Rapid Spread of Transposable *P* Elements in Experimental Populations of *Drosophila melanogaster*

Allen G. Good,*[1] Gerald A. Meister,† Hugh W. Brock,† Thomas A. Grigliatti† and Donal A. Hickey*

**Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5, and †Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A9*

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ABSTRACT

The invasion of *P* elements in natural populations of *Drosophila melanogaster* was modeled by establishing laboratory populations with 1%, 5% and 10% P genomes and monitoring the populations for 20 generations. In one experiment, the ability of flies to either induce or suppress gonadal sterility in different generations was correlated with the amount of *P* element DNA. In a second experiment, the percentage of genomes that contained *P* elements, and the distribution of *P* elements among individual flies was monitored. The ability to induce gonadal dysgenesis increased rapidly each generation. However, the increase in P cytotype lagged behind by five to ten generations. The total amount of *P* element DNA and the frequency of flies containing *P* elements increased each generation. The number of *P* elements within individual genomes decreased initially, but then increased. Finally, the distribution of *P* elements within the genomes of individuals from later generations varied considerably, and this pattern differed from the parental P strain. These results suggest that the interaction between the assortment and recombination of chromosomal segments, and multiplicative transposition could result in the rapid spread of *P* elements in natural populations.

P elements are a family of transposable elements found in *Drosophila melanogaster*. The complete *P* factor is 2.9 kb long, and codes for transposase, a protein required for transposition (BINGHAM, KIDWELL and RUBIN 1982; O'HARE and RUBIN 1983; RIO, LASKI and RUBIN 1986). Some *P* elements are derived from intact *P* factors by deletion and range in size from 0.5 to 2.9 kb (RUBIN, KIDWELL and BINGHAM 1982). The deleted elements do not encode a functional transposase but can be mobilized in the presence of complete *P* factors. Mobilization of *P* elements occurs in the germline of the hybrid progeny of certain crosses. This mobilization of elements induces hybrid dysgenesis, a syndrome which includes temperature sensitive gonadal sterility, high mutation rates, chromosome aberrations and male recombination (KIDWELL, KIDWELL and SVED 1977).

D. melanogaster strains can be phenotypically categorized in the P-M system according to their ability to induce and/or suppress gonadal sterility in hybrid offspring (KIDWELL, FRYDRYK and NOVY 1983). In P strains, males can induce gonadal sterility and females can suppress it. In M strains, males cannot induce gonadal sterility and females are susceptible. Therefore, hybrid dysgenesis occurs only when P strain males are crossed to M strain females. It does not occur in the reciprocal cross (M males to P females) or in P×P or M×M crosses (for reviews, see BREGLIANO and KIDWELL 1983; ENGELS 1983). The nonreciprocal nature of the hybrid dysgenesis phenomena is due to regulatory ability of the female, manifested in her eggs. Individuals that are resistant to the action of *P* elements have been described as having P cytotype, whereas those that are susceptible have been said to have M cytotype (ENGELS 1979).

There is considerable variation in the P-M phenotype beside the P and M strain extremes. Strong P strains have 30–50 copies of P sequences, and as few as 30% of these may be complete *P* factors (O'HARE and RUBIN 1983). Strains that cause less than 10% gonadal sterility among hybrids with M strain females but are also resistant to the dysgenic activity of P strain males are called neutral or Q strains (KIDWELL and NOVY 1979; KIDWELL, FRYDRYK and NOVY 1983). Q strains may have only a subset of P sequences, or may have a reduced number of *P* factors relative to P strains (BINGHAM, KIDWELL and RUBIN 1982). In addition, there are two main subtypes of M strains. True M strains completely lack *P* elements by molecular analysis and have extremely high susceptibility to P strains (KIDWELL 1985); pseudo M (M′) strains contain *P* elements, sometimes many of them, and can show from low to high susceptibility (ENGELS 1984; BOUSSY and KIDWELL 1987). The partial suppression of hybrid dysgenesis exhibited by most Q and M′ strains differs from that exhibited by P strains since it is only chromosomally inherited (KIDWELL 1985; BLACK *et al.* 1987). This partial suppression

[1] Present address: Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Saskatchewan, Canada S7N OW9.

may result from presence of the *KP* elements. However, at least one M′ strain suppresses gonadal dysgenesis, but contains no *KP* elements (BLACK *et al.* 1987; BOUSSY *et al.* 1988).

Variation in the P-M phenotype exists in worldwide populations of Drosophila. Surveys of wild populations from America, Japan, Europe, Asia, Africa and Australia reveal these populations to be P, Q or M′ (ANXOLABEHERE, NOUAUD and PERIQUET 1982; BREGLIANO and KIDWELL 1983; KIDWELL, FRYDRYK and NOVY 1983; KIDWELL 1983; TAKADA *et al.* 1983; YAMAMOTO, HIHARA and WATANABE 1984; ANXOLABEHERE *et al.* 1984, 1985; KIDWELL and NOVY 1985; BOUSSY 1987; BOUSSY and KIDWELL 1987). The P-M variation present among these populations suggests that *P* elements are not at equilibrium in wild populations of Drosophila. Nevertheless, no true M strains have been found in natural populations since 1974.

In contrast to natural populations, long-established laboratory strains are usually true M type (BINGHAM, KIDWELL and RUBIN 1982; KIDWELL 1983; KIDWELL, FRYDRYK and NOVY 1983; BREGLIANO and KIDWELL 1983). Two hypotheses have been proposed to account for this distribution. The stochastic loss hypothesis (ENGELS 1981) suggests that *P* elements have always been present in substantial frequencies in natural populations, and that their absence from long-established laboratory populations is due to loss of *P* elements from these strains by genetic drift. In contrast, the recent invasion hypothesis (KIDWELL 1983) posits that *P* elements did not exist among natural populations prior to the 1950s and that P sequences recently invaded natural populations of *D. melanogaster*, spreading rapidly by replicative transposition.

A prerequisite to the recent invasion hypothesis is that *P* elements can invade true M populations rapidly. KIDWELL, NOVY and FEELEY (1981), using nonmolecular tests (gonadal sterility and cytotype), showed that in the absence of measurable gonadal dysgenesis (20°), mixed P-M populations changed unidirectionally toward P type. Under conditions of strong negative sterility selection (27°), KIYASU and KIDWELL (1984) found that most mixed populations also evolved to P type. These observed increases in the frequency of P type flies provide strong evidence that P sequences can spread once introduced into a true M population.

The use of the dysgenesis phenotype alone as a marker to monitor the spread of *P* elements is complicated by the fact that there are multiple dispersed copies of these elements within each genome. Based on theoretical considerations, it has been argued that the "spread" of such multicopy elements in mixed populations involves several components; namely, the colonization of new genomes, an increase in copy number within genomes, and competition between complete and deleted elements in the mobile element populations (HICKEY 1982; GINSBURG, BINGHAM and YOO 1984; KAPLAN, DARDEN and LANGLEY 1985). The earlier experiments are unable to distinguish between these components since P strains usually contain 30 to 50 elements per individual and only a few elements may be necessary to induce hybrid sterility. This paper describes several experiments undertaken to gain a fuller understanding of both the "dispersal" and "copy number accumulation" aspects of *P* element spread. Low frequencies (0.01, 0.05 and 0.10) of P genomes were introduced into true M populations in the absence of sterility selection. The dispersal of elements as well as their physiological effects were monitored by single fly ovary blots and by testing the ability of samples of male flies to induce and of female flies to suppress gonadal sterility. Quantitative dot blots and single fly Southern blots were used as indicators of copy number accumulation.

MATERIALS AND METHODS

Drosophila strains: (1) Harwich: An inbred wild type P strain collected from the wild in 1967 by M. L. TRACEY, JR. A white-eyed mutant was isolated by M. G. KIDWELL and was used here as one of the P stocks and as the standard P strain to test cytotype (KIDWELL, NOVY and FEELEY 1981). (2) $\pi 2$: An inbred wild type P strain originally collected from Madison, Wisconsin by W. R. ENGELS (ENGELS 1979). (3) Canton-S: A wild type laboratory true M strain that was used as the M stock and to test for *P* element activity.

Experimental populations: Experimental populations were maintained at 21° on Yeast Agar medium (900 ml H_2O, 100 g sugar, 50 g dried yeast, 15 g agar). Populations were initiated with mated Canton-S (true M type) females only. A proportion (2%, 10% or 20%) of these females were mated with P type males, the remainder were mated with Canton-S males. In this way we could introduce a small proportion of P genomes (1%, 5% or 10%) while minimizing the effects of genetic drift and fertility differences in the first generation of the experiment.

In one series of experiments, populations were initiated with either 1% or 10% P genomes, by mating Canton-S females to males from each of two P strains, Harwich and $\pi 2$. Each of these four populations consisted of four replicates and each replicate was maintained in two half pint bottles. At each generation, the flies from both bottles were mixed, and about 400 flies were transferred to fresh bottles to start a new, discrete generation (a total of 1600 flies for each experimental population). In generations 5, 10 and 20, samples of flies from each population were tested for the ability to induce and/or to suppress gonadal sterility. In generations 2, 5, 10 and 20, samples of 200 male and female flies were frozen, DNA was extracted and the relative amount of *P* element hybridizing DNA/genome was estimated using a quantitative dot blot method.

In a separate series of experiments, two populations were established with initial frequencies of 5% $\pi 2$ genomes. Each of these populations was maintained in 25 half-pint bottles, and at each generation the flies were collected, pooled, and mixed. About 60 flies were distributed to each of 25 new bottles to establish the next generation. Presence of *P* elements was monitored at each generation by means of single fly ovary blots. In addition, at even numbered generations between generations 6 and 20, genomic DNA from single

flies was analyzed by Southern blot hybridization to determine the distribution of *P* elements in individual genomes.

Determination of P activity and cytotype: The induction and suppression of GD sterility were used, respectively, to assay for P activity and P cytotype. To determine the mean degree of sterility induced by a sample of flies, five males were mated individually with three or four Canton-S females at 29° (cross A, KIDWELL, KIDWELL and SVED 1977). The F_1 hybrid females were aged for three days, and their ovaries were dissected according to the methods of SCHAEFER, KIDWELL and FAUSTO-STERLING (1979). Ten females from each individual mating were scored for sterility, and the percentage dysgenesis was calculated as the mean number of dysgenic ovaries from the five individual matings.

To determine the mean regulatory ability exhibited by a sample of flies, individual females were mated singly with three Harwich males at 29° (cross A*, ENGELS and PRESTON 1980). The F_1 hybrid females were aged and dissected as described above. Ten females from each individual mating were scored for sterility and the percentage dysgenesis was calculated as the mean number of dysgenic ovaries from five individual matings. None of the strains used in these experiments contain *KP* elements (BLACK *et al.* 1987); therefore, we will refer to the regulatory ability of the lines tested as P or M cytotype.

Quantitative dot blots: DNA was extracted from 200 mixed male and female flies by the method of STRAUSBAUGH and KIEFER (1979). Genomic dot blots were performed using a Schleicher & Schuell Minifold following the procedure of KAFATOS, JONES and EFSTRADIADIS (1979) and the DNA was bound to the filter by UV cross-linking using the technique of CHURCH and GILBERT (1984). Two ^{32}P-labeled probes were used. As a single copy gene internal standard, the 7.2-kb *Xdh* (*xanthine dehydrogenase*) fragment of the C20 plasmid was used (RUBIN and SPRADLING 1983). The 0.84-kb *Hin*dIII fragment from the pπ25.1 plasmid was used as a *P* element probe (SPRADLING and RUBIN 1982; KARESS and RUBIN 1984). This *Hin*dIII fragment encompasses nucleotide positions 39 to 877 of the complete *P* element. This fragment was chosen because it should hybridize to the majority of *P* elements, both complete and internally deleted (O'HARE and RUBIN 1983). Prehybridization and hybridization were carried out at 65° using the protocol of MANIATIS, FRITSCH, and SAMBROOK (1982). For each cross, an estimate was made of the amount of *P* element hybridizing DNA in the samples as a percentage of the amount in Harwich. Autoradiograms were made from dot blots hybridized with the *Xdh* probe. The filters were then extensively washed in 10 mM Na phosphate, 50% formamide at 65° to remove the *Xdh* probe. Finally the blots were rehybridized to the *P* element probe and autoradiograms were made. Each autoradiogram was then scanned using a Vitatron TDL 100 densitometer. Based on a dilution series the relationship between DNA concentration and densitometer reading was found to be linear unless the dots being scanned had saturated the x-ray film. Using three replicate dots, the ratio of *P* element signal to *Xdh* signal was calculated for the Harwich strain and for each of the experimental populations. The percentage of *P* element hybridizing DNA in each cross compared with Harwich was then calculated using the formula:

$$\%\ \text{of Harwich P DNA} = \frac{\left(\begin{array}{c}\textit{Hin}\text{dIII densitometer reading/}\\ \textit{Xdh}\text{ densitometer reading}\end{array}\right)\text{ for experimental stock}}{\left(\begin{array}{c}\textit{Hin}\text{dIII densitometer reading/}\\ \textit{Xdh}\text{ densitometer reading}\end{array}\right)\text{ for Harwich stock}} \times 100\%\ .$$

Because the DNA samples from the Harwich stock were included on all filters as a standard and comparisons were made only within a filter, this method prevents variables such as the amount of probe, radioactive intensity of the probe, and the exposure times from having any effect on the final calculation of the percentage of *P* element hybridizing DNA.

Single fly ovary blot assays: Ovary blot assays were done by a technique similar to the one used to study the distribution of P sequences in natural populations (ANXOLABEHERE *et al.* 1985). Nitrocellulose filters were equilibrated on filter paper that had been soaked in 5% SDS, 0.05 M EDTA. Ovaries were dissected from females onto the nitrocellulose. After 30 min, the nitrocellulose filters were soaked in 0.5 M NaOH for 7 min, then neutralized by soaking them twice in 1 M Tris-HCl (pH 7.4) for 2 min each. Finally, the filters were treated for 4 min with 1.5 M NaCl, 0.5 M Tris-HCl (pH 7.4), and baked for 2 h at 80°. The filters were prehybridized and hybridized under standard conditions (MANIATIS, FRITSCH and SAMBROOK 1982). The same 0.84-kb *Hin*dIII fragment of pπ25.1 used in the quantitative dot blots was used as the probe.

Ovaries from π2 (P) and Canton-S (true M) flies were included on each filter as controls. The amount of probe bound to ovary DNA from experimental flies was scored visually as positive or negative with respect to these controls. Any questionable dots were scored as negative. No attempt was made to quantify the intensity of the signal. However, to test the sensitivity of this assay, ovaries from several strains with known numbers of *P* elements were examined. Among the strains tested were tAP-1, tAP-3 and tAP-5 (GOLDBERG, POSAKONY and MANIATIS 1983). Each of these strains is homozygous for a single, stable *P* element with an *Adh* insert. The ovaries from females of all three of these strains, as well as any strains with more elements, always tested positive when compared to the Canton-S controls. Thus, it appears that this method can routinely detect an individual with only two *P* elements, though we make no claim that it always does so. Presumably we can detect equally well those flies heterozygous for two elements and those homozygous for one element.

Single fly Southern blots: Each fly was homogenized in 100 μl of 0.125 M Tris-HCl (pH 8.5), 0.08 M NaCl, 0.05 M EDTA, 0.16 M sucrose, 0.5% SDS, and incubated at 65° for 30 min. Potassium acetate was added to a final concentration of 1 M, and the solution was kept on ice for 60 min. After centrifugation for 10 min in a microcentrifuge, the supernatant was extracted with phenol/chloroform, and the nucleic acids were precipitated with ethanol. The pellet was resuspended in 10 μl of solution containing *Bam*HI, *Xba*I, and the appropriate buffer, and made to 50 μg/ml with RNase. After digestion at 37° for 2 h, the sample was separated electrophoretically on 0.7% agarose gels. Blotting and hybridization were carried out using standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982). Filters were first hybridized with the 0.84-kb *Hin*dIII fragment of pπ25.1 and an autoradiogram was made. They were then washed extensively to remove the P probe completely. They were rehybridized to sAC-1 (an *Adh* single copy probe; GOLDBERG 1980), and another autoradiogram was made.

RESULTS

We monitored the increase in the ability of the flies of the experimental populations to induce and sup-

press gonadal sterility and simultaneously undertook a molecular analysis of various facets of *P* element dispersal and abundance. However, the results reported in Figures 1–4 were obtained in different populations than the results reported in Figures 5–7. Our assumption is that the results obtained in each series of experiments are directly comparable since the principal difference between the two sets is the size of the populations; the populations in the second series (molecular analyses) were larger than those in the first series.

Evolution of the ability to induce and suppress gonadal sterility: To measure the rate of *P* element spread phenotypically, the ability of males to induce gonadal dysgenesis was scored in several experimental populations. Two populations were initiated with 1% P genomes and another two populations were initiated with 10% P genomes. At each starting frequency, one population contained the Harwich P type genome while the other contained the $\pi 2$ P type genome. There were four replicates of the experiment. At generations 5, 10 and 20, males were selected at random and crossed to true M females to test their ability to induce gonadal dysgenesis in the F_1 female offspring. The results of these experiments are summarized in Figure 1. There is a very rapid increase in the frequency of the ability to induce gonadal sterility, regardless of the initial frequencies or the particular P strain used.

Females from the same populations were tested for their ability to exhibit P cytotype. Figure 2 shows the changes in cytotype of a sample of female flies from populations at generations 5, 10 and 20. By the fifth generation the samples demonstrated very little ability to suppress gonadal sterility. In the tenth generation, three of the four populations still had less than 30% P cytotype, but by generation 20 most of the populations had greater than 80% P cytotype. In all four experiments the increase in the ability of the flies to suppress gonadal sterility occurred much later than did the increase in ability to induce gonadal sterility (compare Figures 1 and 2). However, once the flies began to acquire P cytotype, they developed it fairly rapidly.

Changes in total *P* element hybridizing DNA: To determine whether the amount of *P* element hybridizing DNA in the population actually increased, DNA was prepared from 200 flies at generations 2, 5, 10 and 20, and dot blotted. Dot blots were probed with a single copy gene (*Xdh*), washed, and then reprobed with an internal *P* element probe. Figure 3 shows an example of a dot blot of DNA prepared from generations 2, 5, 10 and 20 from one experimental population initiated with 1% P genomes. Clearly there is an increase in the amount of *P* element hybridizing DNA with increasing generation. The data were normalized to the single copy gene and the percentage of *P* element hybridizing DNA relative to the Harwich control strain was estimated (see MATERIALS AND METHODS). As summarized in Figure 4, the relative amount of signal from the *P* element probe increased essentially monotonically from generation 2 through generation 20 in all populations. This demonstrates that there was a rapid increase in the mass of *P* element hybridizing sequences in each population.

Dispersion of P sequences through a population: The rapid "spread" of the P phenotype (induction and suppression of gonadal sterility) is expected to be correlated with an increase in the frequency of genomes containing P sequences. Two independent replicate populations were established with Canton-S females; 90% of the females had been mated with Canton-S males and 10% had been mated to $\pi 2$ males. The proportion of flies in subsequent generations that had some P sequences was estimated using the single fly ovary blot method (see MATERIALS AND METHODS). At each generation the ovaries were removed from 200 individual female flies. The DNA from each was isolated on nitrocellulose filters and probed with an internal *P* element fragment. The hybridization signal of the experimental flies was compared to the signal obtained with reference true M (Canton-S) and P ($\pi 2$) strain flies, and scored visually as positive or negative. Figure 5 shows typical results from generations 1, 3, 6, 9, 15 and 20. While this method is quite sensitive (see MATERIALS AND METHODS), it is likely to underestimate the number of flies containing *P* elements, since dubious signals were interpreted as negative.

The percentage of flies containing *P* elements at each generation from the two replicate populations initiated with 5% P genomes is shown in Figure 6, panels A and B. The number of flies that have *P* elements increases rapidly until about generation ten, at which point approximately 90% or more of the individuals contain a detectable number of P sequences. Then the rate of *P* element spread diminishes. These results show that, if a few P flies are introduced into a randomly mating true M population, the spread of *P* elements to new genomes can be very rapid.

Changes in P sequences in single flies: The results obtained above indicate both a dispersion and multiplication of P sequences in these experimental populations. To confirm these results, single fly Southern blots were performed. DNA was prepared from single flies of even numbered generations from generation 6 to 20 of the populations initiated with 5% $\pi 2$ genomes. The DNA was digested with *Bam*HI and *Xba*I, separated electrophoretically, transferred to nitrocellulose filters, and probed with an internal *P* element fragment. Since neither of the two restriction enzymes used to cut the genomic DNA cut within the complete *P* element, one would expect this combination of enzymes and probe to yield one band for each hemi-

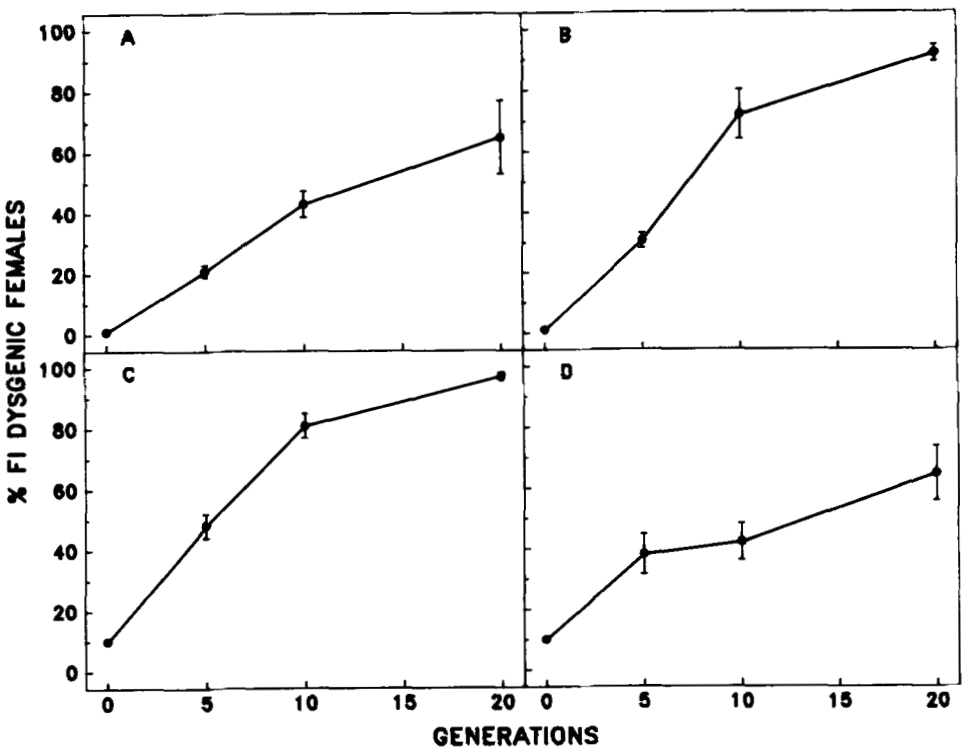

FIGURE 1.—Changes in the mean ability of flies to induce gonadal sterility. Dysgenic potential was assayed by the ability of males to produce dysgenic female progeny. Panel A shows the results for populations initiated with 1% Harwich genotypes; panel B for 1% π2; panel C for 10% Harwich; and panel D for 10% π2. The results shown are the means of four replicate populations in each case (see MATERIALS AND METHODS). Error bars represent one standard error.

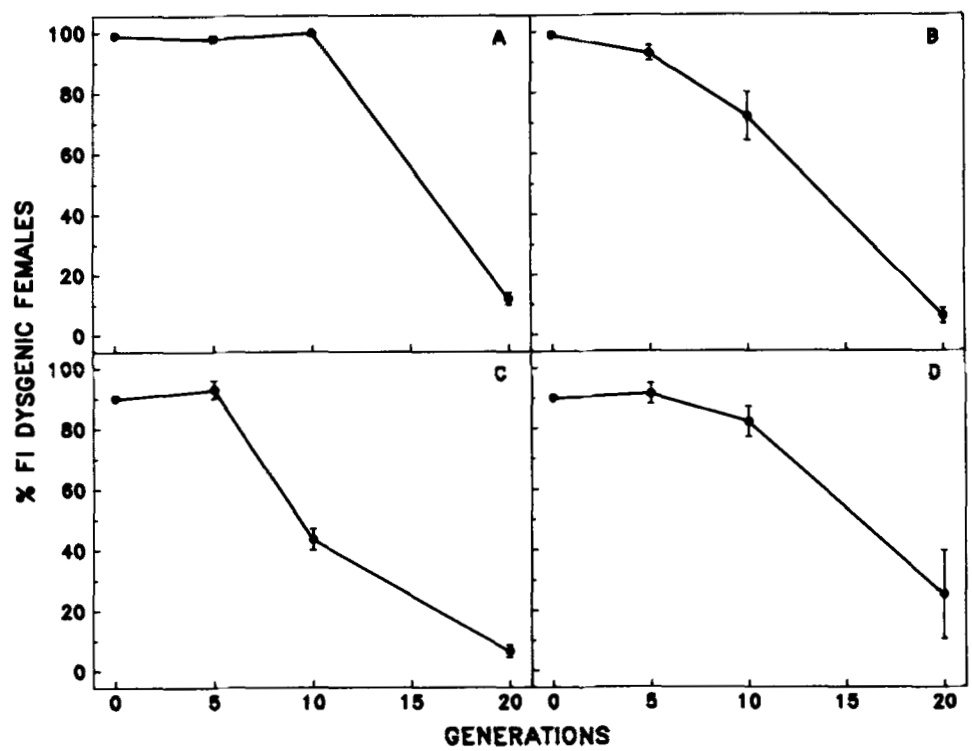

FIGURE 2.—Changes in P cytotype levels within the experimental populations. The decrease in F_1 dysgenic females shown here reflects a corresponding increase in P cytotype (*i.e.*, an increase in the females' ability to suppress gonadal sterility). The initial composition of the experimental populations is as described in Figure 1. Error bars represent one standard error. Error bars are present on all data points, but appear to be absent when the error is low.

zygous or homozygous *P* element. Figure 7 shows a Southern blot containing DNA of flies from generations 6, 14 and 20 of one experimental population, and of the Canton-S (true M) and π2 (P) reference strains. Notice that the π2 controls did not yield as many bands as might have been expected. *In situ* hybridization reveals the π2 strain to have about 30 *P* elements per genome (BINGHAM, KIDWELL and RUBIN 1982) yet only about 12 bands are visible in the π2 control lane of the gel. This result may be partially due to the use of a small internal P fragment probe. Similar results have been found by others even with multiple fly Southern blots. DANIELS *et al.* (1987) detected about 40 bands in Southern blots of Harwich-77 when *in situ* analyses had detected 60 to 65. BLACKMAN and colleagues (1987) also show far fewer *P* element hybridizing bands in Southern blots of π2 than expected. This simply implies that the actual number of elements per fly will be underestimated

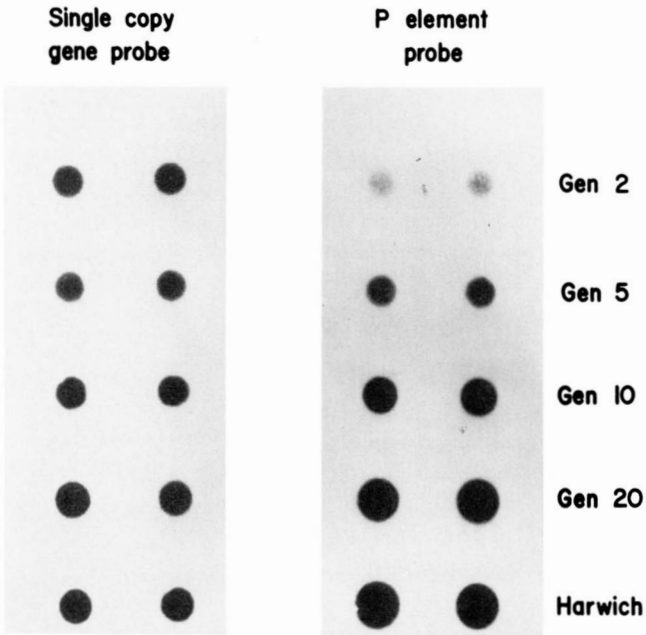

FIGURE 3.—Sample quantitative dot blot. The DNA is from two replicates of population B (Figure 1) and Harwich controls. The blot has been probed with a single copy gene probe (*Xdh*) and an internal *P* element probe. See MATERIALS AND METHODS and GOOD and HICKEY (1987) for details.

using Southern analysis, at least when a large number of elements are present in the genome.

Despite the fact that the single fly Southerns underestimate the actual number of elements per fly, several observations can be made. First, the number of P sequences per individual increases dramatically between generations 6 and 20. Second, the intensity of labelling and the number of bands in generation 20 resemble or are greater than the P strain control. Finally, in later generations, many of the bands are not shared either between individuals of a given generation or with the original P strain. The first two observations demonstrate that P sequences are increasing in number within individual flies. The three observations together suggest that the *P* elements are multiplying and transposing.

DISCUSSION

These experiments model the introduction of low frequencies of P flies into randomly mating true M populations. Figure 1 shows that, with increasing generation, there was a very rapid increase in the amount of dysgenesis induced by a sample of flies. These data confirm earlier results and suggest that *P* elements can rapidly spread in mixed P-M populations in the absence of any imposed selection (KIDWELL, NOVY and FEELEY 1981; KIYASU and KIDWELL 1984; KIDWELL 1986).

The acquisition of P cytotype in the populations, as measured by the ability to suppress gonadal dysgenesis, occurred markedly later than the increase in the ability of flies to induce gonadal dysgenesis (compare Figures 1 and 2). These results support earlier observations that the M cytotype will often be retained for some time in the presence of P sequences, but that eventually the cytotype will change (KIDWELL and NOVY 1979; ENGELS 1981; KIDWELL 1986). The lag in cytotype switching could be explained in terms of complete and incomplete *P* elements present in different generations. Only *P* factors produce the transpos-

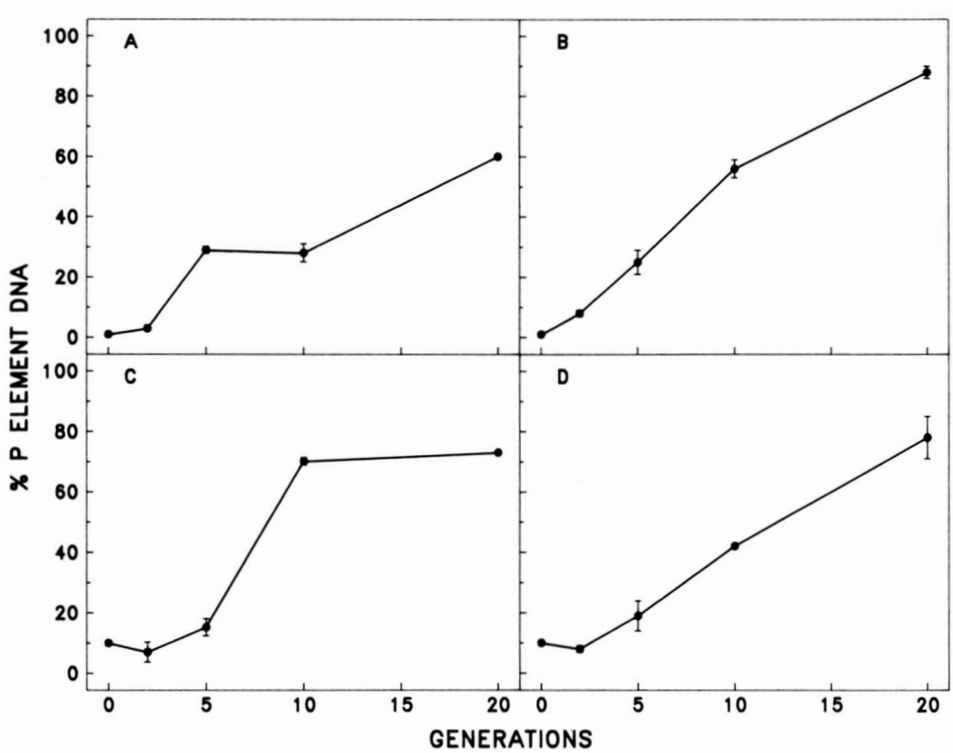

FIGURE 4.—Changes in the amount of *P* element hybridizing DNA. Populations are as described in Figure 1. Results shown are the means of two replicate populations in each case.

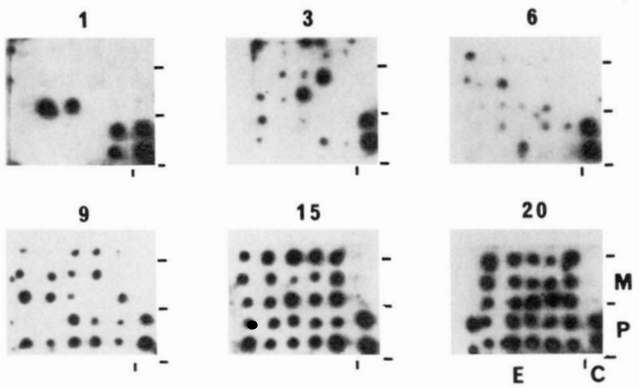

FIGURE 5.—Representative ovary blots from generations 1, 3, 6, 9, 15 and 20. Each blot has the ovary DNA from 25 experimental females (labeled E on last blot) and all were hybridized simultaneously to an internal *P* element probe. The right hand column of each blot (labeled C) has the ovaries of two true M strain (Canton-S) and two P strain (π2) control females.

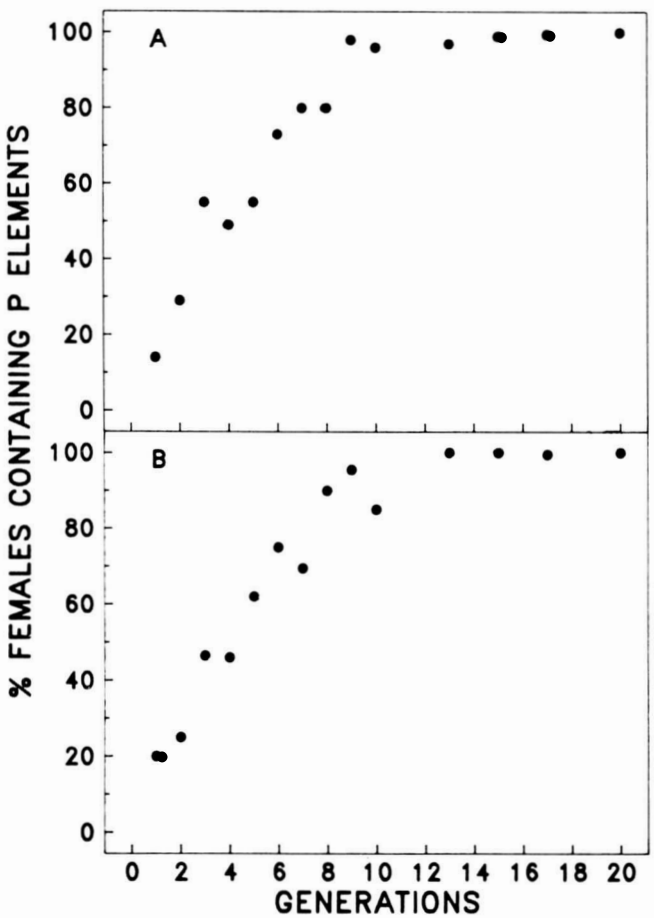

FIGURE 6.—The presence of P sequences in individual females. P sequences were detected by probing ovary blots with an internal *P* element fragment. Panels A and B summarize the results of replicate populations initiated with 5% π2 genomes.

ase that is necessary for transposition and the induction of gonadal dysgenesis (RIO, LASKI and RUBIN 1986). On the other hand, it seems likely that P cytotype may be dependent on incomplete elements. NITASAKA, MUKAI and YAMAZAKI (1987) have demonstrated that a chromosome segment that repressed transposition contained two incomplete *P* elements.

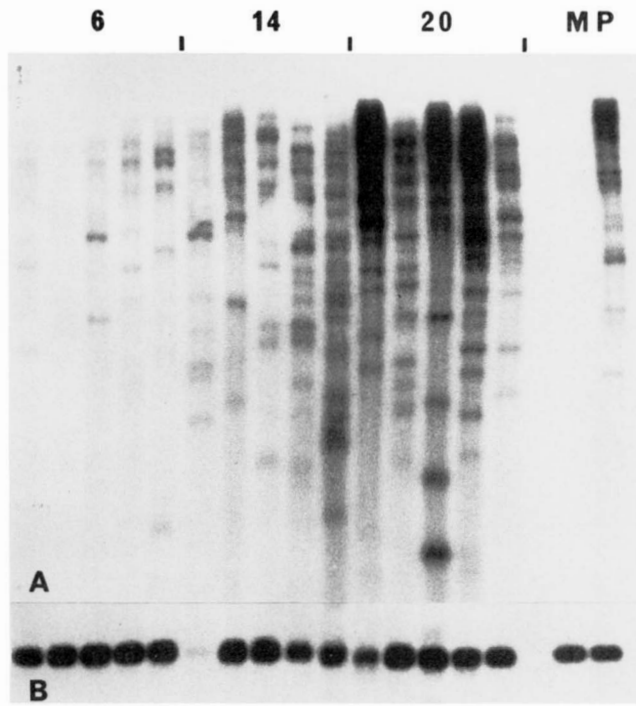

FIGURE 7.—Distribution of P sequences in individual flies. DNA from single flies was digested with *Bam*HI and *Xba*I, Southern blotted and probed for P sequences. Panel A shows results for generations 6, 14 and 20 of a population initiated with 5% π2 genomes. A Canton-S and a π2 lane are included as true M and P controls, respectively. The blot was washed and reprobed with an *Adh* probe. Panel B shows the hybridization obtained to a unique *Bam*HI-*Xba*I fragment of approximately 5 kb in length, located 5′ of the *Adh* gene (starting at nucleotide −660). This can be used to compare the amount of DNA loaded into individual lanes.

They dismissed one as too small to be an important regulatory element, and showed that the other was a deleted *P* element carrying only open reading frames 0 through 2. H. M. ROBERTSON and W. R. ENGELS (personal communication) have shown that the presence of a single element mutated in or near the 2–3 intron or within open reading frame 3 can also substantially reduce hybrid dysgenesis. They hypothesize that chance deletions produce elements that code for a repressor rather than a transposase.

If the induction of hybrid dysgenesis is dependent on the presence of *P* factors, and P cytotype is dependent on incomplete *P* elements, then two models could plausibly explain why acquisition of P cytotype lags behind the ability to induce gonadal dysgenesis. The simplest model is that the number of *P* factors within the genomes of the invading flies is substantially greater than the number of cytotype generating *P* elements. Outbreeding with true M flies would produce some progeny containing *P* factors, but lacking cytotype generating *P* elements. Alternatively, the lag in cytotype switching could be explained if *P* factors undergo replicative transposition at higher rates than the cytotype generating *P* elements. These models are not mutually exclusive. In either case, new cytotype determining elements might have to be generated,

and this process might take many generations, especially if the process is not specific. *P* elements are formed by internal deletions of *P* factors, and it has been suggested that this degenerative process is a consequence of transposition (VOELKER *et al.* 1984; DANIELS, STRASBAUGH and ARMSTRONG 1985; DANIELS *et al.* 1985). If transposition is a function of the number of *P* factors per genome, then as the factors disperse, transposition rates might increase dramatically. Consequently, the probability of generating appropriately deleted *P* elements would also increase. Once individuals having cytotype generating elements appear in the population they might have a selective advantage over their dysgenic siblings. This could explain the observation that once P cytotype begins to appear, the populations acquire it rapidly.

The single fly ovary blots (summarized in Figure 6), show that there is a rapid increase in the number of flies containing some P sequences. This dispersal of *P* elements to new genomes is not surprising. In fact, it would be expected for any element present in high copy number and distributed throughout the genome. One need not invoke replicative transposition or mobilization of any type. Recombination and chromosome assortment would disperse such a high copy number element from a few individuals to many in only a few generations of outbreeding. However, if such simple processes were solely responsible for the spread of *P* elements, then one would predict that the increase in the frequency of genomes with some elements would be accompanied by a corresponding decrease in the number of elements within individual genomes (see discussion by ANXOLABEHERE *et al.* 1986; GOOD and HICKEY 1987). Alternatively, if some multiplicative process also occurred, then the number of elements within individual flies might not be reduced.

There are several lines of evidence which support the latter alternative. First, the dot blot assays on pooled flies (Figures 3 and 4) indicate that the total amount of *P* element hybridizing DNA in the populations increases with increasing generation. Second, while the single fly ovary blots show that the number of individuals with *P* elements increases each generation, they also suggest that the number of *P* elements per individual increases in later generations. While we made no attempt to quantify the single fly ovary blots, the intensity of the signals is probably a rough reflection of the number of elements present within each fly. Note that a single probe was hybridized simultaneously to all filters shown in Figure 5 and that the $\pi 2$ controls have a relatively constant intensity. If outbreeding was wholly responsible for *P* element dispersal, one would expect the intensity of the hybridization per individual fly to decrease with increasing generation. Such a trend is seen in the early generations (compare the intensity of hybridization to ovaries from generations 1, 3 and 6). However, in later generations this trend is reversed. From generation 9 onward the positive signals are not only more frequent, but also, they appear more intense. This observation was made while testing large numbers of ovaries (200 flies each generation) and is consistent with an increase in the number of P sequences per fly in these later generations. Finally, the single fly Southern blots also indicate that the number of elements in individual flies increases considerably in the later generations (Figure 7). All flies tested from generation 6 show fewer bands than the $\pi 2$ control; by generation 14 some of the flies have about as many bands as the control; and by generation 20 the number of bands and the intensity of labelling of all flies tested are at least as great as the control.

The decrease in the intensity of the positive ovary blots and decrease in number of bands in the Southern blots of *P* element containing flies from early generations relative to the initiating $\pi 2$ flies indicate that chromosome assortment and recombination during outbreeding are important to the dispersal of *P* elements. In the early generations after invasion, assortment and recombination probably act quickly to increase the number of individuals with *P* elements, while reducing the average number of *P* elements per genome. We do not mean to imply that a multiplicative process does not act in the first generations after invasion. The data in Figures 3 and 4 show that there is a continuous increase in the total amount of *P* element DNA in the population from generation 2 onwards. Rather, it seems likely that outbreeding contributes most to *P* element dispersal in early generations. In later generations, all the molecular data show that there is an increase in the number of *P* elements per genome, suggesting that a multiplicative process is counteracting any tendency towards decreasing the number of *P* elements per genome produced by outbreeding.

We believe that this multiplicative process is replicative transposition. This model receives support from the single fly Southern analysis. As mentioned above, by generation 20 the number of P homologous bands and the intensity of labelling among individual flies from the experimental populations are at least as great as those of the $\pi 2$ strain which was used to invade the true M populations. In addition, note that few of the P homologous bands are shared either between individuals of the experimental group or with the $\pi 2$ control. This suggests that there are more elements in later generations than the earlier generations in the experimental group, and that many of these elements are located at new positions within the individual genomes. That is, the elements are both multiplying and transposing.

There are two alternative explanations which might explain the increase in the number of elements accom-

panying their spread through the populations. The first alternative explanation that is the chromosome segments containing P sequences may be disproportionately represented in the offspring of P-M crosses due to some form of positive selection. This selection could act on the *P* elements themselves or on associated loci. There is, however, no evidence that *P* elements confer any selective advantage on flies which contain them. To the contrary, there is evidence that the presence of these elements generates a significant genetic load (MACKAY 1986; FITZPATRICK and SVED 1986). Although it would be difficult to exclude the possibility of weak selection for *P* elements or linked loci, it seems unlikely that selection could account for the dramatic increase in *P* element number observed in our experiments. The second alternative explanation that might explain the increase in *P* element sequences is conservative transposition. Conservative transposition coupled with chromosomal assortment can lead to genotypes with increased numbers of transposable elements. Like unequal crossing-over within gene families, however, this process is unbiased. It can generate genotypes with increased or reduced number of elements with equal likelihood. The observed increase in the mean amount of P hybridizing DNA in all populations indicates a strong positive bias and thus argues against this explanation of the increase in *P* element number.

The observed lag in cytotype switching is consistent with the replicative transposition model of *P* element spread. Transposition occurs minimally in populations in which all of the individuals have a P cytotype. If replicative transposition is an important process in the spread of *P* transposable elements, then, the elements must first undergo a phase of transpositional activity before they produce P cytotype. The P cytotype could then act as a form of self-regulation by preventing overreplication and thus could limit the genetic load on the population caused by the *P* elements (HICKEY 1982; CHARLESWORTH and LANGLEY 1986). A balanced system such as this would be assured if *P* factors were necessary for the accumulation of *P* cytotype conferring elements, as they appear to be for the *KP* element accumulation (BLACK *et al.* 1987).

In conclusion, our results suggest that both chromosome assortment and recombination, and multiplicative transposition play an important role in the dynamics of *P* element invasion. Through the combination of these effects, even a few migrants could spread *P* elements very efficiently among natural populations of *D. melanogaster*. Semi-isolated populations, which might remain genetically distinct for non-transposable genetic markers, could thus be rapidly invaded by these self-replicating DNA sequences.

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