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Comparison of the regulation of P elements in M and M′ strains of *Drosophila melanogaster*

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Summary

M and M′ strains of *Drosophila melanogaster* in the P–M system of hybrid dysgenesis were compared in two series of tests, with the following results. (1) The *singed-weak* hypermutability regulation test showed that M′ strains had lower P excision rates than M strains, suggesting that P-elements repression must occur in M′ strains although it is not detectable by gonadal dysgenesis assays. (2) The evolution of mixed P+M and mixed P+M′ populations was compared, using a strong P strain. The P+M cultures invariably evolved in a few generations into strong P cultures, while the P+M′ cultures evolved into P-type cultures with reduced P-factor potentials. However, after 30 generations of culture, both these types of mixed cultures had similar P copy numbers, suggesting that regulation of copy number had occurred in them.

1. Introduction

The P transposable element, which is responsible for the P–M system of hybrid dysgenesis in *Drosophila melanogaster* (Kidwell *et al.* 1977, and see Engels, 1988), has a high capacity for chromosomal contamination (Kidwell, 1983*a*; Kidwell *et al.* 1988). P strains have active P elements in their genomes and an extrachromosomal state which represses P transposition. M strains are devoid of P elements and do not repress P transposition: this allows P–M system dysgenic traits to occur in dysgenic crosses and is referred to as P susceptibility (Kidwell *et al.* 1988). Kidwell *et al.* (1981) and Anxolabéhère *et al.* (1986), have shown that a mixed culture of P- and M-strain individuals evolves towards a P-type strain over a few generations. This illustrates the phenomenon of contamination of the M genome by P factors (Kidwell, 1983*a*) and is accompanied by a rapid loss of P susceptibility.

Some strains collected recently from the wild possess some P copies while showing properties, in the diagnostic gonadal dysgenesis assays of the P–M system, similar to those found for M strains (Anxolabéhère *et al.* 1985). The P copies of these strains, called M′ strains, do not suppress P susceptibility in terms of GD sterility.

In this study, M′ strains were first characterized for P copy number by *in situ* hybridization and for the structure of the P elements using Southern blots. We then compared M and M′ strains using both GD assays, the *singed-weak* hypermutability regulation test and experimental lines initiated by mixtures of a P strain with M and M′ strains. We show that M and M′ strains, giving similar results with GD assays, differ for other phenotypic properties.

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2. Materials and methods

(i) *M and M′ strains used*

M strains, i.e. strains devoid of P copies: Gruta, Hikon, Kochi, Crimea, Charolles, Canton-S (all very old laboratory strains, collected before 1960); Marseillan (France, 1965), Uman-70 (USSR, 1970) (mass-mated populations).

M′ strains i.e. strains having several P copies on their genome and chosen because of their high P susceptibility (see below), which was similar to those of M strains: Kurume (Japan, 1976), Alma-Ata (USSR, 1981), Chimkent (USSR, 1983), Ica (Peru, 1957), Tashkent (USSR, 1981), Uman-83 (USSR, 1983) and Nasr'Allah (Iso-female line; Tunisia, 1983). Whenever possible, each strain (except Nasr'Allah) was derived from more than 30 individuals. They were kept under standard laboratory conditions by mass cultures of about 300 flies. The precise geographical origin of Eurasian strains is shown in Fig. 1.

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(ii) *Gonadal dysgenesis assays*

Gruta was used as the M reference strain (Anxolabéhère *et al.* 1988), Harwich as the strong P reference strain (Kidwell *et al.* 1977); the Harwich stock used here has a mean P copy number per haploid genome of 41·4 (mean of five *in situ* hybridization measures; range: 39–45).

The diagnostic tests used were the standard tests for measuring gonadal (GD) sterility potential (Kidwell, 1983*b*):

Cross A: Gruta females × males of tested strain.

Cross A*: females of tested strain × Harwich males.

For each cross 30 pairs of flies were mated *en masse* in half-pint bottles and immediately placed at 29 °C. Approximately 2 days after the onset of eclosion, F1 progeny were collected and allowed to mature for 2 days at room temperature. At least 50 females were then taken at random for dissection. Dissected ovaries were scored as unilateral (S1 type) or bilateral (S0 type) dysgenic ovaries (Schaeffer *et al.* 1979). The frequency of dysgenic ovaries was calcultated as % GD = % S0 + $\frac{1}{2}$ % S1.

Cross A measures P-activity potential, and Cross A* measures P susceptibility in the tested strain. P susceptibility reflects the ability of the strain to regulate the activity of the P elements and embraces different mechanisms of regulation including cytotype (Engels, 1979*a*) and KP elements (Black *et al.* 1987). M cytotype allows P elements to be active and results in a high P susceptibility (high GD percentage by cross A*), whereas P cytotype represses P-element activity and results in a low or null P susceptibility (less than 5% of GD sterility). KP elements have been shown to reduce gonadal dysgenesis when introduced into experimental mixed lines (Black *et al.* 1987).

(iii) *Hypermutability at the* singed *locus* (*a second measure of P-element regulation*)

Reference stocks. M5/sn^w;π_2 is a P strain with the genetic background of π_2 and produces more than 90% GD sterility in Cross A. This strain has both the Muller-5 balancer chromosome and an *X* chromosome with two defective P elements inserted at the *singed* locus. This mutation, called *singed-weak* (sn^w), causes a slight malformation of the bristles (Engels, 1979*b*, 1981*a*, 1984; Roiha *et al.* 1988). In an unregulated state, sn^w is unstable in the presence of P transposase, mutating to sn^e (a more extreme allele) or sn^+ (apparent wild type). Because the reference strain has the P cytotype, its sn^w allele is stable. The use of this strain allows the regulatory capacity of a given strain for excision to be tested by measuring the mutation rate in appropriate crosses (see below).

w,sn^3: when made heterozygous with the various alleles of sn^w, sn^3 enhances their expression and makes scoring easier; the dominance relations of the *singed* allele are $sn^+ > sn^w > sn^e = sn^3$ (Simmons, 1987).

Mating plan. Thirty tested females were crossed *en masse* to sn^w;π_2 males at 20 °C. Fifty virgin females (F_1 progeny) were crossed *en masse* to fifty *w*,sn^3 males at 25 °C and were allowed to lay eggs in successive bottles for 10 days. Only sn^e and sn^w phenotypes were scored in the F_2 progeny of both sexes. Because it is not possible to tell apart sn^+ from the wild strain tested and a sn^+ revertant, the hypermutability percentage was calculated as follows: % = $sn^e/(sn^e + sn^w)$.

(iv) In situ *hybridization*

P-element copy number was measured by *in situ* hybridization to polytene chromosomes of a tritium DCTP-labelled probe (Pπ25·1; O'Hare & Rubin, 1983) containing the full length P element, plus genomic DNA from the 17C region, in a pBR322 plasmid. Two to five slides were made for each strain, and 5–6 nuclei were analysed per slide. In order to determine the copy number per haploid genome, females of the tested strain were crossed with Gruta strain males (devoid of P copies) and the squash procedure was performed on the F_1 larvae. This analysis was carried out for all M and M′ strains and also at the 30th generation for 18 experimental populations (see below). The 17C label was used as a hybridization-positive control and was, of course, not counted.

(v) *Southern blot analysis*

The structure of the P sequences present in some M′ strains was analysed by Southern blots in order to test for the presence of KP elements. The genomic DNAs were digested with *Ava* II, run on 1% agarose gels, blotted onto nitrocellulose filters and hybridized with the nick-translated Pπ25·7 BWC clone of the P factor which contains almost the full length P element and is devoid of genomic sequences (K. O'Hare, personal communication). In a second analysis the genomic DNAs were digested with *Dde* I, blotted and then hybridized with the nick-translated 0·5 kb *Sal* I fragment of the Pπ25·7 BWC clone. Filters were washed for 30 mn in 2 × SSC, 0·1% SDS at room temperature.

(vi) *Experimental populations*

Two sets of experimental populations were run: the first consisted of mixtures of individuals from a strong P strain (Harwich) and several different M strains, the second set consisted of mixtures of individuals from the same P strain and from several different M′ strains.

Initial crosses:

Cross 'C1': 50 M or M′ strain virgin females were crossed *en masse* with 50 Harwich males.

Cross 'C2': 50 M or M′ strain males were crossed *en masse* with 50 Harwich virgin females.

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These two crosses were carried out for each M or M′ strain. The parents were allowed to lay eggs for two successive periods of five days on normal maize food, seeded with live yeast. The first set of vials formed the '18 °C' subgroup and were maintained at this temperature for all subsequent generations. The second set were transferred to 25 °C after the GD sensitivity period (embryo and first instar larva, Engels, 1981*b*) and were maintained at this temperature for all subsequent generations ('25 °C' subgroup).

In order to follow the dynamics of P-factor expression in mixed cultures, the P-activity potentials in all experimental populations were measured every five generations until generation 30 for the '18 °C' subgroup and until generation 10 for the '25 °C' subgroup (these flies were not tested after this time, because the results were similar to those of the '18 °C' subgroup). The P susceptibility in the experimental populations was also monitored by carrying out cross A* over the first few generations, and at the last generation. As previously shown (Anxolabéhère *et al.* 1986), when a strong P strain was mixed with M or M′ strains, P susceptibility rapidly disappeared after the first few generations and did not reappear. This was also the case in the present experiments and consequently P susceptibility measures are not reported. In order to compare the copy numbers of the P+M and P+M′ combinations, *in situ* hybridization was carried out for 18 mixed lines at the 30th generation.

3. Results

(i) *GD sterility assays and P copy numbers of the strains studied*

Table 1 shows the GD sterility frequencies in crosses A* and A for the M and M′ strains and for Harwich. It also gives the number of P-element copies per haploid genome, as measured by *in situ* hybridization. The M and M′ strains show very similar values for cross A*, all very close to the maximum 100% GD sterility except for Ica (85%). This indicates that P factor regulation, both in the M strains devoid of P copies and in the M′ strains which have 5–20 copies, is absent or not detectable by the GD criterion. The frequencies of cross A GD sterility were all very low (under 5%), as expected for M and M′ strains (Anxolabéhère *et al.* 1988).

(ii) *Geographical origin of the M′ strains*

Fig. 1 provides a summary of the geographical distribution of the Eurasian strains, according to their characteristics in the P–M system (Anxolabéhère *et al.* 1985). It also gives some unpublished data on P copy number in French and German populations. There is clearly a clinal distribution for P copy number from West to East. This cline has previously been suggested by Anxolabéhère *et al.* (1985) on the basis of data produced by the squash-blot technique (Tchen *et al.* 1985) and is confirmed here with the more direct *in situ* technique. Such a cline supports the hypothesis of a West to East spread of the P element on this continent (Anxolabéhère *et al.* 1985).

Table 1. *Comparison of GD sterility and P element copy number in M and M′ strains*

Strain	% GD Cross A*	% GD Cross A	P copy number
Harwich (P)	0	99	41·4
M strains			
Gruta	99	2	0
Hikon	100	0	0
Kochi	99	0	0
Crimea	99	1	0
Charolles	99	0	0
Canton-S	97	0	0
Marseillan	98	2	0
Uman-70	100	4	0
M′strains			
Kurume	100	1	5·0
Alma-Ata	99	0	7·7
Chimkent	96	0	8·8
Ica	85	0	13·0
Tashkent	96	3	13·2
Uman-83	94	3	17·5
Nasr'Allah	98	0	19·5

GD sterility was measured by the dissection of 100 F_1 females. The number of P copies per genome is reported as the mean of 4–6 larvae. The confidence interval at 95% of this mean was in no case greater than 2.

(iii) *Southern blot analysis of M′ strains*

The structure of P elements in M′ strains was analysed using Southern blots in order to detect the presence of KP elements, which have been reported to be typical of M′ natural populations (Black *et al.* 1987; Boussy *et al.* 1988). The Southern blot presented in Fig. 2*a* corresponds to a digestion of the genomic DNA from M′ strains with the *Ava* II restriction enzyme, and was hybridized with the nick-translated almost complete P element from the pπ25·7 BWC clone. The expected fragments generated by the KP element following digestion with *Ava* II are 478 and 628 bp long, and are known to co-migrate in all KP bearing strains (Black *et al.* 1987; Boussy *et al.* 1988). All the M′ strains analysed here, except Kurume (lane 6), clearly show prominent bands at positions expected for the two KP fragments. The case of Kurume is ambiguous because a smaller quantity of DNA was loaded. A new blot (Fig. 2*b*) was performed with equal loading in each lane. The expected 0·48 and 0·63 kb bands are seen for Alma-Ata and Chimkent (lanes 1 and 3) but not for Kurume (lane 2) whose single band, in this region, is

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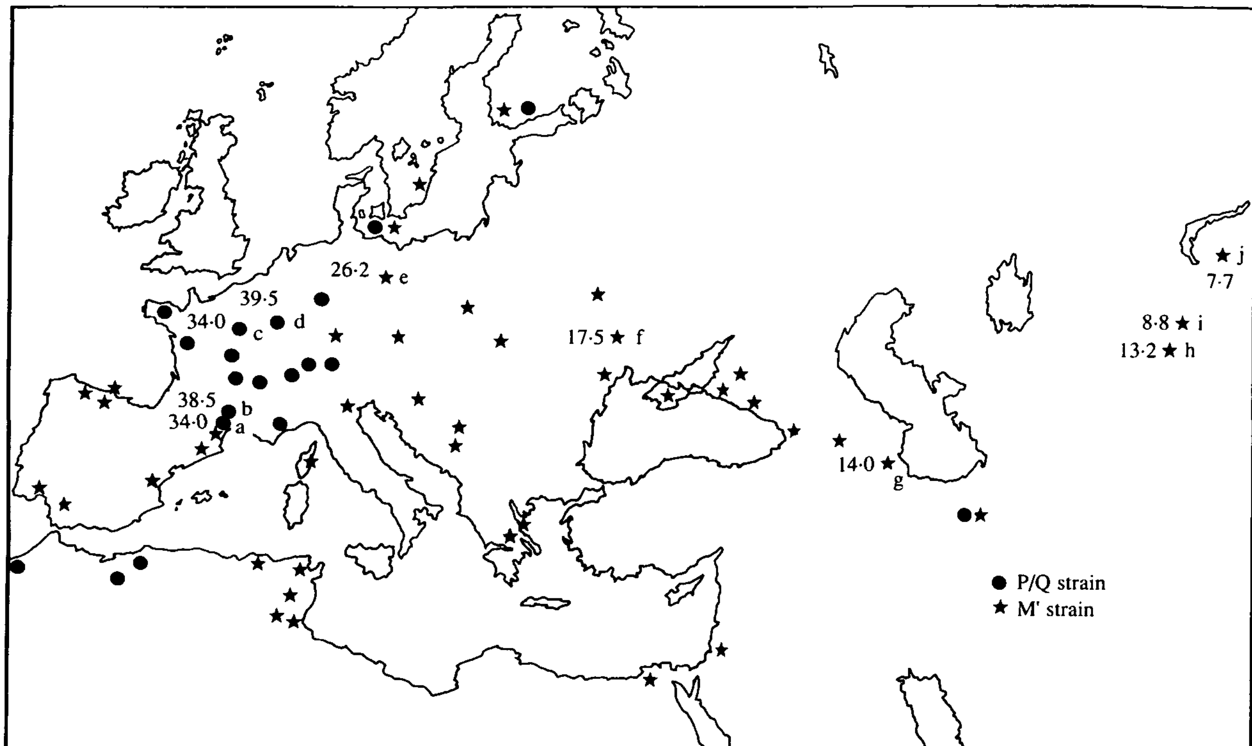

Fig. 1. Geographical distribution of Eurasian populations of *D. melanogaster*. The numbers of P sequences per genome are shown for populations a–j. a, Marseillan (1978); b, Mèze (1978); c, St Geneviève des Bois (1986); d, Epernay (1984); e, Berlin (1983); f, Uman (1983); g, Lerik (1983); h, Tashkent (1981); i, Chimkent (1983); j, Alma-Ata (1981). Characterization of strains as either P/Q (●) or m′ (★) was made on the basis of F_1 GD sterility frequencies from A and A* crosses (Anxolabéhère *et al.* 1985).

slightly but unambiguously below the Alma-Ata and Chimkent bands.

The Southern blot shown in Fig. 2*c* corresponds to a digestion of genomic DNA by the *Dde* I enzyme and was hybridized with a probe which corresponds to half of the last exon of the P element. The fragment expected for the KP element, following the digestion with *Dde* I, is 421 bp long (Black *et al.* 1987). We note that this fragment is detected in all strains except Kurume (lane 5). Both the *Ava* II and *Dde* I digestion have thus allowed us to detect KP elements in all but one (Kurume) M′ strains. The Ica strain was not tested because this strain is being studied separately and has already been found to possess KP elements (D. Anxolabéhère, personal communication).

The restriction fragments produced by a complete P element after digestion with *Ava* II are expected to be 1838, 544 and 478 bp long. The *Ava* II digestion of the complete P element in the pπ25·1 clone was loaded into lane 7 (Fig. 2*a*). The 1·8 kb fragment signal is intense whereas the 0·54 and 0·48 kb show only slight traces. Only the Chimkent strain (lane 3) presents a 1·8 kb fragment which co-migrates with the corresponding fragment of the positive control. The presence of the 544 bp fragment is difficult to see because its intensity is very low, even in the positive control. However, the other strains clearly lack the 1·8 kb fragment. This provides strong evidence for absence of complete P elements.

We conclude that all strains, except Kurume, have some KP elements and that all strains (except perhaps Chimkent) are devoid of complete P elements. Moreover all strains also produce fragments other than those expected from complete P and KP elements (Fig. 2) and therefore must have defective copies which are different from the KP element.

(iv) singed-weak *hypermutability regulatory comparison of M and M′ strains*

The M and M′ strains were tested by the *singed-weak* hypermutability regulatory test. Data shown in Table 2 indicate that all the M′ strains, except Kurume, show lower excision rates than the M strains. The overall difference between the M and M′ groups is significant (even taking into account Kurume) when tested by the Mann–Whitney non-parametric test ($P < 0{\cdot}025$). On the contrary Kurume shows a high rate of mutability, as high as that of Canton-S and three times as high as other M′ strains. According to this assay, Kurume is an M-like strain, very different from the other M′ strains, in which the excision rate is reduced: note that Kurume was found to have a low copy number and to be devoid of KP elements. Harwich is a P strain which has the P cytotype: it provides a negative control and, as expected, it produces a very low excision rate.

Previous studies, carried out on the 'Sexi' strain (Jongeward *et al.* 1987) and the M5/Birmingham strain (Simmons *et al.* 1987), have shown that some

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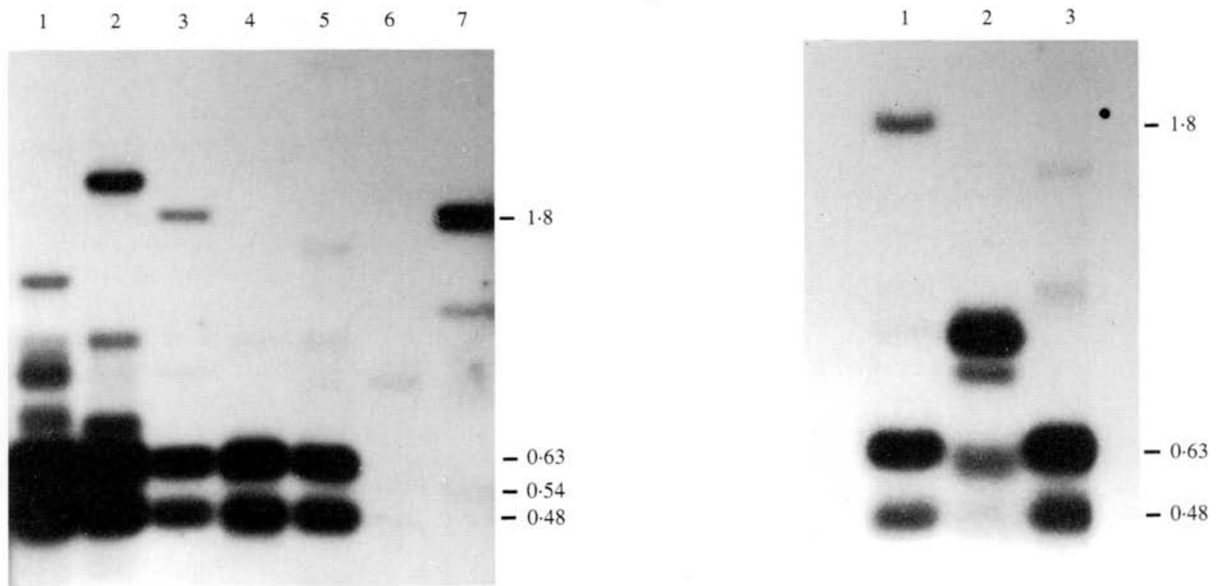

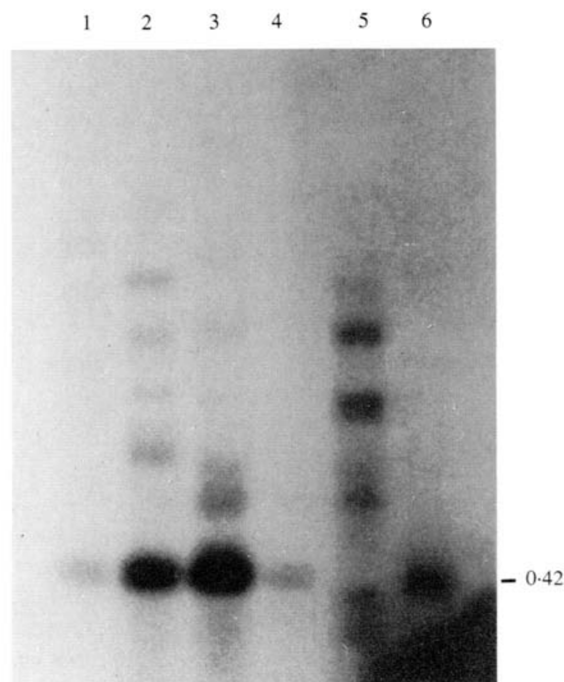

(*c*)

Fig. 2. Structure of P elements from natural populations. (*a*, *b*) Genomic DNAs were digested with *Ava* II and hybridized with Pπ25·7 BWC and assigned to lanes as follows: (*a*) 1, Nasr'Allah (1983); 2, Uman (1983); 3, Chimkent (1983); 4, Tashkent (1981); 5, Alma-Ata (1981); 6, Kurume (1976); 7, Pπ25·1 clone digested with *Ava* II. (*b*) 1, Chimkent (1981); 2, Kurume (1976); 3, Alma-Ata (1981). (*c*) Genomic DNAs were digested with *Dde* I, hybridized with the 0·5 kb *Sal* I fragment of Pπ25·7 BWC and assigned to lanes as follows: 1, Uman (1983); 2, Chimkent (1983); 3, Tashkent (1981); 4, Alma-Ata (1981); 5, Kurume (1976); 6, Nasr'Allah (1983). The sizes (in kb) of the expected fragments from KP and complete P elements (see text) are indicated.

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Table 2. singed-weak *hypermutability regulation test. The scores of males and females were pooled*

Strain	sn^e	sn^w	%
Harwich (P)	5	2090	0·2
M strains			
Gruta	202	2419	7·7
Hikon	260	3668	6·6
Kochi	68	891	7·1
Crimea	206	2737	7·0
Charolles	55	648	7·8
Canton-S	592	5204	10·2
Marseillan	130	1444	8·3
Uman-70	129	1388	8·5
M′ strains			
Kurume	193	1383	12·2
Alma-Ata	120	2804	4·1
Chimkent	120	2748	4·2
Ica	112	2238	4·8
Tashkent	155	2883	5·1
Uman-83	125	3090	3·9
Nasr'Allah	88	1815	4·6

The hypermutability percentage was calculated as $\% = sn^e/(sn^e+sn^w)$

M′ strains reduce sn^w hypermutability. However these strains did not show strong P susceptibility (33 and 31 % GD sterility, respectively, by the Cross A* test). We show here that some M′ strains, phenotypically comparable to M strains by the GD assays, can reduce the P excision rate at the sn^w hypermutable locus. No direct comparison between excision rates of our strains and of Sexi can be made because of different mating plans. A test of the M5/Birmingham strain (Simmons *et al.* 1987) gave a 3% mutation rate (130 sn^e and 4275 sn^w). This value is a little smaller than the values of the M′ strains analysed here (see Table 2); this is not surprising given the reduced P susceptibility of M5/Birmingham, using the GD criterion.

(v) *Comparison of mixed lines*: *GD assays*

Tables 3 and 4 show Cross A GD sterility frequencies of the experimental populations for the two reciprocal initial crosses (C1 and C2 respectively) as a function of test generation and rearing temperature. For the M strain group, a very high level of P activity potential was observed from the 5th generation onwards; this group remained at this level until the 30th generation. No differences were found between the two rearing temperatures or between the two initial crosses. For the M′ group the situation was less straightforward: the different M′ strains did not all react in the same way. One strain (Kurume) showed a similar evolution to that of the M strain group: a very high P potential was found at both temperatures and in all generations. However, the other M′ mixed populations showed much lower levels of P activity potential with no effect of population rearing temperature. C1 and C2 lines both showed some isolated variation depending on the generation, but the overall tendency of the M′ group was to remain at a lower level or even to decrease during the last generations of the test.

To enable comparisons to be made between and within the M and M′ groups, the mean of the last three tests of P-activity potential (generations 20, 25, 30) is shown in Table 5 (the two columns on the right). Comparisons of the M and M′ groups clearly show the difference between these two groups, and also show, within the M′ group, the difference between Kurume and the other lines. The overall difference between the M and M′ groups is significant (even taking into account the Kurume mixed lines) when tested by the Mann–Whitney non-parametric test ($P < 0{\cdot}01$ for C1 and for C2). Comparison between the C1 and C2 populations showed no significant differences (except for the Alma-Ata lines).

(vi) *Comparison of mixed lines*: in situ *data at the 30th generation*

Table 5 shows the mean number of P copies detected per haploid genome at the 30th generation, in 18 experimental populations, together with the P copy number of the M′ strains from Table 1. These data show at most only small differences between P+M and P+M′ mixed populations. The magnitude of the differences in each case is smaller than that expected under a simple model of additivity between the copy numbers in Harwich and M′ strains. Under this model, the expected copy number of an experimental population from an M′ strain mixed with the Harwich strain should be the average copy number obtained in experimental populations from the M strains mixed with the Harwich strain, plus the number of copies due to the M′ strain. In any case, the final expected copy number should be greater in M′+Harwich populations than in M+Harwich populations. This is not the case here. The different mixed populations of Harwich with Gruta, Hikon, Kochi and Uman-70 are similar, giving a mean of 39·0 for C1 and 36·0 for C2. In all cases, except Alma-Ata/C1, the values obtained in the M′ group are close to these values. For example, there are 17 copies in the Uman-83 strain and we found, when this strain was crossed with Harwich flies, 39·7 copies for C1 and 41·3 for C2. Moreover, the mean values obtained for the P+M′ mixed populations are 39·6 for C1 and 38·9 for C2, values which are also very close to those of the P+M mixed populations. This shows that copy number regulation is effective, resulting in each case in a copy number close to that of the Harwich strain (around 40 copies).

4. Discussion

In the P–M system of hybrid dysgenesis, M′ strains can have similar GD assay properties to those of M

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Table 3. *Cross A GD sterility percentage for the C1 mixed populations as a function of generation number and rearing temperature. A minimum of 50 females were dissected for each GD measure*

Generation...	F_1	F_5		F_{10}		F_{15}	F_{20}	F_{25}	F_{30}
Temperature (°C)...	18°	18°	25°	18°	25°	18°	18°	18°	18°
M strains									
Gruta	59	96	96	97	91	96	100	99	100
Hikon	74	99	98	92	96	93	100	99	94
Kochi	45	95	99	98	98	94	99	100	92
Crimea	69	91	97	89	83	89	99	95	98
Charolles	62	97	96	83	98	88	99	99	99
Canton-S	60	92	93	88	92	90	95	93	96
Marseillan	61	81	88	80	95	83	100	100	97
Uman-70	64	98	99	96	100	98	98	92	90
Mean	61·8	93·6	95·8	90·4	94·1	91·4	98·8	97·1	95·8
M′ strains									
Kurume	70	90	89	97	86	98	99	97	100
Alma-Ata	61	76	76	55	76	64	50	29	27
Chimkent	63	67	59	60	57	54	32	32	45
Ica	40	61	57	56	70	53	65	77	63
Tashkent	45	33	50	57	32	32	71	77	67
Uman-83	55	54	68	69	53	65	45	37	37
Nasr'Allah	46	62	64	62	52	44	67	65	68
Mean	54·3	63·3	66·1	65·1	60·9	58·6	61·3	59·1	58·1

Table 4. *Cross A GD sterility percentage for the C2 mixed populations as a function of generation number and rearing temperature. A minimum of 50 females were dissected for each GD measure*

Generation...	F_1	F_5		F_{10}		F_{15}	F_{20}	F_{25}	F_{30}
Temperature (°C)...	18°	18°	25°	18°	25°	18°	18°	18°	18°
M strains									
Gruta	91	95	—	98	—	92	97	98	100
Hikon	86	94	99	95	97	91	100	100	97
Kochi	93	98	88	94	98	99	100	98	100
Crimea	90	91	95	97	97	95	100	94	95
Charolles	88	86	84	88	98	97	100	94	99
Canton-S	95	86	93	90	100	94	95	96	97
Marseillan	81	90	—	87	—	90	83	93	98
Uman-70	92	92	94	97	100	98	97	96	91
Mean	89·5	91·5	92·2	93·3	98·3	94·5	96·5	96·1	97·1
M′ strains									
Kurume	92	97	94	99	98	96	98	97	100
Alma-Ata	83	93	78	95	86	97	80	78	83
Chimkent	82	78	82	82	68	71	37	48	64
Ica	69	57	76	60	62	60	87	71	56
Tashkent	83	63	59	65	55	61	68	69	62
Uman-83	78	64	88	64	71	56	56	35	40
Nasr'Allah	80	67	86	66	56	91	92	—	37
Mean	81·0	74·1	80·4	75·9	70·9	76·0	74·0	66·3	63·1

strains despite having several P elements. Most of these strains were recent stocks derived from wild populations. We have studied a set of these M′ strains for their P-element copy number and structure. We have compared them to M strains using a hypermutability regulation test (which is another measure of P susceptibility) and by following P-activity potential in mixed lines.

We have found that M′ strains can have from five to 20 copies, as detected by *in situ* hybridization, and yet show complete P susceptibility as measured by Cross A*. All the M′ populations that we have examined from Eurasia, except for Kurume, were found to carry KP elements, as one would expect from the fact that this specific defective P element is widespread in Eurasia (Black *et al.* 1987), Australia

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Table 5. *P-element copy number and GD sterility frequencies in the C1 and C2 mixed populations, after 30 generations*

	Original strains	Experimental populations			
	Initial number of	Number of P copies[a]		Cross A GD sterility[b]	
Name	P copies	C1	C2	C1	C2
M strains					
Gruta	0	36·7	37·0	99·7	98·3
Hikon	0	35·0	31·3	97·7	99·0
Kochi	0	41·3	37·7	97·0	99·3
Crimea	0	—	—	97·3	96·3
Charolles	0	—	—	99·0	97·7
Canton-S	0	—	—	94·7	96·0
Marseillan	0	—	—	99·0	91·3
Uman-70	0	43·0	38·0	93·3	94·7
Mean	—	39·0	36·0	97·2	96·6
M′ strains					
Kurume	5·0	38·5	41·5	98·7	98·3
Alma-Ata	7·7	48·3	33·0	35·3	80·3
Chimkent	8·8	37·5	40·7	36·3	49·7
Ica	13·0	—	—	68·3	71·3
Tashkent	13·2	34·0	38·1	71·7	66·3
Uman-83	17·5	39·7	41·3	39·7	43·7
Nasr'Allah	19·5	—	—	66·7	64·5
Mean	—	39·6	38·9	59·5	67·7

[a] Number of P copies per genome at the 30th generation expressed as the mean of 2–3 slides. The confidence interval at 95% of this mean was never greater than 4.
[b] Mean of cross A GD sterility measure at generations 20, 25, and 30.

(Boussy *et al.* 1988) and China (Anxolabéhère and Periquet, personal communication). All the M′ strains analysed here appear to be devoid of complete P elements (except perhaps Chimkent).

Both methods of comparison showed that M and M′ strains have different properties, although Kurume behaves like an M strain. The hypermutability tests indicate that regulation occurs in the germ-line of M′ strains which decreases the P excision rate. Further, the behavior of P factors is modified in P+M′ lines as the P-type strain 'reconstitution' does not happen in the same way as in the P+M lines.

The differences between the P+M′ and the P+M mixed cultures could be due to the fact that selection is taking place, on the basis of which the Harwich chromosomes eliminate the M chromosomes, but do not have the same effect on the M′ chromosomes; M strains have been maintained for longer in the laboratory and could have lower adaptative values. However, under this hypothesis the differences in the results found for mixed cultures involving Ica 1957 and Marseillan 1965 or Uman 1970 should be in the opposite direction to those which were observed.

Using *in situ* hybridization, we tested for the presence of a specific P site (site 74F) which was found in all the tested larvae of the Harwich strain. We found that for the two kinds of mixed cultures, this site occured in approximately half the chromosomes observed, as expected under random chromosomal segregation (data not shown). Moreover, the way in which the populations were set up (direct crosses between the strains) allows recombination to take place, and the genome to be mixed, from the first generation onwards. The difference between the P+M and P+M′ mixed cultures cannot be explained simply by chromosomal selection.

In fact, both for sn^{w} hypermutability and for the results from mixed lines, the differences between M and M′ strains must be due to the different P elements in M′ strains. These include KP elements which produce a 0·84 kb transcript and whose potential 207 amino-acid polypeptide may interfere negatively with P transposase function (Black *et al.* 1987). Such differences may also be due to other kinds of elements; P elements defective for the last exon have been shown to exert a repressor effect on complete P factors (H. Robertson, unpublished data described in Engels, 1988; Nitasaka *et al.* 1987). The Kurume strain behaves like an M strain; this can be interpreted as the consequence of both a small copy number (5) and the absence of KP elements.

The P+M′ lines reached a similar number of copies to that of the P+M lines, whilst having a reduced P-activity potential (except the case of Kurume). The

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presence of the defective elements has therefore modified the relative proportion of complete and deleted elements without altering the total number of elements in the line. The first point is in agreement with Black *et al.* (1987) and with the results of M. Jackson (personal communication) who found, using Southern blots on mixed lines, an increase in the number of copies for the KP family coupled with a drastic decrease in the number of complete P elements. The second point indicates that, whether or not additional copies are introduced by M′ chromosomes, and irrespective of the number and the structure of these copies, a copy number close to that of Harwich is reached. Such a phenomenon would be expected if the quantity of transposase provided by the Harwich chromosomes is a limiting factor which allows the establishment of a given number of copies during chromosomal contamination processes. 'Transposase titration' effects have already been detected using sn^{w} hypermutability, by genetical methods (Simmons & Bucholz, 1985) and by DNA microinjection assays (W. Eggleston, M. Fortini and Engels, cited in Engels, 1988). The similar numbers of copies, reached here by P+M or P+M′ lines, suggests that transposase titration may also play a role in P-copy number regulation.

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