Genet. Res., Camb. (1994), **63**, *pp.* 27–38 *With 7 text-figures*

Differences in *P* element population dynamics between the sibling species *Drosophila melanogaster* and *Drosophila simulans*

KIYOSHI KIMURA* AND MARGARET G. KIDWELL†
Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, USA

(*Received 7 June 1993 and in revised form 3 November 1993*)

Summary

Patterns of *P* element establishment and evolution were compared in populations of *D. melanogaster* and *D. simulans*. For each species, mixed populations were initiated with M strain flies lacking *P* elements together with *P* strain flies having similar *P* element copy numbers and phenotypes. The mixed populations were subsequently maintained under similar environmental conditions. On the basis of gonadal sterility assays, *P* elements tended to be significantly more active in *D. melanogaster* than in *D. simulans* populations. This activity difference between the two species was positively associated with *P* element copy number, determined by restriction enzyme analysis, and transposition frequency, as determined by a transposition assay. Host factors are the most likely explanation for the observed species variation. Difficulty of establishment may be a factor determining the absence of *P* elements in natural populations of *D. simulans*.

1. Introduction

Although transposable elements (TEs) have been identified in a wide variety of organisms and the structural properties of many of them have been extensively described (see, for example, Berg & Howe, 1989), very little is known about their population dynamics and phylogenetic distributions. Improvement of knowledge in this area is likely to be far from a trivial task. Because different TE families differ in their properties and TE–host genome interactions may be important, it will be necessary to study the dynamics of the distribution of a number of TE families, each in several hosts. Another complicating factor is that the rate of movement of many TEs is often so low that large, and possibly prohibitively expensive studies may be required for an adequate evaluation.

Because of its high transposition rate, the *P* family of transposable elements in *Drosophila* provides an opportunity for surmounting some of these hurdles. This high transposition potential of *P* elements in the absence of element-encoded repressors has been demonstrated in several laboratory experiments under controlled conditions (e.g. Bingham, Kidwell & Rubin, 1982; Preston & Engels, 1989). High transposition rates must also have been an important factor contributing to the rapid world-wide invasion of natural populations of *D. melanogaster* during the last half century (Kidwell, 1983; Anxolabéhère *et al.* 1988).

D. simulans is an excellent candidate species for the study of *P* element dynamics in comparison with its sibling species *D. melanogaster*. The two species are believed to have diverged from one another about 3 MY ago (Lachaise *et al.* 1988). Like other *D. melanogaster* sibling species, *D. simulans* does not naturally harbour *P* elements in the wild (Brookfield *et al.* 1984). Therefore, no *P*-element-encoded repressors are initially present to retard transposition. Furthermore, a limited number of previous studies have suggested that *P* element dynamics differ in the two sibling species (Scavarda & Hartl, 1987; Daniels, Chovnick & Kidwell, 1989; Montchamp-Moreau, 1990).

Here we describe the results of experiments in which *P* elements were monitored simultaneously for a number of generations in populations of *D. melanogaster* and *D. simulans* maintained under similar conditions. Differing patterns of evolution in the two species were found to be associated with differing transposition rates.

* Present address: Laboratory of Apiculture, Department of Animal Genetics, Institute of Animal Industry, Tsukuba Norin Danchi, P.O. Box 5, Ibaraki 305, Japan.
† Corresponding author.

2. Materials and methods

(i) D. melanogaster *strains*

Harwich-*w* (HW) is a strong P strain used as a reference strain for the P–M system; the original strain was collected by Dr M. L. Tracey in Harwich, Massachusetts in 1967; the *white* mutation carried by this strain was isolated by M.G.K. from the original Harwich strain.

Canton S (CS) is a long-established laboratory strain that carries no *P* elements (Bingham, Kidwell & Rubin, 1982) and is used as an M reference strain for the P–M system. The original strain was collected at Canton, Ohio in the 1940s.

T series lines (88-4-1 (84), 88-63-1 (86), 88-72-1 (87), and 88-283-3a (82)) are *P*-element-transformed lines constructed by Daniels *et al.* (1987).

Para Wirra (PW) is a strong P strain collected in Para Wirra, Australia in 1972 (Colgan & Angus, 1978).

AS57, AS125, AS237 and AS273 are single marked *P* element insertion strains obtained from the *Drosophila* Stock Center at Bloomington, Indiana. These are autosomal recessive lethal strains maintained by the use of balancers. Each was originally transformed using a pUChsneo plasmid that carried a *P* element marked with a *neomycin* resistance gene (*neo*^{r}).

(ii) D. simulans *strains*

8DS (8D) is a standard strain of *D. simulans*, which was provided by Dr H. Krider; this strain is used as an M reference strain for the P–M system in *D. simulans*.

Table 1. *Percent GD sterility in the progeny of A and A* crosses involving the parental strains used in Experiments 1 and 2*

Parental strains	Cross A[1]	Cross A*[2]
D. melanogaster		
Harwich-*w*	100·0	0·0
Para Wirra	95·0	0·0
88-4-1	100·0	0·0
88-63-1	90·0	4·5
88-72-2	80·0	18·0
88-283-3a	88·5	14·0
D. simulans		
DsPπ5C8	86·0	14·0
DsPπ5C12	70·0	12·5
DsPπ5C32	20·5	15·0
DsPπ5C35	85·5	8·5
DsPπ5C17	89·0	6·0
DsPπ5C27	95·0	0·0

[1] Harwich-*w* and DsPπ5C27 were used as P reference strains for *D. melanogaster* and *D. simulans* respectively.
[2] Canton S and 8DS were used as M reference strains for *D. melanogaster* and *D. simulans* respectively.

DsPπ5 series [DsPπ5C8 (08), DsPπ5C12 (12), DsPπ5C17 (17), DsPπ5C27 (27), DsPπ5C32 (32), and DsPπ5C35 (35)] are *P*-element-transformed lines of *D. simulans* constructed using the 8DS strain as a transformation recipient by Daniels, Strausbaugh & Armstrong (1985); the DsPπ5C27 line of this series is used as a P reference strain for the P–M system in *D. simulans*.

DS9, DS12, DS18, and DS26 are single marked *P* element insertion strains that were synthesized for this study. The plasmid pUChsneo (Steller & Pirotta, 1986) was coinjected into flies with pUChs$\pi\Delta$2-3 (Laski, Rio & Rubin, 1986) as helper. The white riverside ($w^{riverside}$) mutant strain (a gift from Dr J. Coyne) was used as the transformation recipient strain. F_1 larvae were fed on a G418 supplemented medium. All F_1 survivors were backcrossed to the parental strain and were again grown on the G418 supplemented medium. Putative transformed lines were backcrossed to the parental strains for two more generations. By means of these procedures, all neomycin-sensitive flies were killed and all flies that survived should have been heterozygous for pUChsneo and consequently neomycin resistant. Each of the putative transformed lines was checked by Southern blotting for the presence of insertions and all were found to carry just a single insert. The results of GD sterility tests are shown in Table 1 and indicate the status of some of these strains in the P–M system of hybrid dysgenesis.

(iii) *Gonadal sterility tests*

The procedure used for the GD sterility tests is as described by Kidwell (1986). DsPπ5C27 and Harwich-*w* were used as P reference strains and 8DS and Canton S red were used as M reference strains, for *D. simulans* and *D. melanogaster*, respectively.

(iv) *Southern blotting procedures*

For the population experiments, genomic DNA was extracted from 100 adult flies (50 females and 50 males), according to the protocol described by Daniels & Strausbaugh (1986). The Southern blotting procedure followed the protocol suggested by Schleicher & Schuel (S & S, Keene, NH). The plasmid pπ25.7BWC (Daniels *et al.* 1988) was used as a *P* element probe. Plasmids were labelled by random-primed incorporation of digoxigenin-labelled deoxyuridine-triphosphate using the Genius kit (Bohringer and Mannheim, Indianapolis, IN) and the protocol supplied by the manufacturer. The hybrids were detected by a colouring reaction using X-phosphate and NBT. The reaction was stopped when the background started to show up (normally at about two days).

For the transposition assay, the same procedure for Southern blotting was followed except that in order to detect movement of single *P* elements, 10 μg of DNA

https://doi.org/10.1017/S0016672300032055 Published online by Cambridge University Press

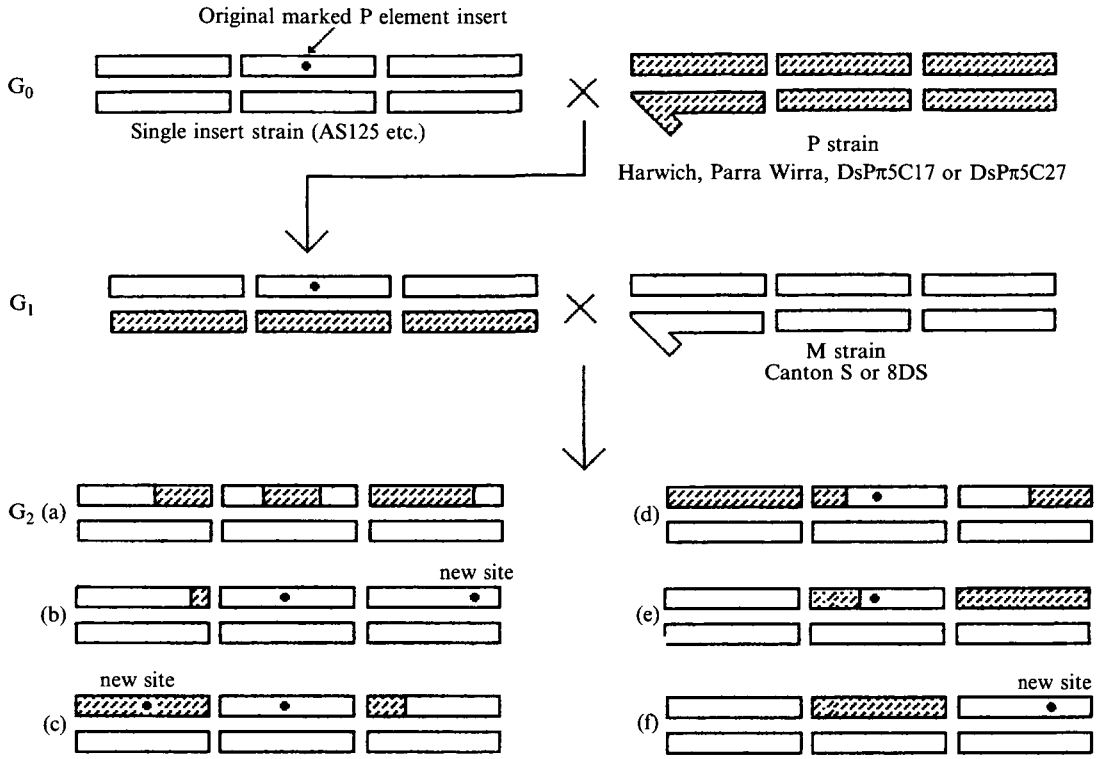

Fig. 1. Mating scheme for transposition assay for *D. melanogaster* and *D. simulans*. Various kinds of progenies are shown in the G_2 generation. Only female individuals are shown in the example. Individuals (b), (c) and (f) carry marked *P* elements that have transposed to new genomic sites. Individuals (a) and (f) do not carry the original inserts because of segregation or recombination.

was loaded in each lane, instead of 1–2 μg. Furthermore, in order to decrease the stringency, the second wash was performed with a high salt concentration (1 M-NaCl), at 42 °C, instead of at 65 °C. An *Xho* I/*Bam* HI fragment cut from the plasmid 'pMC1neo poly A', purchased from Stratagene (La Jolla, CA), was used as the probe. The neomycin resistance gene in this plasmid is identical to the one in the pUChsneo plasmid.

(v) *Construction of* D. simulans *strains with single marker inserts*

D. simulans strains with a marked *P* element carrying a neomycin resistance gene were constructed using a standard transformation procedure (Spradling & Rubin, 1982). The plasmid, pUChsneo, was kindly supplied by Dr Pirrotta (Steller & Pirrotta, 1986). pUChs$\pi\Delta$2-3 (Laski, Rio & Rubin, 1986) was used as a helper plasmid for transformation. Four transformed strains were constructed and tested in the transposition assay. Each carried a single homozygous, autosomal insert.

(vi) *Mating scheme for the transposition assay*

Ten females from a single insertion strain were crossed with 10 males from a P strain as shown in the mating scheme in Fig. 1. Each of these paternal strains provided a source of transposase. The combination of one maternal strain and one paternal strain constituted one set, each of which was duplicated. Flies were kept on regular medium, supplemented with G418, so that only those larvae that carried the *neo*^{r} gene survived. For the controls, ten G_1 female flies were crossed with 10 male flies from an M strain (Canton S for *D. melanogaster* or 8DS for *D. simulans*) and maintained on food without a G418 supplement. In the G_2 generation, 200 females and 200 males were selected, at random, from each set of matings. Each was then divided into four groups. Four extractions per cross of genomic DNA were made. Each extraction was from 100 G_2 flies. Southern blots were performed using the procedure described above. The transposition of a marked *P* element was indicated by a single faint band on a blot. These bands were counted in order to estimate transposition frequencies.

(vii) *Classification of strains in the P–M system of hybrid dysgenesis*

Drosophila populations and strains are characterized on the basis of two properties related to the phenotypic effects of their *P* elements. Different strains may vary in their potential for mobilizing *P* elements when they are in an unregulated or susceptible state. This property, here referred to as P activity, is measured by the cross A tests described below. Strains may also vary in their ability to regulate or suppress the activity of the *P* elements present in their genomes as measured in the Cross A* tests described below. The capacity

https://doi.org/10.1017/S0016672300032055 Published online by Cambridge University Press

for strong regulation is often referred to as P cytotype and that for weak or no regulation as M cytotype (Engels, 1989).

Based on these two properties, strains of *D. melanogaster* are divided into two broad types, P strains and M strains, according to their phenotypic characteristics in the A and A* test crosses. P strains have the potential for P activity, but the level of this activity may vary from high to low. P strains also have P cytotype. A subset of P strains that produce less than 10% gonadal sterility on the basis of standard phenotypic tests are called Q strains. M strains have no significant level of P activity. There are two main subtypes of M strains, true M and M′ (pseudo M). True M strains carry no *P* elements and have M cytotype. M′ strains typically carry multiple *P* elements, most of which are incomplete. The ability of M′ strains to regulate the activity of *P* elements varies from high to moderately low.

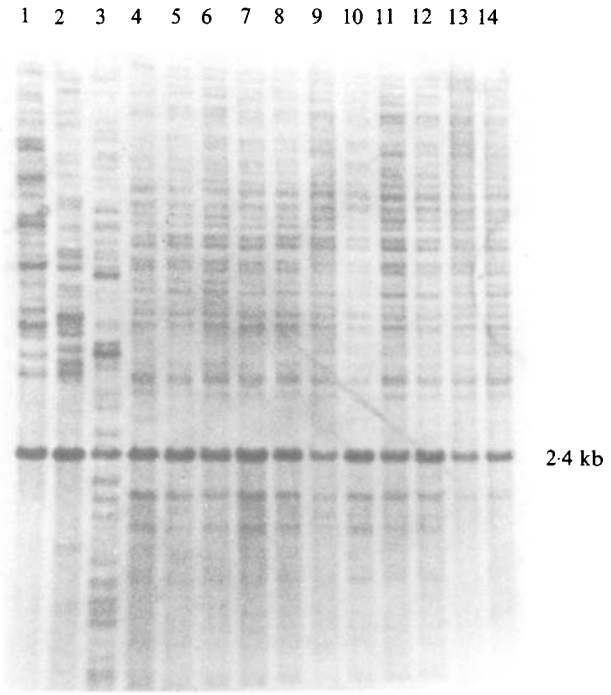

Fig. 3. Southern blot banding patterns over ten generations in mixed populations of Canton S and Harwich-*w* (line CSHWA) compared with *D. simulans* and *D. melanogaster* P strain controls. About 2 μg of genomic DNA was digested by *Acc* I and probed with pπ25.7BWC. Lanes are as follows: 1: DsPπ5C27; 2: DsPπ5C17; 3: Para Wirra; 4: Harwich-*w*; 5–14: CSHWA generations 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, respectively.

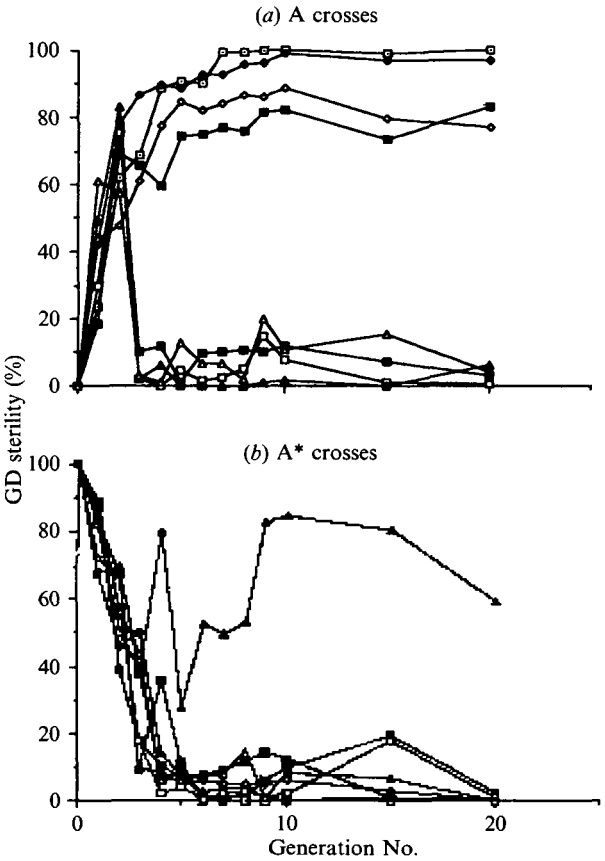

Fig. 2. The results of GD sterility tests in Experiment 1 for generations 1 through 20. Each graph plots the mean values for two replicates. (*a*) *D. melanogaster* and *D. simulans* A crosses provide a measure of P activity potential. (*b*) *D. melanogaster* and *D. simulans* A* crosses provide a measure of *P* element regulation. (⊡), *D. melanogaster* Harwich A; (◆), *D. melanogaster* Harwich B; (■), *D. melanogaster* Para Wirra A; (◆), *D. melanogaster* Para Wirra B; (■), *D. simulans* 5C17 A; (□), *D. simulans* 5C17 B; (▲), *D. simulans* 5C27 A; (△), *D. simulans* 5C27 B.

3. Results

(i) *Mixed population experiments*

This set of experiments was designed to compare the activity and distribution of *P* elements in *D. melanogaster* and *D. simulans* populations initiated by mixtures of P and M strains with similar properties. Four sets of experiments were performed. Mixed populations were started with 16 P-strain flies (8 males and 8 females) and 64 M-strain flies (32 males and 32 females) giving a 1:4 ratio of P to M. Each original mixed P–M strain combination was duplicated. In every generation, 40 males and 40 virgin females per mixed population were selected at random to produce the next generation. All populations were maintained in half pint milk bottles.

Mixed population Experiment 1

In the first set of experiments, the parental *D. melanogaster* P strains Harwich-*w* and Para Wirra and the *D. simulans* strains DsPπ5C17 and DsPπ5C27 were chosen for their similar *P* element copy number and strong potential for P activity. Judging from a restriction enzyme analysis, using an *Acc* I digest, all these four strains carried at least 30 *P* element copies (data not shown). The parental M strains used were Canton S in *D. melanogaster* and 8DS in *D. simulans*.

https://doi.org/10.1017/S0016672300032055 Published online by Cambridge University Press

Table 2. *Percent GD sterility in progeny of Crosses A and A* in Experiments 1 and 2 at generations 5 and 10*

Paternal strain	Expt. no.	Generation 5		Generation 10	
		A	A*	A	A*
D. melanogaster[1]					
Harwich-*w* A	1[2]	91·0	8·0	100·0	0·0
Harwich-*w* B		88·5	7·5	99·0	0·0
Para Wirra A		74·5	7·0	82·0	12·5
Para Wirra B		84·5	5·0	88·5	6·0
88-4-1 A	2	54·8	19·8	62·0	15·0
88-4-1 B		52·0	2·5	60·0	8·0
88-63-1 A		47·8	25·2	45·0	60·0
88-63-1 B		36·7	47·1	46·0	72·0
88-72-1 A		11·2	83·9	7·0	88·5
88-72-1 B		26·7	82·3	6·5	90·5
88-283-3a A		51·2	49·2	45·0	35·0
88-283-3a B		45·2	32·7	39·0	36·0
D. simulans[3]					
DsPπ5C17 A	1[2]	1·0	11·5	12·0	9·5
DsPπ5C17 B		4·0	4·0	17·0	2·0
DsPπ5C27 A		0·0	27·5	1·5	83·0
DsPπ5C27 B		13·0	6·0	11·0	8·0
DsPπ5C8 A	2	18·0	60·5	5·0	50·5
DsPπ5C8 B		0·0	40·5	0·0	40·5
DsPπ5C12 A		14·5	71·5	10·0	80·5
DsPπ5C12 B		1·0	1·0	5·0	5·0
DsPπ5C32 A		0·0	94·0	0·0	98·5
DsPπ5C32 B		0·0	94·0	0·0	97·5
DsPπ5C35 A		0·0	50·0	0·0	32·0
DsPπ5C35 B		5·0	49·0	5·0	18·0

Each percentage is an average of duplicate trials.
[1] Canton S was used as the M parental strain for all *D. melanogaster* mixed populations.
[2] The results for generations 15 and 20 of Experiment 1 are not included.
[3] 8DS was used as the M parental strain for all *D. simulans* mixed populations.

The absence of *P* elements in both M strains was confirmed by Southern hybridization.

Each mixed population was maintained by 40 randomly sampled flies of each sex for each of 20 generations. At generations 1 through 10, 15 and 20, samples of 50 males and 50 females per population were sampled at random for GD sterility tests and 40 males and 40 virgin females were similarly taken for DNA extraction and restriction enzyme analysis (see Materials and methods).

The values plotted in the graphs in Fig. 2 are the mean frequencies of GD sterility in the progeny of test crosses for each of the four pairs of duplicate populations. In the *D. melanogaster* A crosses, GD sterility frequencies increased rapidly from low to very high levels in less than ten generations, indicating a rapid increase in P activity potential. In the A* crosses, on the other hand, GD sterility decreased from initially high frequencies to very low levels in later generations, indicating a change from low to high levels of regulation of P activity.

In *D. simulans*, GD sterility frequencies in the A cross again increased sharply in the first two generations. However, in contrast to the *D. melanogaster* populations, these frequencies all dropped precipitously after generation 3 and remained low in later generations. With the exception of one strain, GD sterility in the *D. simulans* A* crosses showed the same decreasing trend as those in *D. melanogaster*. These results suggest that strong P regulation tends to evolve quickly in both species.

In summary, the results indicate that all the *D. melanogaster* P–M mixed populations evolved to P strains during the 20-generation period of observation. In contrast, the *D. simulans* mixed populations evolved to Q or M′ strains during the same period. In both species, a trend usually appears to be established for each individual population by about the fifth generation, with no major changes occurring subsequently.

Fig. 3 shows a sample of a typical set of Southern blots, cut with *Acc* I, for a single *D. melanogaster* mixed population, over the first 10 generations. It can be seen that the hybridization patterns of the Southern bands of this CSHWA population are quite prominent and did not change significantly over time. However, the intensity of hybridization of each band did

https://doi.org/10.1017/S0016672300032055 Published online by Cambridge University Press

sometimes vary from generation to generation. Similar observations (data not shown) were made for the other mixed populations. In general, the Southern banding patterns did not show any consistent trends through time. This suggests that the total numbers of *P* element sites in each population tended not to change, but that the number of *P* elements in each site may have varied from one generation to another. It is interesting to note that GD sterility frequencies showed significant changes over time, despite the lack of any consistent change in Southern banding patterns.

Mixed population Experiment 2

Although naturally occurring P strains were available for *D. melanogaster*, in Experiment 1 we necessarily had to use P-transformed strains from *D. simulans* for comparison. Consequently, any differences between natural and transformed strains were confounded with possible species differences. In order to check this possibility, transformed P strains of both species were used in Experiment 2. The P-transformed strains (T series) available for *D. melanogaster* were only moderately strong with respect to P activity (see Table 1). Therefore, we also used P-transformed strains of *D. simulans* with similar moderate levels of P activity. The mixed populations were sampled for GD sterility tests and Southern analysis at generations 5 and 10 only.

Table 2 presents the GD sterility frequencies observed in generations 5 and 10 of the P–M mixed populations in Experiment 2, in comparison with those for the same generations in Experiment 1. There was considerably more variability in sterility among strains within species for both the A and A* crosses in Experiment 2 than in Experiment 1. The majority of the *D. melanogaster* mixed populations showed moderate to high GD sterility frequencies in the A crosses. No consistent pattern appeared in the A* crosses. In *D. simulans*, only population 8D12B evolved to a typical Q type but all other populations became M′ types or were intermediate between M′ and Q types.

The changes in most of the mixed populations between generations 5 and 10 are not large, but there are a few exceptions (CS86A and B, and 8D35A and B). The absence of major changes after the first few generations is consistent with the results of Experiment 1, in which the major changes occurred before generation 5. Although four lines in Experiment 2 did show unusual patterns, it is possible that these populations would have evolved further after generation 10, if the experiment had continued longer.

In summary, even though in Experiment 2 the evolutionary trends are not as clear as in Experiment 1, the overall patterns of evolution in the *D. simulans* mixed populations again showed distinct differences from those of *D. melanogaster*. As in Experiment 1, only a low level of P activity was established in *D. simulans* lines. Although only moderate levels of

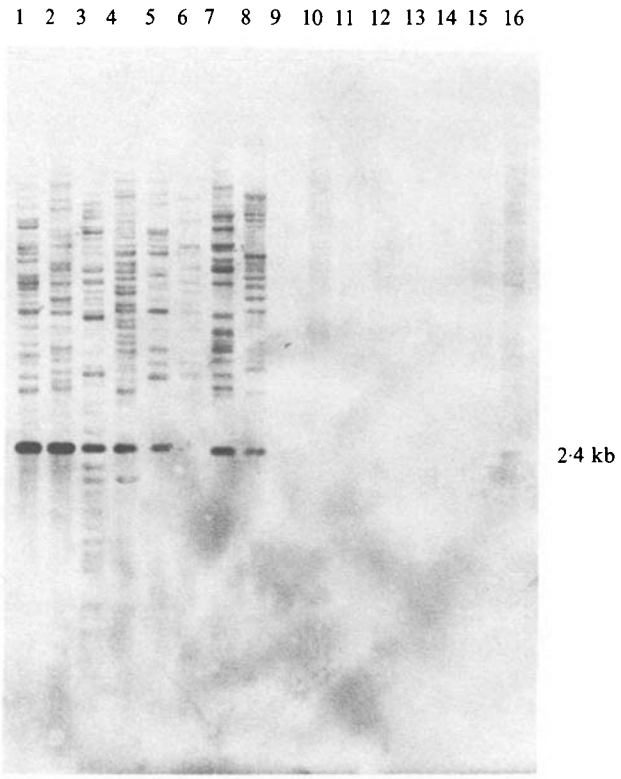

Fig. 4. Hybridization pattern of Southern blots of mixed population strains of Experiment 2 at generation 5. About 1 μg of genomic DNA was digested by *Acc* I and probed with pπ25.7BWC. Lanes are as follows: 1: DsPπ5C27 (control); 2: DsPπ5C17 (control); 3: Para Wirra (control); 4: Harwich-*w* (control); 5: DsPπ5C35 (control); 6: DsPπ5C32 (control); 7: DsPπ5C12 (control); 8: DsPπ5C8 (control); 9: 8D35B; 10: 8D35A; 11: 8D32B; 12: 8D32A; 13: 8D12B; 14: 8D12A; 15: 8D08B; 16: 8D08A.

P activity were observed in the *D. melanogaster* mixed populations derived from P-transformed lines, as in Experiment 1 after 10 generations these were consistently markedly higher than those of the corresponding *D. simulans* lines. Therefore, the differential use of transformed versus untransformed P lines does not appear to account for the species differences observed in Experiment 1.

Unlike Experiment 1, in Experiment 2 the amount of total *P* element DNA evident in the Southern blots was quite small in some of the mixed population strains of *D. simulans* (Fig. 4). In some of the experimental lanes (9–16), hybridization is barely visible, but there are obvious bands in other lanes. The faintness of the hybridizing bands often made it impossible to discern changes of pattern. Consistent with these observations, no hybridization at all could be detected, under the stringency conditions applied to the filters, for the 8D12B line in generation 10 (not shown). It is interesting to note that all *D. simulans* lines evolved into M′ types except for this one which had the functional characteristics of the Q type. In contrast, just as in Experiment 1, changes in the Southern banding patterns within each *D. melanogaster* line showed no consistent temporal trends.

https://doi.org/10.1017/S0016672300032055 Published online by Cambridge University Press

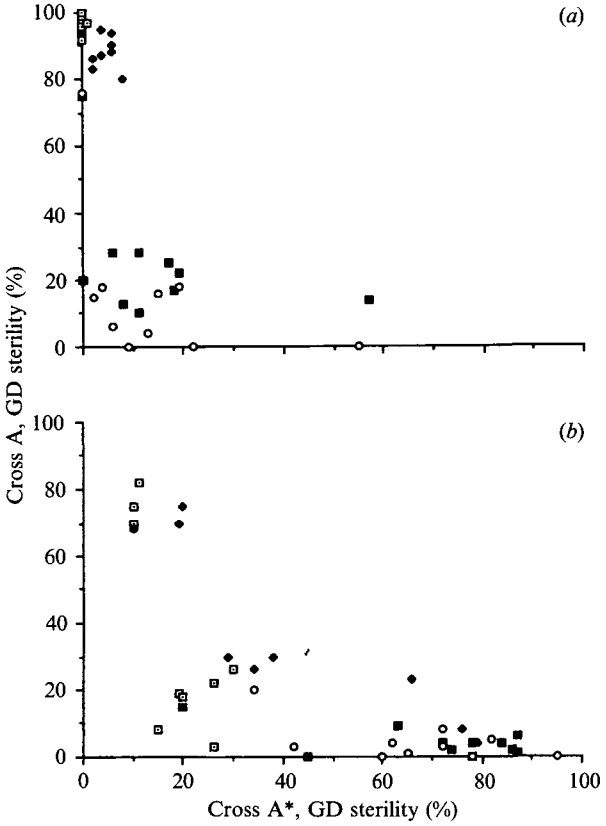

Fig. 5. The results of Experiment 3 GD sterility tests at generation 5. (*a*) Mixed population lines derived from strong P strains: (⊡), CS × HW *D. melanogaster*; (◆), CS × PW *D. melanogaster*; (■), 8DS × 5C27 *D. simulans*; (○), 8DS × 5C17 *D. simulans*. (*b*) Mixed population lines derived from relatively weak P strains: (⊡), CS + 88-4 *D. melanogaster*; (◆), CS + 88-72 *D. melanogaster*; (■), 8DS + 5C35 *D. simulans*; (○), 8DS + 5C8 *D. simulans*.

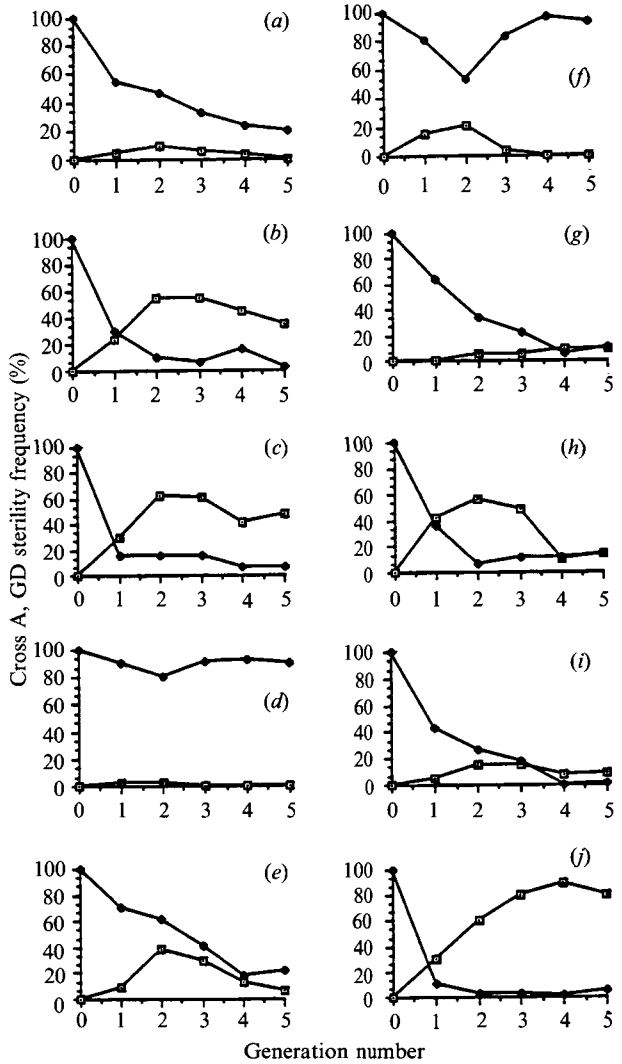

Fig. 6. The results of Cross A and Cross A* GD sterility tests of Experiment 4 for generations 1–5. The results from each mixed population line are shown in separate graphs (*a*)–(*j*).

Mixed population Experiment 3

In Experiments 1 and 2, each combination of parental strains was replicated only twice (A and B). It is possible that these duplicates did not adequately represent the trends of evolution of the P–M mixed population strains because the size of the experiments was too limited. We checked whether experimental size influenced the observed trends by employing the same parental combinations as in Experiments A and B. However, in this experiment, each combination was replicated 10 times. Four *P* parental strains of each species were used. As in Experiment 1, Canton S and 8DS were used as the M parental strains, for *D. melanogaster* and *D. simulans*, respectively. At generation 5, these 80 mixed populations were subjected to a GD sterility test.

Fig. 5 shows that the general trend of the results is similar to those observed in Experiments 1 and 2. All mixed populations initiated with strong P strains of *D. melanogaster* quickly evolved to the P type. Although almost all *D. simulans* lines evolved to the Q type, two exceptional lines evolved to the P type and two lines evolved to the M′ type. The outcome was not as clear in lines initiated with relatively weak P strains (Fig. 5*b*), as it was in lines initiated with strong P strains (Fig. 5*a*), but the direction of the trends observed were similar to those seen in Experiment 2. From the results of this experiment, it is concluded that the results of Experiments 1 and 2 are indicative of repeatable differences between the two species rather than being the outcome of random processes.

Mixed population Experiment 4

In Experiment 1, the evolutionary trends observed in the first five generations of the *D. simulans* mixed population strains were very pronounced and unexpected on the basis of previous results with mixed *D. melanogaster* populations. In order to test the repeatability of this result the experiment was conducted a second time using the *D. simulans* DsPπ5C27 and 8DS parental strains, each in ten separate replicates. GD sterility tests were performed during each of the first five generations.

https://doi.org/10.1017/S0016672300032055 Published online by Cambridge University Press

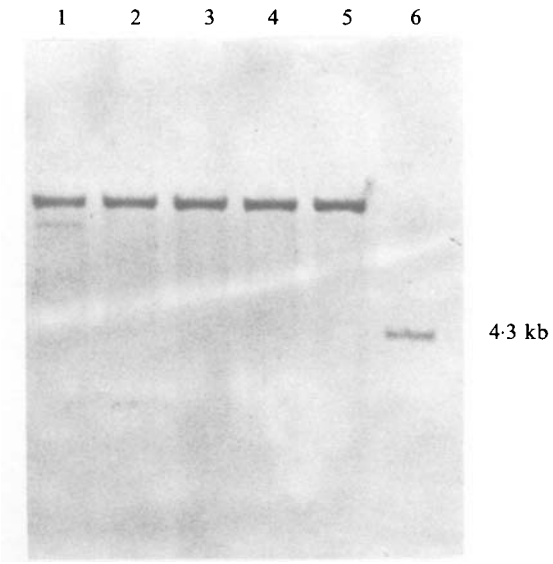

Fig. 7. A sample of Southern blots from the transposition assay. 10 μg of genomic DNA, extracted from a sample of 100 flies, was loaded into each lane and probed with the *Xho* I/*Bam* HI fragment cut from the pMC1neo poly A plasmid. Lanes are as follows: 1–4: DNA from G_2 flies from DS26 × DsPπ5C27 crosses, cut with *Bam* HI/*Eco* RI (duplicates); 5: DS26 DNA cut with *Bam* HI/*Eco* RI; 6: DS26 DNA cut with *Hin* dIII.

From Fig. 6 it can be seen that the majority of *D. simulans* mixed populations appeared to be evolving towards the Q type after 5 generations. One line (Fig. 6*j*) exhibited the characteristics of the strong P type. Two lines (Figs 6*d* and 6*f*) evolved M′ characteristics. At least half of the lines showed the tendency observed in Experiment 3 for an early increase in P activity, followed by a subsequent decline to low levels. Although considerable variation in the patterns of change were observed among the replicates, the overall trends were in agreement with those of Experiment 3, but the pattern observed in Experiment 1 appears to underestimate the extent of individual variability.

(ii) *Transposition assay*

As described in Materials and methods, four single *P* insert lines were synthesized for each of the two species. As shown in Fig. 1, a number of genotypes were generated in the G_2 individuals. About half of these were expected to carry out the original single insert. The other half were not expected to do so because of segregation, recombination, or excision.

Figure 7 shows a sample of the Southern blots from this transposition assay. Each lane contains 10 μg of DNA from 100 adult flies produced by the cross DS26 × DsPπ5C27 and digested with *Bam* HI/*Eco* RI. The dense bands, in lanes 1 through 5, represent the original insert. A faint band in lane 1, just below the dense band, indicates a transposition event. Lane 6 shows a band representing a 4·3 kb fragment from

Table 3. *Transposition frequencies of marked* P *elements*

Single insert strains	Paternal source of transposase	Number of new sites in duplicate trials[1]		Transposition in duplicate trials[2]	
D. melanogaster					
AS59	Harwich	3	6	0·75	1·50
	Para Wirra	5	6	1·25	1·50
AS125	Harwich	12	13	3·00	3·25
	Para Wirra	12	14	3·00	3·50
AS247	Harwich	4	4	1·00	1·00
	Para Wirra	4	6	1·00	1·50
AS273	Harwich	3	3	0·75	0·75
	Para Wirra	3	4	0·75	1·00
Total	Harwich	48	—	1·53	—
	Para Wirra	54	—	1·69	—
Grand total		102	—	1·61	—
D. simulans					
DS9	DsPπ5C17	3	4	0·75	1·00
	DsPπ5C27	4	3	1·00	0·75
DS12	DsPπ5C17	1	0	0·25	0·00
	Dspπ5C27	1	0	0·25	0·00
DS18	Dspπ5C17	2	1	0·50	0·25
	Dspπ5C27	2	1	0·50	0·25
DS26	Dspπ5C17	1	2	0·25	0·50
	Dspπ5C27	2	5	1·25	0·50
Total	Dspπ5C17	14	—	0·44	—
	Dspπ5C27	18	—	0·56	—
Grand total		32	—	0·50	—

[1] Number of faint bands on Southern blots per 400 G_2 flies.
[2] Number of new sites divided by 400, multiplied by 100.

https://doi.org/10.1017/S0016672300032055 Published online by Cambridge University Press

genomic DNA digested with *Hin* dIII. A fragment of this size is expected because *Hin* dIII cuts near both ends of the P-neo region of pUChsneo. Other blots were similar to this sample. The results of the complete assay are summarized in Table 3. Differences between duplicate samples tested from the same line were not statistically significant. Mean transposition frequencies were more than three times higher in *D. melanogaster* than in *D. simulans*. With only one exception, all observed *D. melanogaster* single line transposition frequencies were higher than those of *D. simulans*. Within-species variability was also high. An Analysis of Variance (not shown) indicated that the components of variance due to species and maternal strain inserts are both highly significant ($P < 0{\cdot}0001$). However, the variance due to paternal strains was not statistically significant.

4. Discussion

The results of all four mixed populations experiments comparing *D. simulans* and *D. melanogaster* showed strikingly different evolutionary tendencies with respect to the *P* element family. Although there were exceptions, mixed populations of *D. melanogaster* tended to evolve towards strong or moderately strong P strains, while those of *D. simulans* evolved towards weak P, or M′, strains. The *D. melanogaster* and *D. simulans* parental lines were carefully selected for use in these experiments, not only on the basis of similarity in phenotypic tests, but also on their similarity with respect to *P* element genomic copy number. Therefore, initial copy number differences in the two species cannot account for the different evolutionary patterns. Furthermore, an hypothesis based solely on total numbers of elements appears to be naive, since we know that different sizes of element appear to have different roles (reviewed by Engels, 1989).

The high degree of repeatability of the results of Experiment 1 suggest that the species differences observed are not just the result of random effects due to a limited number of trials. The agreement of the *D. melanogaster* results with those of previous mixed population studies (Kidwell, Novy & Feeley, 1981; Kiyasu & Kidwell, 1984; Good *et al.* 1989) add further support to this conclusion. The transformation experiments of Scavarda & Hartl (1987), Daniels, Chovnick & Kidwell (1989) and Montchamp-Moreau (1990) are also consistent with this hypothesis. They all demonstrated that *P* elements can be introduced into *D. simulans* by micro-injection, and that the number of *P* elements usually increases over a number of generations. If, in addition, selection is performed to increase copy number, the number of *P* elements in *D. simulans* can reach a level as high as that of strong P strains of *D. melanogaster*. However, as suggested in the present experiments, in the absence of selection, *P* elements do not normally increase as quickly in *D. simulans* as they do in *D. melanogaster*.

The *D. melanogaster* transposition frequency of 1·59% per site per generation which was obtained in this study is within the range of 0·29–12·63% per site per generation observed by Berg & Spradling (1991) using the jump starter element, *P[js]*. An earlier study in *D. melanogaster*, using hybrid dysgenesis, gave higher estimates of transposition rates around 25% per site per generation (Bingham, Kidwell & Rubin, 1982). The Southern blots from the present mixed population experiments indicated that hybridization patterns changed rather slowly from one generation to the next, suggesting that transposition frequencies were not extremely high in either species.

The results of the present transposition assay indicated that the transposition frequencies in the two species are statistically significantly different from one another. These give strong support to the idea that a higher transposition frequency in *D. melanogaster* than in *D. simulans* may be an important factor in determining the observed differences in *P* element dynamics. However, it cannot be ruled out that factors that are indirectly related to transposition rate differences might also be responsible. For example, there might be an increased production of deleted forms in *D. simulans* than in *D. melanogaster*. Alternatively, there might be greater reduction in fitness associated with transposition and hybrid dysgenesis in *D. simulans* than in *D. melanogaster*.

This and other considerations strongly suggest that the observed species differences in *P* element evolution have a genetic basis and, further, that host genes that influence transposition frequency are likely to be involved. Support for a role of host factors in *P* element regulation comes from a number of sources. A non-*P* element-encoded inverted repeat binding protein (IRBP) has been identified and purified from tissue culture (Rio & Rubin, 1988); it binds to the outer 16 bp of both the *P* element terminal inverted repeats. Preliminary data from gel shift assays corroborate this result; specific binding of a host factor in nuclear protein extracts from embryos of a *P*-element-free strain to the 5′ and 3′ ends of the *P* element has been demonstrated (K. R. Peterson, unpublished results). The results of a *P* element excision assay of a number of Drosophilid and non-drosophilid species (O'Brochta & Handler, 1988) are also consistent with the idea that non-conserved, host-encoded functions, other than *P* element transposase, are necessary for *P* element mobility. Additional support for the existence of host factors comes from the demonstration of variability among *D. melanogaster* M lines in their ability to repress *P* factor activity (Daniels *et al.* 1987).

As mentioned earlier, in contrast to *D. melanogaster*, no *P* elements have been observed in the genomes of natural populations of *D. simulans* (Brookfield *et al.* 1984). The *P* elements of *D. melanogaster* were likely derived from *D. willistoni* by horizontal transfer in the last four decades (Daniels *et al.* 1990; Kidwell, 1992).

https://doi.org/10.1017/S0016672300032055 Published online by Cambridge University Press

The geographical distributions of both *D. melanogaster* and *D. simulans* presently overlap that of *D. willistoni*, and in the region where the three occur together in Central and Southern America, *D. simulans* is often found to be the dominant species, relative to *D. melanogaster* (Dobzhansky & Pavan, 1950; Belo & deOliveira Filho, 1976; David & Tsacas, 1980). Therefore, at least recently, the two species seem to have had similar geographical opportunities for *P* element horizontal transfer.

The most parsimonious explanation for the absence of *P* elements in *D. simulans* is that horizontal transfer is likely to be a very rare event, and so, by chance, these elements may simply not have been introduced into *D. simulans*. There is a possibility (Johnston, 1913; see also review by Engels, 1992) that *D. melanogaster* was introduced to the Americas from Africa as recently as about 200 years ago. If the same is true for *D. simulans*, the period of overlap of geographical range of *D. simulans* and *D. willistoni* during which horizontal transfer could have been possible is similarly very recent.

On the other hand, it may be that horizontal transfer of mobile elements occurs more frequently than non-mobile genes, and more frequently than had previously been speculated. Not only is it plausible that transposons by their very nature are disposed to these sorts of events, but, also, evidence for horizontal transfer of a number of different mobile elements has recently started to accumulate (for review see Kidwell, 1993). In addition, as described earlier, it is well-documented that *D. simulans* can be transformed relatively easily with *P* elements by the microinjection technique. Therefore, the explanation for the species differences may lie, not in a difference in probability of transfer, but in a difference between the two species in facility of establishment following transfer. We argue that the results of the present study provide evidence for this hypothesis.

The result of our mixed population studies and the *P* element transformation experiments of Montchamp-Moreau (1990) show that *P* element copy number can increase in *D. simulans* populations, but, unlike *D. melanogaster*, it is difficult to develop strong P strains in this species, even in small bottle populations. In order for a successful invasion of *P* element to occur it may be essential first to develop a strong P population. Our bottle population studies showed that *D. simulans* mixed populations rarely evolve into P populations, but are more likely to evolve into Q or M′ populations, even though strong P strains were used as parental strains for the mixed populations. These populations will tend to lose *P* elements by recombination and drift and also by mixture with other M populations, because *P* elements do not transpose very well in the genome of *D. simulans*.

Selection might increase the frequency of *P* elements in a population by hitchhiking. This apparently occurred in the study of Daniels, Chovnick & Kidwell (1989). However, no evidence has yet been presented, in either species, to suggest that *P*-bearing flies have any adaptive advantage relative to M flies. We conclude that the difficulty of establishment of *P* elements in the *D. simulans* genome may be the primary reason for their absence in natural populations of this species.

It is possible that different species may have evolved different adaptations to minimize transposable element activity. An interesting possible example of the evolution of such a barrier is provided by species in the obscura group of *Drosophila*. Miller *et al.* (1992) and Paricio *et al.* (1991) have independently reported the existence of an array of tandemly repeated *P*-homologous sequences in several closely related species of the obscura group of *Drosophila*, *D. subobscura*, *D. guanche*, and *D. madeiriensis*. These *P*-related sequences have apparently lost their mobility. However, in *D. subobscura*, a polyadenylated mRNA has been identified similar to that of a *D. melanogaster* *P* element repressor mRNA (R deFrutos, personal communication). The explanation for the presence of these *P*-homologous sequences is currently not clear, but it is speculated that a *P* repressor sequence may have been amplified as a molecular defence strategy against *P* element transposition (Paricio *et al.* 1991). According to this scenario, the tandemly repeated unit may represent an adaptation of an ancestral *Drosophila* species to provide some type of 'immunity' against *P* element invasion. This could be a fascinating example of the appropriation by the *Drosophila* host of a mechanism provided by the TE invader (a modified repressor sequence) as a defence weapon against reinvasion by active elements of the same TE family. Alternatively, it may be argued that once all the active *P* elements were lost, the selection pressure associated with the maintenance of repression would have been too weak to maintain the open reading frames of the tandemly repeated elements, unless these elements had subsequently adopted a new, unknown function.

The proportion of middle repetitive DNA in *D. simulans* has been estimated to be only about one third that of *D. melanogaster* (Dowsett & Young, 1982). A large proportion of middle repetitive DNA is thought to be made up of transposable genetic elements or their remains (Young & Schwartz, 1981). It might be argued that the differences in the amount of middle repetitive DNA between these sibling species, and the observations reported in the present study, might reflect an evolutionary feature of *D. simulans*, not possessed by *D. melanogaster*, that has the effect of restricting the acquisition of new TE sequences by this species. However, contrary to this idea, it can be argued that most transposable elements are retrotransposons that are very different in structure to *P* elements and likely to be subject to different regulatory mechanisms. It is, therefore, difficult to

imagine that there are many host functions that restrict the acquisition of both major classes of elements in a similar way in *D. simulans*. The elucidation of the fascinating questions raised by these observations presents an interesting challenge for future investigations of the evolution of transposable elements at the genomic level.

The authors gratefully acknowledge the help of Jonathan Clark and Rosa deFrutos in discussions and for suggestions on the manuscript. This work was partially supported by grants from the National Institutes of Health and the National Science Foundation.

References

Anxolabéhère, D., Kidwell, M. G. & Périquet, G. (1988). Molecular characteristics of diverse populations are consistent with the hypothesis of a recent invasion of *Drosophila melanogaster* by mobile *P* elements. *Molecular Biology and Evolution* **5**, 252–269.

Belo, M. & deOliveira Filho, J. J. (1976). Especies domesticas de *Drosophila*: V. Influecies de factores ambientais no mumero de individuos capturados. *Revistas Brasileira de Biologia* **36**, 903–909.

Berg, D. E. & Howe, M. (eds) (1989). *Mobile DNA*. Washington, D.C.: American Society of Microbiology Publications.

Berg, C. A. & Spradling, A. C. (1991). Studies on the rate and site-specificity of *P* element transposition. *Genetics* **127**, 515–524.

Bingham, P. M., Kidwell, M. G. & Rubin, G. M. (1982). The molecular basis of P–M hybrid dysgenesis: the role of the *P* element, a P strain-specific transposon family. *Cell* **28**, 995–1004.

Brookfield, J. F. Y., Montgomery, E. & Langley, C. (1984). Apparent absence of transposable elements related to the *P* elements of *D. melanogaster* in other species of *Drosophila*. *Nature* **310**, 330–332.

Colgan, D. J. & Angus, D. S. (1978). Bisexual hybrid sterility in *Drosophila melanogaster*. *Genetics* **89**, 5–14.

Daniels, S. B., Clark, S. H., Kidwell, M. G. & Chovnick, A. (1987). Genetic transformation of *Drosophila melanogaster* with an autonomous *P* element: phenotypic and molecular analysis of long-established transformed lines. *Genetics* **115**, 711–723.

Daniels, S. B., Boussy, I. A., Tukey, A., Carillo, M. & Kidwell, M. G. (1987). Variability among 'true M' lines in P–M gonadal dysgenesis potential. *Drosophila Information Service* **66**, 37–39.

Daniels, S. B., Peterson, K. R., Strausbaugh, L. D., Kidwell, M. G. & Chovnick, A. (1990). Evidence for horizontal transmission of the *P* transposable element between *Drosophila* species. *Genetics* **124**, 339–355.

Daniels, S. B. & Strausbaugh, L. D. (1986). The distribution of *P* element sequences in *Drosophila*: the *willistoni* and *saltans* species groups. *Journal of Molecular Evolution* **23**, 138–148.

Daniels, S. B., Strausbaugh, L. D. & Armstrong, R. A. (1985). Molecular analysis of *P* element behavior in *Drosophila simulans* transformants. *Molecular and General Genetics* **200**, 258–265.

David, J. R. & Tsacas, L. (1980). Cosmopolitan, subcosmopolitan and widespread species: Different strategies within the *Drosophila* family (Diptera). *C.R. Soc. Biogeogr.* **57**, 11–26.

Dobzhansky, Th. & Pavan, L. (1950). Local and seasonal variations in relative frequencies of *Drosophila* in Brazil. *Journal of Animal Ecology* **19**, 1–14.

Dowsett, A. P. & Young, M. W. (1982). Differing levels of dispersed repetitive DNA among closely related species of *Drosophila*. *Proceedings of the National Academy of Sciences, USA* **79**, 4570–4574.

Engels, W. R. (1989). *P* elements in *Drosophila*. In: *Mobile DNA* (ed. D. E. Berg and M. M. Howe), pp. 437–484. Washington, D.C.: American Society of Microbiology Publications.

Engels, W. R. (1992). The origin of *P* elements in *Drosophila melanogaster*. *BioEssays* **14**, 681–686.

Good, A. G., Meister, G., Brock, H., Grigliatti, T. A. & Hickey, D. (1989). Rapid spread of transposable *P* elements in experimental populations of *Drosophila melanogaster*. *Genetics* **122**, 387–396.

Johnson, C. W. (1913). The distribution of some species of *Drosophila*. *Psyche* **20**, 202–205.

Karess, R. E. & Rubin, G. M. (1984). Analysis of *P* transposable element functions in *Drosophila*. *Cell* **38**, 135–146.

Kidwell, M. G. (1983). Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **80**, 1655–1659.

Kidwell, M. G. (1986). P–M mutagenesis. In: *Drosophila: A practical approach* (ed. D. B. Roberts), pp. 59–81. Oxford: IRL Press.

Kidwell, M. G. (1990). Evolutionary aspects of hybrid dysgenesis in *Drosophila*. *Canadian Journal of Zoology* **68**, 1716–1726.

Kidwell, M. G. (1993). Lateral transfer in natural populations of eukaryotes. *Annual Review of Genetics* **27**, 235–256.

Kidwell, M. G., Novy, J. B. & Feeley, S. M. (1981). Rapid unidirectional change of hybrid dysgenesis potential in *Drosophila*. *Journal of Heredity* **72**, 32–38.

Kimura, K. (1991). Species specific factors affecting the population dynamics of *P* transposable elements in *Drosophila melanogaster* and *Drosophila simulans*. Ph.D. Dissertation, University of Arizona.

Kiyasu, P. K. & Kidwell, M. G. (1984). Hybrid dysgenesis in *Drosophila melanogaster*: the evolution of mixed P and M populations maintained at high temperature. *Genetical Research* **44**, 251–259.

Lachaise, D., Cariou, M., David, J. R., Lemeunier, F., Tsacas, L. & Ashburner, M. (1988). Historical biogeography of the *Drosophila melanogaster* species subgroup. *Evolutionary Biology* **22**, 159–227.

Laski, F. A., Rio, D. C. & Rubin, G. M. (1986). Tissue specificity of *Drosophila P* element transposition is regulated at the level of mRNA splicing. *Cell* **44**, 7–19.

Miller, W. J., Hagemann, S., Reiter, E. & Pinsker, W. (1992). *P* element homologous sequences are tandemly repeated in the genome of *Drosophila guanche*. *Proceedings of the National Academy of Sciences, USA* **89**, 4018–4022.

Montchamp-Moreau, C. (1990). Hybrid dysgenesis in *P*-transformed lines of *Drosophila simulans*. *Evolution* **44**, 194–203.

O'Brochta, D. A. & Handler, A. M. (1988). Mobility of *P* elements in Drosophilids and non-drosophilids. *Proceedings of the National Academy of Sciences, USA* **85**, 6052–6056.

Paricio, N., Pérez-Alonso, M., Martinez-Sebastian, M. J. & deFrutos, R. (1991). *P* sequences of *Drosophila subobscura* lack exon 3 and may encode a 66 kd repressor-like protein. *Nucleic Acids Research* **19**, 6713–6718.

Preston, C. R. & Engels, W. R. (1989). Spread of *P* transposable elements in inbred lines of *Drosophila melanogaster*. In: *Progress in Nucleic Acid Research and Molecular Biology*. Hollaender Symposium Proceedings **36**, 71–85.

Rio, D. C. & Rubin, G. M. (1988). Identification and

purification of a *Drosophila* protein that binds to the terminal 31-base-pair inverted repeats of the *P* transposable element. *Proceedings of the National Academy of Sciences, USA* **85**, 8929–8933.

Scavarda, N. J. & Hartl, D. L. (1987). Germ line abnormalities in *Drosophila simulans* transfected with the transposable *P* element. *Journal of Genetics* **66**, 1–15.

Spradling, A. C. & Rubin, G. M. (1982). Transposition of cloned *P* elements into *Drosophila* germ line chromosomes. *Science* **218**, 341–347.

Steller, H. & Pirrota, V. (1986). *P* transposons controlled by the heat shock promoter. *Molecular Cell Biology* **6**, 1640–1649.

Young, M. W. & Schwartz, H. E. (1981). Nomadic gene families in *Drosophila*. *Cold Spring Harbor Symposia on Quantitative Biology* **45**, 629–640.