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Hybrid dysgenesis in natural populations of *Drosophila melanogaster* in Japan. II. Strains which cannot induce *P-M* dysgenesis may completely suppress functional *P* element activity

NOBUKO HAGIWARA[1], ETSUKO NAKAMURA, ETSUKO T. MATSUURA AND SADAO I. CHIGUSA[2]

Department of Biology, Ochanomizu University, Bunkyo-ku, Tokyo 112, Japan

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Summary

Many inbred and isofemale lines derived from wild populations of *Drosophila melanogaster* were tested for gonadal dysgenic sterility, male recombination and sn^w secondary mutation. Among them, we have found strains whose dysgenic offspring show negligible sterility, and undetectable male recombination and sn^w mutation. They can be considered to be neutral strains in the strict sense. Such neutral strains appear to carry only defective *P* elements in their genomes. Taking the observations of Karess & Rubin (1984) into account, it is suggested that some defective *P* elements retain the function necessary for *P* cytotype. Cytotype determination mechanisms are discussed.

1. Introduction

The *P* element is one of the families of transposable elements in *Drosophila*. The intact 2907 bp *P* elements carries a 31 bp inverted repeat at both ends (O'Hare & Rubin, 1983) and appears to encode transposase and a factor which regulates transposition (Karess & Rubin, 1984; Laski *et al.* 1986; Rio *et al.* 1986). The *P* element transposes at extremely high rates under certain conditions. It induces the syndrome of *P-M* hybrid dysgenesis, which occurs non-reciprocally in hybrid offspring of dysgenic crosses between *M* females lacking functional *P* elements and *P* males. The syndrome includes gonadal dysgenic (GD) sterility, male recombination, chromosomal rearrangements and enhanced mutability (for reviews see Bregliano & Kidwell, 1983; Engels, 1983).

Hypermutable *singed-weak* was also obtained in *P-M* hybrid dysgenesis (Engels, 1979*a*). This results from two small, defective *P* elements inserted at the *singed bristle* locus on the *X* chromosome (Karess & Rubin, 1984). The insertional visible mutations caused by *P* elements are usually unstable and revert at a rate of about 10^{-3} in the dysgenic state (Rubin *et al.* 1982). However, *singed-weak* (sn^w) mutates secondarily to the wild type (sn^+) or to the extreme singed allele (sn^e) at extremely high rates, up to 5×10^{-1} in hybrid offspring of dysgenic crosses. With one copy of the intact *P* element in the genome, it mutates at a rate of 1·7–8·1 % (Karess & Rubin, 1984). It is stable in the offspring of the reciprocal crosses (Engels, 1979*a*) and in the offspring of dysgenic crosses unless a functional or intact *P* element is present (Engels, 1984; Karess & Rubin, 1984). Therefore, using this hypermutation system, the presence of the intact *P* element can be detected by genetic analysis. It seems reasonable to consider the secondary mutation rate as dependent on transposase activity encoded by the *P* element (Spradling & Rubin, 1982; Rubin & Spradling, 1982).

According to O'Hare & Rubin (1983), defective *P* elements outnumber the intact ones by approximately 2:1 in the genome of π_2, a strong *P* strain. Defective *P* elements may account for a major proportion of *P* elements carried by *Q* strains, where the term 'defective' is used with respect to their function and structure. *Q* strains do not produce sterile offspring when crossed to either *P* males or *M* females (Kidwell, 1979), but do induce other dysgenic traits to some extent (Engels & Preston, 1981; Simmons *et al.* 1980,

[1] Present address: Department of Molecular, Cellular, and Developmental Biology, Campus Box 347, University of Colorado, Boulder, CO 80309.

[2] To whom correspondence should be addressed.

1984*a*,*b*). Strains ν_6 and Mt Carmel, derived from natural populations in North America, have been frequently used as *Q* or weak *P* strains, defined originally on the basis of GD sterility (Engels & Preston, 1981). They have been reported to carry intact *P* elements (Simmons *et al.* 1984*a*), at least two in the ν_6 strain (Simmons *et al.* 1984*b*). However, these results do not necessarily lead to the conclusion that all *Q* strains must carry intact *P* elements.

We report here data indicating that certain *Q* strains derived from natural populations do not carry any intact *P* elements. These particular strains possess the *P* cytotype which completely represses GD sterility caused by *P*-element movement, and therefore may be termed completely neutral strains. It is suggested that *P*-cytotype strains do not necessarily possess intact *P* elements. Our evidence is based on genetic analysis of gonadal dysgenic (GD) sterility, male recombination, and *singed-weak* secondary mutation. We discuss possible implications of the present results for models of *P*-cytotype determination

2. Materials and Methods

(i) Drosophila *strains*

Canton-S. A standard *M* strain (Kidwell, 1979).

C(1)*DX*;*bw*;*st*. An attached-X *M* strain.

cn bw. An *M* strain with recessive markers, *cn* and *bw*. This stock was used to measure male recombination frequency in dysgenic hybrids.

Harwich. A standard *P* strain (Kidwell, 1979).

y sn^w(*M*)/*y*$^+$*Y*;*bw*;*st*. An *M* cytotype strain provided by W. R. Engels, containing no *P* element except those inserted at or near the *sn* locus. This stock was used for testing the presence of transposase-producing *P* elements by scoring the mutational instability of the *sn*^{w} gene (Engels, 1979*a*,*b*).

Strains derived from natural populations

Sixteen, 9 and 1 inbred lines were derived from natural populations of Ishigaki, Naha and Chichijima (Japan), respectively. They were maintained by brother–sister mating for about ten successive generations and subsequently kept as mass cultures.

Isofemale lines were derived from natural populations of various countries and islands such as India (2), Tonga (2), Pakistan (2), South Korea (2), Ponape (2), and Akita, Japan (3). The number of lines derived for each geographical population is indicated in parentheses. These flies from various parts of Asia were collected by O. Kitagawa.

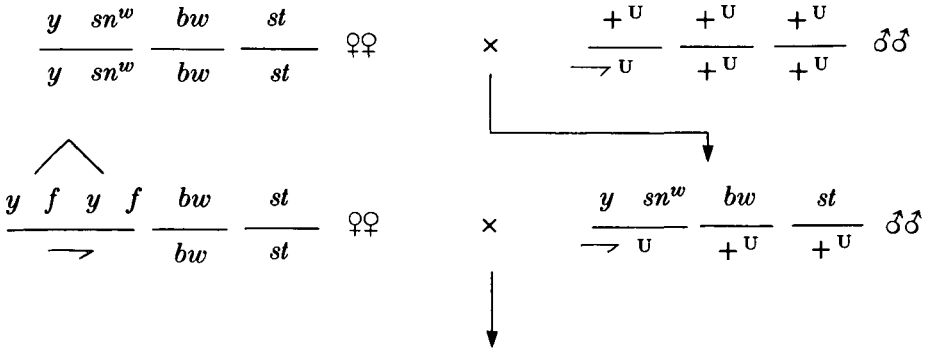
Isogenic second chromosome lines were established by the *SM*1 (*Cy*) method from flies collected in Izumo and Akita, Japan. In these isogenic strains the chromosomes other than the second were substituted with those of the *M* strain, Samarkand. These chromosomes have undergone dysgenic crosses during the chromosome extraction processes and, as a consequence, may have been modified by hybrid dysgenesis.

Worldwide surveys of the *P-M* system made by our colleagues were referred to in selecting these strains from our laboratory stocks for the purpose of the present experiment (Takada *et al.* 1983; Kato *et al.* 1984; Ohishi *et al.* 1982).

Refer to Lindsley and Grell (1968) for symbols and phenotypes of mutant markers.

(ii) *Stability test of sn*^{w}

In order to test the activity of *P* elements, the following crosses were made:



The *sn*^{w} chromosomes were placed in the dysgenic state by crossing *sn*^{w}(*M*) females with U (unknown) males at 25 °C. All F_2 males emerging by the 16th day were scored for their *sn* phenotypes. Approximately 50 F_1 dysgenic males were sampled for each strain under consideration and were crossed to attached-X females, and then a total of approximately 2000 F_2 males were scored for their *sn* phenotypes. The proportion of F_2 male offspring showing mutation from *sn*^{w} to *sn*$^+$ or *sn*^{e} was used as a 'genetic' estimate of transposase activity of whole *P* elements located in the *Y* chromosome and the autosomes of each strain. These *P* elements can be considered as helpers for excising the defective *P* elements inserted in tandem at the *sn* locus (Karess & Rubin, 1984).

In the present experiment, the *sn*^{w} secondary mutation rate was measured only through the *male*

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germ line. It is possible that a different rate would be obtained through the *female* germ line (Kocur *et al.* 1986; Simmons *et al.* 1985).

(iii) *Gonadal sterility test*

To make the parental genome of dysgenic females identical to that of males tested for sn^w mutability, 3–5 males of each strain were singly crossed to *C*(1) *DX*; *bw*; *st* females (*M* cytotype). These crosses were referred to as cross A for Y and autosomes.

Next, to test the cytotype-determining ability of the same sets of chromosomes as above, the *Y* chromosomes and the autosomes of *C*(1) *DX*; *bw*; *st* females were substituted with those of the strains under consideration following the procedure described by Engels (1979*b*). Mass matings of approximately 20 males and 20 females were set up at 22·5 °C for each strain. When progeny emerged, virgin females were collected for each cross and about 20 of them were mated again to new males of the corresponding strain for the next generation. This scheme was repeated for 6 generations. The females of each strain thus obtained were designated as *C*(1) *DX*; $+^U$; $+^U$. They were kept at 25 °C with mass matings in vials (100 × 30 mm) thereafter, and crossed to Harwich males to test their cytotypes. These crosses were referred to cross A* for Y and autosomes. Therefore:

Cross A for Y and autosomes = *C*(1) *DX*; *bw*; *st* ♀ × U ♂,
Cross A* for Y and autosomes = *C*(1) *DX*; $+^U$; $+^U$ ♀ × Harwich ♂.

Cross A for Y and autosomes was made to estimate the level of sterility-inducibility by unknown males (U ♂) and cross A* for Y and autosomes was made to test the cytotype of attached-X females (*C*(1) *DX*; $+^U$; $+^U$ ♀). These matings were kept at 29 °C for 7 days. All F_1 progeny emerging by the 11th day after the initial cross were transferred to a new vial with fresh media for ageing three or more days. Fifteen females from each vial were dissected for examination of their ovaries. The proportion of rudimentary ovaries to total ovaries tested was taken as a measure of sterility-inducibility in cross A for Y and autosomes, and the proportion of normal ovaries to total ovaries tested was taken as a measure of cytotype level in cross A* for Y and autosomes.

In these experiments the *P* elements on the *Y* chromosome and the autosomes were considered with respect to inducibility of gonadal dysgenesis, ability to determine cytotype, *singed-weak* mutability and male recombination. However, when entire parental genomes were tested for dysgenic potential, we conducted the standard A and A* gonadal dysgenesis tests, according to the following mating schemes (Kidwell, 1979):

Cross A = Canton-S ♀ × U ♂,
Cross A* = U ♀ × Harwich ♂.

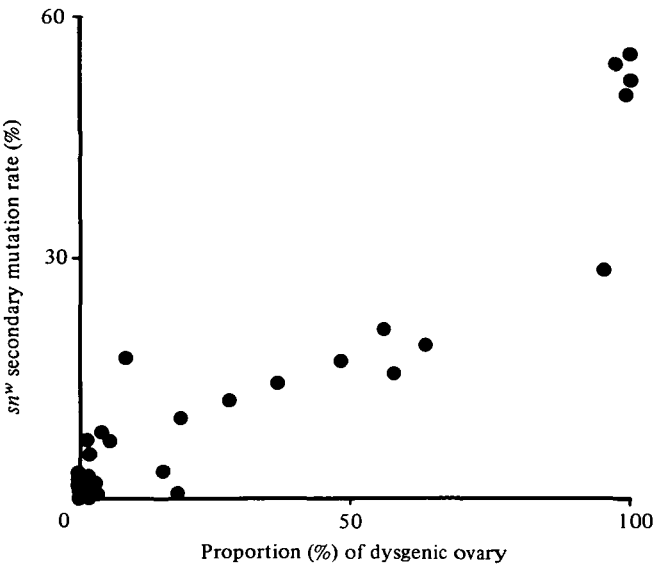

Fig. 1. The relationship between GD sterility and sn^w mutability. The sn^w secondary mutation rate was estimated by the ratio of the mutated males (sn^e, sn^+) to the total males (sn^w, sn^e, sn^+). For the sterility test, males from each strain were mated to females of *C*(1) *DX*; *bw*; *st* at 29 °C. To keep the parental genomes carried by dysgenic females identical to those of dysgenic males of sn^w mutability tests, *M* cytotype attached-X females were used. The ordinate represents the percentage of dysgenic ovaries in crosses A for Y and autosomes. In this experiment 35 strains were examined.

(iv) *Male recombination test*

Male recombination frequency was measured by crossing males from each strain to *cn bw* females. Heterozygous male progeny were then backcrossed to *cn bw* females. Both crosses were made at 25 °C. All progeny emerging by the 16th day were scored for recombinants. Note that the parental gnome composition in this test is the same as that in the test of sn^w instability described above.

3. Results

(i) *Transposase activity and other dysgenic traits*

Fig. 1 shows the relationship between the sterility-inducibility measured by cross A for Y and autosomes and the sn^w secondary mutation rate. A strong relationship was observed between the two measurements. Engels (1984) has reported essentially the same results as ours based on a similar experiment, in which sterility increased monotonically with mutation rate throughout the whole range. In our data, however, transposase activity lower than that reported by Engels appears to be sufficient to induce the same level of sterility. In addition, a rapid increase in sterility appeared near the highest mutation rate in our experiment, though the highest values of the sn^w mutation rates shown by strains producing full sterility were approximately 50% in both experiments. One possible reason for this difference between the two

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Table 1. *The* sn^w *secondary mutation, male recombination and sterility test by cross A for Y and autosomes under the* P-M *system*

Strain	sn^w secondary mutation		Male recombination		Cross A for Y and autosomes	
	Mutation rate(%)	Total number of male progeny observed	Recombination frequency (%)	Total number	% of dysgenic ovaries	Total number of ovaries
Harwich	54·9	2169	1·2	7076	100	150
IG280–1	17·7	1937	0·61	20649	8·9	150
IG280–8	14·4	1985	0·16	14758	36	150
AT2404[a]	10·6	2028	0·42	15936	15·3	180
SN2025[a]	10·2	1733	0·48	12206	0·83	120
AT2420[a]	5·28	2216	0·17	10568	8·7	150
IG575-8	2·19	1589	0·13	15592	0	90
OOQ27	0	3900	$9·6 \times 10^{-3}$	10415	0·83	120
IG489-6	0	3276	0	21413	1·1	90
Canton-S	0	1488	$4·4 \times 10^{-3}$	22777	2	150

[a] Isogenic strains of the second chromosome derived from Akita and Izumo natural populations. The data of these strains are not included in Fig. 1.

experimental results may lie in the sterility-sensitivity of the females used as *M* cytotype strains. Our experimental conditions have revealed that a threshold value may exist for transposase activity to induce complete sterility.

Next we examined the relationship between male recombination and sn^w secondary mutation. The experimental results are summarized in Table 1. It should be noted that the parental genomes carried by dysgenic F_1 males of the two crosses are identical (see the mating schemes described in Materials and methods). A close positive correlation between the male recombination frequency and the sn^w mutation rate was clearly observed. Male recombination is a main feature of *P-M* hybrid dysgenesis and has a positive correlation with GD sterility (Engels & Preston, 1980). These observations seem to imply that the transposase encoded by *P* elements also plays an important role in the production of male recombination. The lethal mutation rate has also been reported to correlate highly with the sn^w secondary mutation rate (Raymond & Simmons, 1981; Amemura *et al.* in preparation). These experimental data indicate that many dysgenic traits can be quantitatively characterized in terms of the sn^w secondary mutation rate.

In predicting the presence of intact *P* elements, the cross A test for the induction of female sterility may not be necessarily good. Strain IG575-8 was classified as a *Q* strain based on standard crosses A and A* (0/120 dysgenic ovaries/total ovaries and 150/150 normal ovaries/total ovaries, respectively). To get comparable data with another *Q* strain, ν_6, reported by Engels & Preston (1981), Canton-S was specifically used in the present dysgenic crosses. However, IG575-8 showed a male recombination frequency similar to that of ν_6. According to Engels & Preston (1981), ν_6 is a strong *Q* strain (cross A, 4/427 sterile females/total females; cross A*, 2057/2071 fertile females/total females), and it also produced male recombinants at a frequency of 0·3%, and sn^w secondary mutations at a rate of about 3% (Engels, 1984). Both IG575-8 and ν_6 may be more properly termed weak *P* strains, since these strains retain the ability to induce male recombination and to destabilize sn^w.

On the other hand, we found another type of *Q* strain which hardly shows male recombination and sn^w secondary mutation. These were strains OOQ-27 and IG489-6, derived from Chichijima (Japan) and Ishigaki (Japan) natural populations, respectively. By standard cross A tests, the proportions of rudimentary ovaries among all ovaries of OOQ-27 and IG489-6 dissected were 0% (0/120) and 0% (0/120), respectively; and by standard cross A* tests, the proportions of normal ovaries among total ovaries of OOQ-27 and IG489-6 dissected were 100% (90/90) and 100% (120/120), respectively. The sn^w secondary mutation rates and male recombination frequencies of these two strains are presented in Table 1, where the estimated values of the two strains are substantially lower than those of IG575-8. These results suggest that GD sterility tests by crosses A and A* alone may not be sufficient to define properly *Q* strains with different transposase activity.

(ii) *Existence of completely neutral strains*

Cross A* for Y and autosomes assesses the effect of the *Y* chromosome and the autosomes on *P* cytotype determination in the synthesized strains. Fig. 2 shows the relationship between the *P* cytotype level measured by cross A* for Y and autosomes and the *P*-element transposase activity based on the rates of the sn^w secondary mutation. The genomic compositions were

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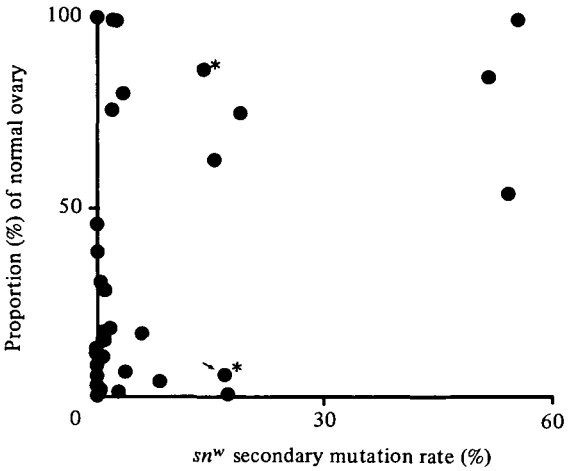

Fig. 2. Relationship between sn^w mutability and ability to convert cytotype from the *M* type into the *P* type. The chromosomal substitution procedure was applied to the strains in Fig. 1. * Strains whose cytotype levels were measured at the second generation (G2) after the chromosome substitution procedure was completed. The cytotype level of the strain marked with a small arrow increased to 40% at G5. The data of the sn^w secondary mutation are the same as shown in Fig. 1.

the same for the two measurements. One conspicuous point at the upper-left corner in Fig. 2 represents a *Q* strain, which produces no sn^w mutation, but appears to have complete *P* cytotype. This complete repression of *P*-element activity was also observed when $sn^w; \pi_2$ males were crossed to females of this strain (Hagiwara, unpublished data). The *Y* chromosome and the autosomes of this strain originated with IG489-6 whose entire genome was examined in the previous section. This strain, newly obtained through successive back crosses, is designated as *C*(1) *DX*; IG489-6.

Based on cross A* for Y and autosomes we found the cytotype of *C*(1) *DX*; OOQ-27 to be *M*, while OOQ-27 itself showed the complete *P* cytotype as described in the previous section. The sn^w mutability on the basis of the whole genome was tested for OOQ-27 (for experimental procedure, see Karess & Rubin, 1984), and no sn^w mutant (0/5545) was observed. It is not certain why the entire genome is necessary for OOQ-27 to have the complete *P* cytotype, but this may be due to the localization of *P* elements with the function of *P*-cytotype determination to the *X* chromosome. OOQ-27 can be considered as a second completely neutral strain.

The cytotype levels of all strains shown in Fig. 2 were later retested at G5 and G12. Most intermediate *P*-cytotype levels remained unchanged for these generations. The stability of intermediate *P*-cytotype level has also been reported by Kidwell (1985). This was also true for most of the *M*-cytotype strains having the lowest *P*-cytotype level. In one strain indicated by a small arrow in Fig. 2, however, the cytotype level changed to about 40%.

At present there is no doubt that both the *P* cytotype and the sn^w secondary mutation rate are controlled by the *P* elements on the chromosomes. We observe in Fig. 2 that strains with various cytotype levels shared a similar mutation rate. This means that strains with a similar number of intact *P* elements could have various levels of the *P* cytotype. How can one interpret this kind of *P* cytotype level variation? A clue may be found by looking at the broad distribution of the *P*-cytotype levels from 0–100% among the strains having the sn^w mutation rate of 0%. It is suggested that such strains lack the intact *P* element in their genomes. These exceptional strains demonstrating uncoupling of these two manifestations of hybrid dysgenesis lead us to a hypothesis concerning the defective *P* element and *P*-cytotype determination described in Discussion.

4. Discussion

The completely neutral strains, *C*(1) *DX*; IG489-6 and OOQ-27, have a strong *P* cytotype, but produce neither sn^w mutation nor male recombination (Table 1). These features indicate that the intact *P* element is *not necessary* to determine the *P* cytotype. We see a peculiar strain in Fig. 2 which is of *M* cytotype but can induce rather high (17%) sn^w secondary mutation rate. In subsequent tests of this strain, its cytotype has remained an *M* type (1/120 normal ovaries/total ovaries in A* cross for Y and autosomes), although the rate of sn^w secondary mutation decreased and reached about 0·9% at G24. It is suggested that some intact *P* elements may have existed in this strain, but they were *not sufficient* to convert the cytotype. This particular result is consistent with the unsuccessful attempt of Karess and Rubin (1984) to change the *M* cytotype to the *P* cytotype, initially using only one intact *P* element. Taking these findings into account, a certain type of defective *P* element appears to play an important role in creating the *P* cytotype.

Several models have been proposed for cytotype-determining mechanisms (Engels, 1980; O'Hare & Rubin, 1983; Simmons & Bucholz, 1985; Simmons *et al.* 1985). They essentially assume that at least one intact *P* element is needed to produce the *P* cytotype. To explain complete suppression of *P*-element activity, the existence of a transposase-producing *P* element has often been postulated to supply an extrachromosomal *P* element pool. In their titration model Simmons & Bucholz (1985) assign a regulatory role to the *P* element itself. In the present experiment, however, we found completely neutral strains with strong *P* cytotype, without any transposase-producing *P* elements. It may not be plausible to explain these observations by either model described above. In a two-component model proposed by O'Hare & Rubin (1983), they assumed two types of molecules, transposase and regulator, encoded by different open reading frames. However, Karess and Rubin (1984)

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found that all of the four open reading frames are necessary for coding the transposase.

The existence of the OOQ-27 and *C*(1)*DX*; IG489-6 strains also appears to support the modified two-component model discussed by Kidwell (1985), though the characteristics of the regulatory molecules remain unanswered. Rio *et al.* (1986) have identified the two 87 and 66 kDa proteins, encoded by *P* element in different splicing patterns. They have reported that both of them retain the region showing homology with the bacterial DNA-binding proteins, but that only the 87 kDa protein has transposase activity. They have suggested that the 66 kDa protein may function as a repressor.

Here we propose a new model for the cytotype-determining mechanism *without any intact* P *elements* in the genome. Satta *et al.* (1985*a*,*b*) reported that ORF1 of the *P* element has sequence homology with the *Tn*3 resolvase-coding region and that the entire region of the *P* element has sequence homology with the *Tn*3 transposase (*TnpA*) coding region. The resolvase is a DNA-binding protein and has dual functions (Grindley, 1983). DNA-binding residues appear to be preserved to some extent in ORF1 of the *Drosophila P* element. It may be supposed that partially deleted *P* elements retaining their ORF1 and some additional regions can encode defective enzymes with DNA-binding ability. Such defective enzymes could compete with the intact transposase protein for *P*-element recognition sites on the chromosomes, lower the transposase activity and lessen the movement of *P* elements, though this is not the only molecular mechanism possible for the regulatory function of defective proteins (also see Rio *et al.* 1986 and the case of *Tn*5, Johnson *et al.* 1982; Isberg *et al.* 1982). Therefore, the strains having a higher number of such defective *P* elements than of intact *P* elements may be able to construct the *P* cytotype. A completely neutral strain could be expected to have the *P* cytotype with a smaller number of such partially defective *P* elements and to repress *P*-element movement more efficiently than a strong *P* strain. Kidwell (1981) has reported that chromosomes from *Q* strains in particular had rapid cytotype-switching ability in F_1 hybrids of *M* females and *Q* males. These predictions are being examined in our laboratory.

In the present paper, we have predicted absence of the intact *P* elements in the genomes of OOQ-27 and *C*(1)*DX*; IG489-6 solely based on the results of genetic experiments. Our preliminary data from Southern blot analysis have not indicated the presence of the intact *P* element in these neutral strains. More genetic and molecular experiments are necessary to gain a deeper insight into the *P-M* system of hybrid dysgenesis.

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Note added. Engels reported (in 'EMBO Workshop of Developmental Biology in *Drosophila*', 1986), that certain defective *P* elements produced a repressor in his microinjection study. His results are consistent with our prediction based on the present genetic study.

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