arms of the individuals tested from each Harwich subline is shown in Table 5, where 5A presents the data of the parent Harwich sublines and 5B the findings with the derived attached-X stocks. As can be seen in Table 5A, the mean number of *P* elements per chromosome arm and per individual was very similar in the three sublines, although the estimates of sites per chromosome arm differed somewhat among the individuals of each subline (data not shown). It should be noted that the number of *P*-element sites per diploid genome is expected to be greater than the

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Table 4. *Suppression of the phenotype of the P-insertion allele* vg^{21-3} *in hybrids derived from different Harwich sublines*

Subline tested	Progeny genotype (female or male)		P chromosome tested	Wing suppression		
				Complete	Partial	None
H^w	*M*	*cn vg bw M*	X and 3	509	22	1
H^s	*P*	*cn vg bw P*		338	14	0
H^r				360	8	0
H^w	*M*	*cn vg bw M*	X	271	32	2
H^s	*P*	*cn vg bw Sb*		240	24	2
H_r				285	21	8
H^w	*M*	*cn vg bw M*	3	497	13	0
H^s	*Y*	*cn vg bw P*		312	9	1
H^r				272	17*	4
H^w	*B*	*cn vg bw M*	3	229	12	1
H^s	*M*	*cn vg bw P*		103	2	0
H^r				99	26**	2
H^w	*B*	*cn vg bw M*	3	200	8	7
H^s	*Y*	*cn vg bw P*		220	10	0
H^r				298	38**	3
H^w	*M*	*cn vg bw M*	None	8	13	359
H^s	*Y*	*cn vg bw Sb*		12	2	341
H^r				5	14	154*
H^w	*B*	*cn vg bw M*	None	38	22	152**
H^s	*M*	*cn vg bw Sb*		8	7	313**
H^r				4	19	117*
H^w	*B*	*cn vg bw M*	None	42	17	140**
H^s	*Y*	*cn vg bw Sb*		7	8	289**
H^r				3	21	124**

The derivation of the genotypes is shown in Fig. 2. *M* are M-strain-derived chromosomes; *P*, P-strain-derived chromosomes; *B* is the Muller-5 X chromosome balancer. *Sb* is the M-strain-derived dominant marker, *stubble bristles*. The hybrids were reared at 25 °C. Partial suppression comprises the wing phenotypes ranked 2–5 on a scale of 1–6 where 1 is wild type and 6 is not suppressed (Williams *et al.* 1988). * $P > 0{\cdot}05$; ** $P > 0{\cdot}01$ or $P > 0{\cdot}001$ in analysis of subline differences within each genotypic class. Significant differences shown next to each subline indicate the analysis of all three pairwise tests, whereas a P value next to only one subline, namely H^r, indicates that these hybrids differed significantly from those of the other two sublines.

haploid number because of site heterozygosity in mass mated inbred cultures, as was demonstrated in the Harwich strain used by Shrimpton, Mackay & Leigh Brown (1990). Another similarity among the sublines was the presence of *P* elements in the heterochromatin. One to three *P* elements per individual were detected in 3R heterochromatin of all sublines, and 3 *P* elements were present in the X heterochromatin of the H^w and the H^r subline. The H^r subline differed most from the other Harwich sublines with regard to the number of *P*-element sites on 2L and 2R where the mean number on 2L (11) and 2R (8) was the reverse of that found in the H^w and H^s sublines. The mean number of sites per chromosome arm and the 67–68 hybridization sites we detected per individual are similar to the estimates reported by Daniels *et al.* (1987) for the Harwich-77 strain. Our estimates are higher than the number reported by Shrimpton *et al.* (1990). They detected 58 hybridization sites per individual averaged over all the inbred selection lines and the same number in the Harwich parent strain. Our estimates of the sites per individual per chromosome arm is however consistent with that reported by Shrimpton *et al.* (1990) with regard to the X, 2R and 3L. Similarly to their findings, we also noted a great variation in the chromosome arm site occupancy among individuals of one subline as well as among the different sublines. Since only 2–3 individuals were tested per line, the variance in the sites occupied in each subline was not determined. The total number of cytological sites occupied per chromosome arm was 31 and 36 on the X chromosome of the H^s and the H^r subline, respectively. The occupancy of sites on 3R was 30 and 31 for the same sublines. This occupancy profile based on only three individuals tested per subline is remarkably similar to that observed for the X (32·8) and 3R (30·9) based on 20 individuals tested per subline by Shrimpton *et al.* (1990) and attests to the mobility of *P* elements within each subline and thus in the P cytotype. The *in situ* hybridization results

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Table 5. *Localization by* in situ *hybridization of P elements on chromosome arms of Harwich sublines with different P-activity-inducing ability*

Subline	Number individuals tested	Number of P element insertions Chromosome arms X	2L	2R	3L	3R	4	Mean no. per individual
A								
H^w	2							
Mean/arm		17·5	7·5	12·5	11·5	17	0·5	66·5
H^s	3							
Mean/arm		18·3	8	11	11·5	18	1·7	68·3
H^f	3							
Mean/arm		16·7	11·3	8·7	11·3	18·7	1	67·7
B								
XXY;H^w	2							
Mean/arm		22	6	8·5	7·5	13·5	0·5	58
XXY;H^s	2							
Mean/arm		18	8	10	11·5	16·5		64
XXY;H^f	3							
Mean/arm		19	11	11	10	14	1	62

XXY are the attached-X stocks derived from each respective subline as described in Materials and methods.

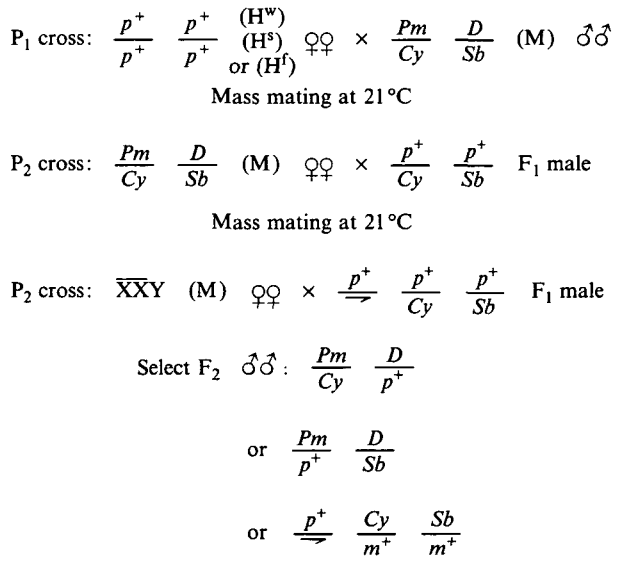

Fig. 3. Mating scheme for the derivation of males with only one major P-strain chromosome, 2, 3, or the X, derived from the Harwich subline. The males were used for estimates of complete *P* elements by Southern blot analysis (Fig. 5). The designation of P-strain chromosomes and M-strain chromosome markers is as in Fig. 1.

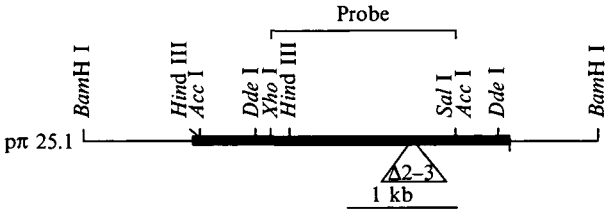

Fig. 4. A partial restriction map of the 2·9 kb *P* element in pπ25.1. The 1·65 kb *Xho* I/*Sal* I restriction fragment was used as the probe for all Southern blots. The *Acc* I sites are at positions 53 and 2412; the *Dde* I sites are at positions 587 and 2763. Δ2–3 refers to the deletion extending from position 1948-2137 (Laski, Rio & Rubin, 1986) of the *P* element present in the Δ2–3 strains used as a reference standard for Southern blot analysis (Fig. 5).

do not reveal any obvious relationship between the number of *P*-element sites per autosome and the activity of the autosome in *sn^w* mutability and *vg* suppression.

(*b*) *Southern blot analysis.* To estimate the number of 'full size' *P* elements on the X and the major autosomes the mating scheme shown in Fig. 3 was used. The quantitation was based on digestion of genomic DNA from 50 F_2 males of the appropriate genotype with *Acc* I which has two restriction sites within the intact *P* element, as shown in Fig. 4. The digestion produces a 2·36 kb fragment from every *P* element which is intact in the region spanned by these sites. The putative number of complete *P* elements was also estimated by digestion with *Dde* I, which generates a 2·2 kb internal fragment (Figure 4). It should be noted that any deletions outside of the *Acc* I or *Dde* I sites would not be detected and these elements would also be included in the estimates. With this reservation in mind and the fact that Southern analysis does not provide the resolution for detection of very small deletions, we will refer to our estimates as full-size or complete elements, an approach also utilized by other investigators (Daniels *et al.* 1987; Biemont *et al.* 1990; Simmons *et al.* 1990). The method for estimating the number of full-size *P* elements, similar to that employed previously (Daniels

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et al. 1987), was to use dilutions of known amounts of digested DNA from samples of F_2 males derived from each Harwich subline and to compare the signal from the different dilutions to a reference DNA standard. The reference standard employed was a strain which carries the Δ2–3 *P*-element insertion in heterozygous conditions (strains 13 and 14, respectively, in Materials and methods). This construct contains two *P* elements, one of which produces a 2·16 kb fragment instead of the expected 2·36 kb fragment upon digestion with *Acc* I because of the engineered internal deletion in the Δ2–3 element (Laski, Rio & Rubin, 1986). The second lower-molecular weight fragment is due to a second deletion that arose later in one of the *P* elements within the Δ2–3 construct (H. Robertson, personal communication). Digestion with *Dde* I results, as expected, in a single band in the 2 kb position (not shown). The representative Southern analyses for chromosome 3, 2, and the X chromosome are shown in Figure 5(*a–c*), respectively, and the number of full-size elements assessed from Southern blots by densitometry is shown in Table 6. It is important to point out that these estimates are approximate, since not all degenerate elements may be detectable and the amount of DNA represented by the complete element band must be adjusted with respect to the signal in the degenerate elements. Thus the relative differences between chromosomes of the sublines on one blot are likely to be more reliable than any absolute differences. As a basis of comparison, genomic DNA was isolated from the standard Harwich subline, H^w, and the number of complete elements present on all the chromosomes was determined by digestion with *Acc* I (last lane of Fig. 5*a*) or with *Dde* I (not shown). Table 6 also includes the number of full-size elements estimated in the derived attached-X P-cytotype strain, XXY;H^w, after digestion with the same restriction enzymes (not shown).

The number of putative full-size elements per genome was 15 in the H^w subline, when estimated on the individual chromosomes (with the exception of the dot chromosome 4, in which *P* elements were detected by *in situ* hybridization), and 38 per diploid genome, based on genomic DNA of the parental subline. Moreover, since similar estimates were obtained from blots of *Acc* I and *Dde* I digests of parental DNA, it argues in favour of a fairly correct assessment (Biemont *et al.* 1990). The analysis showed that the number of putative complete elements per genome of the weak Harwich subline, H^t, was the same as in the standard subline, H^w, whereas in the high hybrid dysgenesis subline, H^s (Margulies, 1990; Griffith & Margulies, 1991), the estimated number of full size *P* elements per genome was 19. All of these estimates were lower than the number reported in the Harwich-77 strain, namely 50 per diploid genome (Daniels *et al.* 1987). These differences may be due to the specific method used for estimating the number of complete elements, such as the resolution of at least some degenerate elements in the same blot. The average number of complete *P* elements in P strains is thought to be about $\frac{1}{3}$ of the total number of elements (Rio, 1990) but is obviously dependent on the strain.

A comparison of the contribution of the major autosomes to sn^w mutability in hybrids of each subline (Table 1) and the estimated number of full-size *P* elements per chromosome (Table 6) shows a correlation only in the case of the H^w subline. Here the greater number of full-size *P* elements on chromosome 3 was twice the number found on chromosome 2, and corresponded to the much higher mutation rate induced in the presence of chromosome 3 in hybrids of this subline. Such correlation is absent, however, in the case of the H^s and the H^t sublines where the number of full-size elements was, respectively, much lower or the same on chromosome 2 *vs.* chromosome 3, whereas the induction of sn^w mutability by each autosome was respectively the same or higher in F_2 hybrids derived from these sublines. A comparison across the sublines does show a positive correlation, since the sublines with the relatively higher number of complete elements on chromosome 3 also showed higher activity by this chromosome with regard to sn^w mutability.

The experiments were also designed to test the activity of chromosomes in *vg* suppression in relationship to the number of full-size *P* elements. In this regard, the lesser degree of *vg* suppression induced by chromosome 3 in hybrids of the H^t subline relative to hybrids of the other sublines (Table 4) also correlates with the lower estimated number of full-size *P* elements on chromosome 3 of this subline. Moreover, the lack of any significant differences in the contribution of the X to *vg* suppression among the sublines also correlated with a similar estimate of full-size elements on the X chromosome of the three sublines. It should be noted that numerous species of degenerate elements were also visible in Southern blots. The similar and dissimilar species of degenerate elements among the sublines are particularly notable on chromosome 3 (Fig. 5*a*). Because some of the deleted versions may not be detectable, as is obvious when the relative intensity is determined of all the signals of the degenerate elements versus the full-size elements on the chromosomes of each subline, no attempt was made to qualitatively or quantitatively assess the incomplete elements. Thus no correlation could be established with regard to the contribution of degenerate elements to sn^w mutability or *vg* suppression. The bands that are seen above the 2·36 kb size (Fig. 5*c*) probably represent fragments due to the elimination of one *Acc* I restriction site and a second cut within the genomic sequence. We do not think they are due to incomplete digestion because of the long time and amount of enzyme used for restriction. The number of these elements was not estimated, but the densitometric tracing adjusted for the relative amount of DNA in these bands.

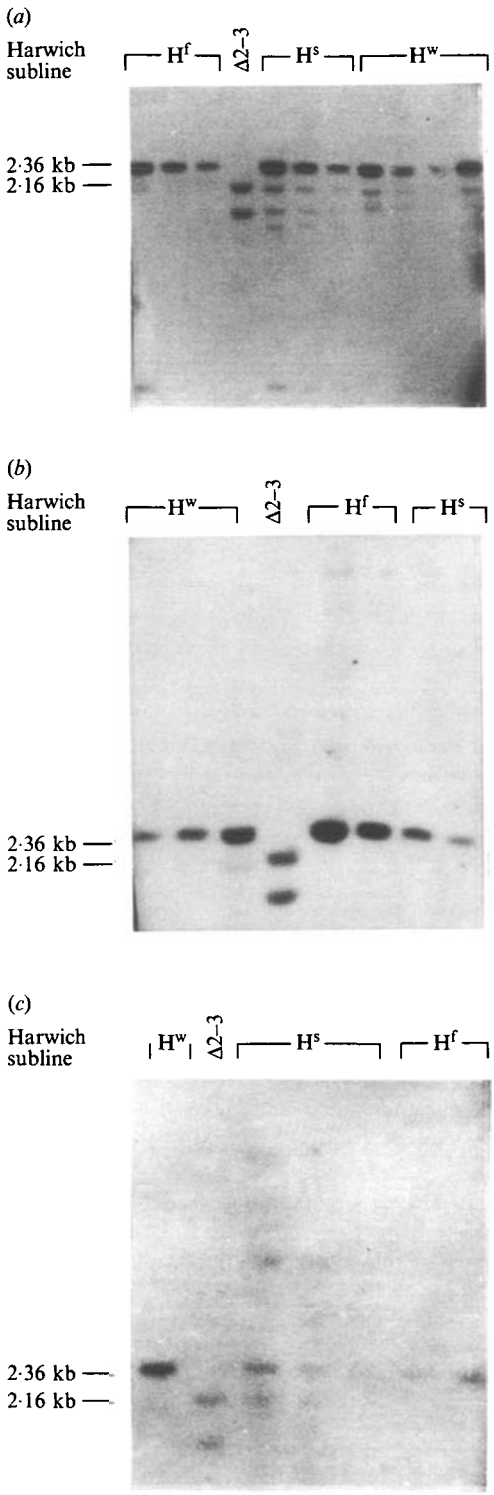

Fig. 5. Southern blot analysis of the number of full-size *P* elements in each major chromosome or the genome of Harwich sublines. Genomic DNA was digested with *Acc* I and probed with the 1·6 kb *Xho* I/*Sal* I fragment from pπ25.1. (*a*) Shows full-size *P* elements on chromosome 3, as well as on all the chromosomes of the parental H^w subline (last lane), (*b*) on chromosome 2; (*c*) on the X chromosome. To estimate the approximate number of full-size *P* elements, a densitometric comparison was made of the signal in the 2·36 kb bands, corresponding to full-size *P* elements, to the 2·16 kb band of the Δ2–3 reference DNA representing one *P* element, after adjusting for the signal in the degenerate (incomplete) *P* element bands. In (*a*) the three lanes corresponding to each Harwich subline had, respectively, 1, 0·5 and 0·25 μg DNA loaded per lane; 6 μg of the reference standard Δ2–3 DNA was loaded in lane 4. The last lane, no. 11, had 0·25 μg genomic DNA loaded from the parental H^w subline. The amount of DNA loaded per lane shown in (*b*) was – H^w lanes: 0·5, 1, 2 μg; Δ2–3, 6 μg; H^f lanes: 2, 1 μg; H^s lanes: 1, 0·5 μg. In (*c*) the DNA loaded per lane was – H^w: 1 μg; Δ2–3, 2 μg; H^s, 1, 0·5 μg; H^f: 0·25, 0·5 μg.

Table 6. *The number of full-size P elements per chromosome and per male genome in Harwich sublines with different P activity ability*

Subline	Chromosomes of males tested: X	2	3	
				Number estimated per genome
H^w	3	4	8	15
H^s	3	4	12	19
H^f	3	6	6	15
				Number estimated per diploid genome
H^w	(All chromosomes)			38
XXY;H^w	(All chromosomes)			34

Estimates from Southern blot analysis are based on genomic DNA digested with *Acc* I or *Dde* I and probed with a 1·54 kb *Xho* I/*Sal* I P-element probe, using the 2–3 P-insertion strain as a reference standard. The derivation of males with a single chromosome derived from a particular Harwich P strain is shown in Fig. 3. Representative Southern blots and methodology are depicted in Fig. 5*a–c*. The estimates of full-size P elements per genome based on individual chromosomes tested does not include chromosome 4 which can harbour from 1–3 P elements as shown by *in situ* hybridization analysis in Table 5. XXY;H^w is an attached-X female stock derived from H^w as described in Materials and methods.

Southern blot analysis of genomic DNA from males of the P cytotype attached-X strain, XXY;H^w, derived from this subline through 18 generations of backcrosses showed a somewhat lower number of full-size elements per diploid genome than in males of the parental subline (Table 6). This estimate also correlates with a smaller number of *P* element insertion sites assessed in males of this strain by *in situ* hybridization (Table 5). It was important to estimate the number of full-size elements in at least one of the attached-X derived strains, since non-dysgenic crosses using attached-X P-cytotype females from each one of these derived stocks produced an unexpectedly high *sn^w* mutability (Table 2). It seems that this high activity in non-dysgenic hybrids is not due to an excessive number of full-size, *P*-transposase-coding, elements in this P-strain-derived stock.

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4. Discussion

The results presented here provide multiple evidence for induction of dysgenic traits in hybrids derived from non-dysgenic crosses. The frequency of occurrence of sn^w mutability is much above the level commonly believed to occur in offspring of P-cytotype mothers. Thus the generalization that P cytotype almost completely represses *P*-element activity in hybrids originating from Harwich P strains seems to hold only for GD sterility. Indirect evidence for the lack of repression of *P* transposition by P cytotype in hybrids from non-dysgenic crosses was reported in experiments which monitored the induction of polygenic traits (Mackay, 1986, 1987; Torkamanzehi, Moran & Nicholas, 1988; Lai & Mackay, 1990). It is noteworthy, however, that this failure of regulation by P cytotype with regard to sn^w mutability was evident only in male hybrids. A possible explanation for this apparent restriction based on sex is the nature of the P-cytotype female. Whereas the P-strain mother of female hybrids was from the parental P strain, male hybrids originated from a P-cytotype attached-X female which was derived from a dysgenic cross. Such females could have become defective in controlling the repression potential. Lack of repression, an important mechanism for curtailing *P*-element activity and thus sn^w hypermutability (Engels, 1979*b*, 1981; Simmons *et al.* 1987; Rio, 1990) is a likely explanation for our observations. It has been suggested (Rio, 1990) that P-cytotype control may be dependent on the specificity of insertion sites of the defective *P* elements which have negative regulatory effects on *P* activity. This specificity may have been affected by the induced mobility of *P* elements in dysgenic hybrids during the derivation of the attached-X stocks. There still remains, however, an unresolved question, namely, that the P cytotype of the derived attached-X females was capable of repressing GD sterility in both sexes, but was very ineffective in repression of sn^w mutability in male hybrids. These observations indicate that GD sterility, the hallmark of hybrid dysgenesis, may not be regulated by the same mechanism as other dysgenic traits. An alternative explanation is the postulate (Rio, 1990) that a different threshold of transposase activity may be required for the various dysgenic traits which would make it easier to detect suppression of GD sterility, as was observed in hybrids of some M′ strains. The possibility also exists that *P*-element mobility must be extremely high for the manifestation of even a moderate level of GD sterility. This is a less likely explanation in view of the findings (Simmons *et al.* 1990) that M′ strains with only one or just a few complete *P* elements induced considerably higher levels of GD sterility than we have noted in non-dysgenic cross hybrids. Indirect evidence that GD sterility is not always correlated with *P* element instability comes from numerous observations that dysgenic hybrids reared at 18 °C are fertile but manifest *P* element mobility in their germ line. The high sterility of H^s subline-derived dysgenic hybrids reared at low temperature (Margulies, 1990) is the exception rather than the rule.

In this study some correlation was observed between the number of complete *P* elements on chromosome 3 of a particular Harwich subline and the relative activity of that chromosome in sn^w mutability and *vg* suppression. The correlation was not good for chromosome 2 and sn^w mutability. These observations reinforce the importance of interaction between particular species of defective and complete *P* elements in suppression and thus in the control of induction of dysgenic traits as emphasized in other studies (Simmons *et al.* 1990; Biemont *et al.* 1990; and reviews by Engels, 1989 and Rio, 1990). The role of repressor-encoding *P* elements is particularly important in *vg* suppression which occurs in somatic cells (Williams *et al.* 1988*a*), although *vg* suppression is cytotype independent. The observation that Harwich sublines with different *P* activity potential, as measured by GD sterility and sn^w mutability, as well as by partial X chromosome loss (Balter, Griffith & Margulies, 1992) had a similar number of *P* elements per genome and per chromosome arm, indicates that, although the total number of transposable elements is conserved, the specific species and their site distribution must be critical in induction of hybrid dysgenesis traits.

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