# Hybrid Dysgenesis in *Drosophila simulans* Lines Transformed With Autonomous *P* Elements

**Stephen B. Daniels,*[1] Arthur Chovnick† and Margaret G. Kidwell***

**Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, and †Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06268*

Manuscript received February 19, 1988
Accepted for publication October 25, 1988

ABSTRACT

The molecular and phenotypic analysis of several previously described *P* element-transformed lines of *Drosophila simulans* was extended in order to determine whether they had the potential to produce a syndrome of P-M hybrid dysgenesis analogous to the one in *Drosophila melanogaster*. The transformed line with the highest number of *P* elements at the beginning of the analysis, DsPπ-5C, developed strong P activity potential and *P* element regulation, properties characteristic of *D. melanogaster* P strains. The subsequent analysis of sublines derived from 34 single pair matings of DsPπ-5C revealed that they were heterogeneous with respect to both their *P* element complements and P activity potentials, but similar with respect to their regulatory capabilities. The subline with the highest P activity, DsPπ-5C-27, was subsequently used as a reference P strain in the genetic analysis of the *D. simulans* transformants. In these experiments, the reciprocal cross effect was observed with respect to both gonadal sterility and male recombination. As in *D. melanogaster*, the induction of gonadal sterility in *D. simulans* was shown to be temperature-dependent. Molecular analysis of DsPπ-5C-27 revealed that it has approximately 30 *P* elements per genome, at least some of which are defective. The number of potentially complete *P* elements in its genome is similar to the number in the *D. melanogaster* P strain, Harwich-77. Overall our analysis indicates that *P*-transformed lines of *D. simulans* are capable of expressing the major features of P-M hybrid dysgenesis previously demonstrated in *D. melanogaster* and that *P* elements appear to behave in a similar way in the two sibling species.

THE phenomenon of hybrid dysgenesis was first reported in *Drosophila melanogaster* (KIDWELL, KIDWELL and SVED 1977) and has been defined as a syndrome of aberrant genetic traits that is induced in the hybrid progeny of certain intraspecific crosses, usually in one direction only (KIDWELL 1979). In this species, the P-M (KIDWELL, KIDWELL and SVED 1977; ENGELS 1988) and I-R (BREGLIANO *et al.* 1980; FINNEGAN 1988) systems have been described in considerable detail. In each of these systems, the manifestations of hybrid dysgenesis have been correlated with the destabilization of a specific transposable element family. In the P-M system, the activity of the *P* element is responsible for a number of associated traits including temperature-dependent sterility, male recombination, chromosomal aberrations, segregation distortion, elevated rates of mutation, nondisjunction and female recombination. In the I-R system, the activation of the *I* element also produces a group of traits which includes mutation, chromosomal aberrations and a distinctive type of temperature-dependent sterility. A third system associated with the *hobo* element has been reported recently (BLACKMAN *et al.* 1987; YANNOPOULOS *et al.* 1987), but, currently, many questions remain about the phenotypic properties of *hobo* and its contribution to the phenomenon of hybrid dysgenesis (BLACKMAN and GELBART 1988).

P-homologous sequences are widely distributed among all the species groups that comprise the subgenus *Sophophora* (DANIELS *et al.* 1984; DANIELS and STRAUSBAUGH 1986; STACEY *et al.* 1986; ANXOLABÉHÈRE, NOUAUD and PÉRIQUET 1987; S. B. DANIELS, unpublished results), although, interestingly, they are not found in the species most closely related to *D. melanogaster*, including its sibling species *D. simulans* (BROOKFIELD, MONTGOMERY and LANGLEY 1984). The explanation for this disjunct distribution is not completely clear, but there is a growing body of evidence that supports the hypothesis that *P* elements have only recently been introduced into the *D. melanogaster* genome (within the past 40 yr) presumably by horizontal transmission from some as yet unknown source (KIDWELL 1983; ANXOLABÉHÈRE, KIDWELL and PÉRIQUET 1988). The other *P*-bearing species in the genus have apparently harbored *P* element sequences for a much longer evolutinary period (DANIELS and STRAUSBAUGH 1986; LANSMAN *et al.* 1987; S. B. DANIELS, unpublished results), but it is not presently known whether any of these species contain the active element found in *D. melanogaster*.

[1] Present address: Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06268.

Evidence for P-M hybrid dysgenesis has not been documented in *P*-bearing sophophorans other than *D. melanogaster*.

With the advent of an efficient method to transduce cloned *P* elements, or their derivatives, into Drosophila germline chromosomes (RUBIN and SPRADLING 1982), it is now possible to introduce autonomous *P* elements into the genomes of species that do not naturally carry them. Such transformants would provide the material to determine whether *P* elements behave in other species as they do in *D. melanogaster*.

There have been several studies in which successful interspecific gene transfer has been achieved. For example, SCAVARDA and HARTL (1984) transformed a $ry^-$ strain of *D. simulans* with a selectable $ry^+$-bearing *P* element vector and BRENNAN, ROWAN and DICKINSON (1984) introduced an autonomous *P* element into the germline of *Drosophila hawaiiensis*, suggesting that, at least within the genus, there are no species barriers to prevent the initial insertion event. In other experiments, DANIELS, STRAUSBAUGH and ARMSTRONG (1985) focused on a molecular analysis of *P* element behavior in a number of *D. simulans* transformed lines during the first 12 generations following germline integration. They observed that *P* element copy number increased over time in some of the lines and that this increase was accompanied by transposition of elements to new genomic sites. Similar results were obtained with the *D. hawaiiensis* P transformants (BRENNAN, ROWAN and DICKINSON 1984).

Despite the molecular evidence that *P* elements can integrate and multiply in the genomes of species other than *D. melanogaster*, evidence for the phenotypic manifestations of hybrid dysgenesis has been sketchy and largely unsubstantiated. In this paper, we have continued the analysis of the *D. simulans* P transformants generated by DANIELS, STRAUSBAUGH and ARMSTRONG (1985), focusing particularly on whether the features characteristic of P-M hybrid dysgenesis arise in a newly invaded species.

## THE EXPERIMENTAL SYSTEM

The *P* family of transposable elements consists of members that are homologous in sequence but heterogeneous in size (O'HARE and RUBIN 1983). *P* elements can be divided into two types, complete or autonomous elements (sometimes called *P* factors) and defective, nonautonomous elements. Complete elements are 2.9 kb in length. Nonautonomous ones are smaller and variable in size and are derived from autonomous elements by internal deletions. The frequency of such deletions is high under conditions of active transposition (VOELKER *et al.* 1984; DANIELS *et al.* 1985; SEARLES *et al.* 1986; TSUBOTA and SCHEDL 1986). Autonomous elements are able to catalyze their own transposition and can also act *in trans* to promote the transposition of nonautonomous ones.

In the P-M system of hybrid dysgenesis, most strains of *D. melanogaster* can be classified as either P, M or Q. Classification is based on the phenotypic properties of the $F_1$ progeny that are produced from specific reference crosses (see MATERIALS AND METHODS for details). Gonadal (GD) sterility is one dysgenic trait that is frequently used to measure the level of P activity. It is characterized by the failure of one or both gonads to develop beyond a rudimentary stage (ENGELS and PRESTON 1979; SCHAEFER, KIDWELL and FAUSTO-STERLING 1979).

P strains exhibit P activity potential (*i.e.*, they produce hybrid dysgenesis when males are crossed to M strain females). They also possess a strong ability to regulate *P* element expression which is often referred to as the P cytotype (ENGELS and PRESTON 1979). M strains, on the other hand, rarely have any significant level of P activity potential, but have high P susceptibility (M cytotype), *i.e.*, they are not able to regulate completely the activity of *P* elements. Q, or neutral, strains show little or no dysgenesis when crossed to either M or P strains, irrespective of direction.

At present, the regulation of *P* element movement is not well understood. The nonreciprocal nature of the dysgenic response implicates certain maternally transmitted cytoplasmic "factors" as being important components in the regulatory process involving cytotype (ENGELS 1979; ENGELS and PRESTON 1979). Other regulatory systems apparently lack these cytoplasmic determinants (KIDWELL 1985; BLACK *et al.* 1987). For more detailed information, see ENGELS' (1988) recent review of *P* elements.

## MATERIALS AND METHODS

***D. simulans* strains employed:** All nontransformed stocks used in this study were found to be devoid of *P* element sequences by Southern blot analysis.

8DS is a laboratory strain provided by H. KRIDER at the University of Connecticut; it was used as the injection recipient in the original transformation experiments (DANIELS, STRAUSBAUGH and ARMSTRONG 1985).

14021-0251.2, -0251.3, -0251.5 and -0251.8 are wild-type lines obtained from the National Drosophila Species Resource Center at Bowling Green State University.

DsTuc2, DsTuc4, DsTuc6, and DsTuc8 are lines established from single gravid females captured from the wild in Tucson, Arizona, in 1985.

*f*; *pm net*; *st e* is a multiply marked strain homozygous for the following recessive markers: forked (*f*; I, 56); plum (*pm*; II, 103); net (*net*; II, 0); scarlet (*st*; III,42); ebony (*e*, III, 61) (STURTEVANT 1929; J. S. F. BARKER, personal communication).

DsP$\pi$-1, -3, -4, -5, -9, -14, -15, -22, -25, -42, -46 are the 11 P-transformed lines originally described by DANIELS, STRAUSBAUGH and ARMSTRONG (1985).

DsP$\pi$-5A, -5B, -5C, -25A, -25B, -25C are single pair sublines derived from two of the original transformed lines, DsP$\pi$-5 and DsP$\pi$-25, at the third generation following transformation (DANIELS, STRAUSBAUGH and ARMSTRONG 1985).

DsPπ-5C-1 through -34 are single pair sublines that were established from the DsPπ-5C line 16 months following transformation.

***D. melanogaster* strains employed:** Canton-S and $ry^{506}$ are M strains that do not carry any *P* elements.

Harwich-77 is an inbred, strong P strain obtained in 1977 as a subculture from the original Harwich line. See DANIELS *et al.* (1987b) for details concerning the molecular characterization of the *P* element composition of this strain.

Agana is an inbred line collected at Agana, Guam, in the 1960s. It is classified as a moderate P strain on the basis of standard phenotypic tests.

**Plasmids** pπ25.1 contains an autonomous *P* element that is capable of catalyzing its own transposition from plasmid to germline DNA and is flanked by genomic sequences from polytene chromosome region 17C; for further details see SPRADLING and RUBIN (1982) and O'HARE and RUBIN (1983).

pπ25.7BWC ("both wings clipped") contains a *P* element that lacks 39 bp from its 5′ end and 23 bp from its 3′ end. This plasmid was constructed by K. O'HARE and does not contain any flanking genomic DNA.

**Generation and culturing of transformants.** All transformants were generated in February of 1984 by embryo microinjection (SPRADLING and RUBIN 1982), and a separate line was established from each (DANIELS, STRAUSBAUGH and ARMSTRONG 1985). For the first 9 months, the original lines were cultured in half-pint bottles; thereafter, they were maintained in duplicate vial cultures. The single pair sublines that were derived from DsPπ-5 and DsPπ-25 (see above) were propagated in essentially the same manner. All cultures were grown on a cornmeal-sucrose-agar-Brewer's yeast medium seeded with live yeast and were maintained at room temperature (21–25°).

**Southern blotting analysis:** Samples for mass extraction of genomic DNA were routinely prepared from approximately 100 adult flies by the method described by DANIELS and STRAUSBAUGH (1986). Procedures for restriction enzyme digestion, agarose gel electrophoresis, gel blotting, preparation of nick-translated probes and filter hybridization were essentially as described in RUSHLOW, BENDER and CHOVNICK (1984).

***In situ* hybridization:** Salivary gland chromosomes were hybridized and labeled with biotinylated BWC DNA (ENGELS *et al.* 1986).

**Gonadal sterility tests:** The specific reference crosses that were used to characterize the P-M phenotypic properties of the transformed *D. simulans* lines are analogous to those used to classify strains of *D. melanogaster* (*e.g.*, KIDWELL and NOVY 1979; ENGELS and PRESTON 1979; DANIELS *et al.* 1987b). P activity potential was measured by crossing males from the line in question to females from a strain free of *P* elements (cross A). In *D. melanogaster*, the standard tests to measure *P* element regulation (*i.e.*, crosses of the A* type) employ males from reference strains having known high levels of P activity. At the beginning of our experiments with *D. simulans*, a strong P strain was obviously not available and the Cross A* analysis was only made possible after the DsPπ-5C-27 strain had developed a strong ability to induce GD sterility (see RESULTS). Therefore, in the interim, other ways to assess *P* element regulation had to be devised.

One measure of regulation was provided by frequencies of intrastrain sterility at high temperature. Also, cross B, the reciprocal of cross A, provided an indication of regulatory potential. If there is strong regulation present in the tested strain, progeny from both intrastrain and cross B matings are expected to show much lower sterility than do those from cross A. Conversely, in the absence of regulation, progeny from these matings are expected to show relatively high sterility. Thus, like cross A*, cross B and the intrastrain mating serve as indices of a line's ability to suppress *P* element activity. Cross B*, which is the reciprocal of cross A*, was employed as a control cross to detect the action of any confounding factors, unrelated to *P*, that might cause a condition similar to GD sterility.

For each reference cross, 20–30 pairs of flies were mated *en masse* in half-pint bottles and immediately placed at 29°. Two to three days later, parents were removed from the culture. Approximately 2 days following the onset of eclosion, $F_1$ progeny were collected and allowed to mature for 3–5 days at room temperature. In most of these crosses, 25–50 $F_1$ females were then taken at random for dissection. In some cases multiple tests were performed, enabling the calculation of standard errors. Dissected gonads were scored as either normal or dysgenic (unilateral or bilateral). Ovaries with at least one developed ovariole were scored as nondysgenic. The frequency of gonadal sterility was calculated by dividing the number of dysgenic gonads by the total number of gonads scored.

In several experiments, phenotypic tests were performed on *D. melanogaster* strains for comparison. These tests were conducted essentially as outlined above with the exception that 10–15 pairs of flies were used to establish the $F_1$ cultures.

**Male recombination tests:** Male recombination was measured under dysgenic, nondysgenic and control conditions. In order to establish the dysgenic state, a cross of the A type was made (see above), in which P-transformed males were mated to virgin females from the multiply marked *f; pm net; st e* stock. For the nondysgenic condition, a cross of the B type was made, in which *f; pm net; st e* males were mated to P-transformed females. For both types of crosses, two separate experimental series were established: transformants from DsPπ-5C were used in one, transformants from DsPπ-5C-27 in the other. As a control, nontransformed males from the parental 8DS stock were crossed to *f; pm net; st e* females. In all cases, crosses were made by mass mating 15–20 pairs in half-pint bottles at 23°. The resulting $F_1$ males were mated singly in vials to approximately seven *f; pm net; st e* females, and 3–4 broods were established by turning parents onto new food every 2–3 days. All of the progeny from each male were screend for exceptional (recombinant) phenotypes.

Two different, but related, estimates of male recombination were computed: the *mean frequency of male recombination,* and the *mean minimum frequency of independent events* (KIDWELL and KIDWELL 1976). The former expresses the total number of recombinants observed as a percentage of the total progeny count. The latter adjusts for clusters of recombinants and is an estimate of the minimum number of recombinant events, also expressed as a percentage of the total progeny count. For this calculation, only one event is scored when more than one recombinant chromosome within a given interval is recovered.

## RESULTS

**Derivation and preliminary molecular analysis of *D. simulans P* element transformants:** *D. simulans P* element transformants were generated by microinjecting pπ25.1 plasmid DNA into the posterior cytoplasm of embryos from the *D. simulans* strain, 8DS. Eleven independent transformed lines were initially established; three generations later, three single-pair

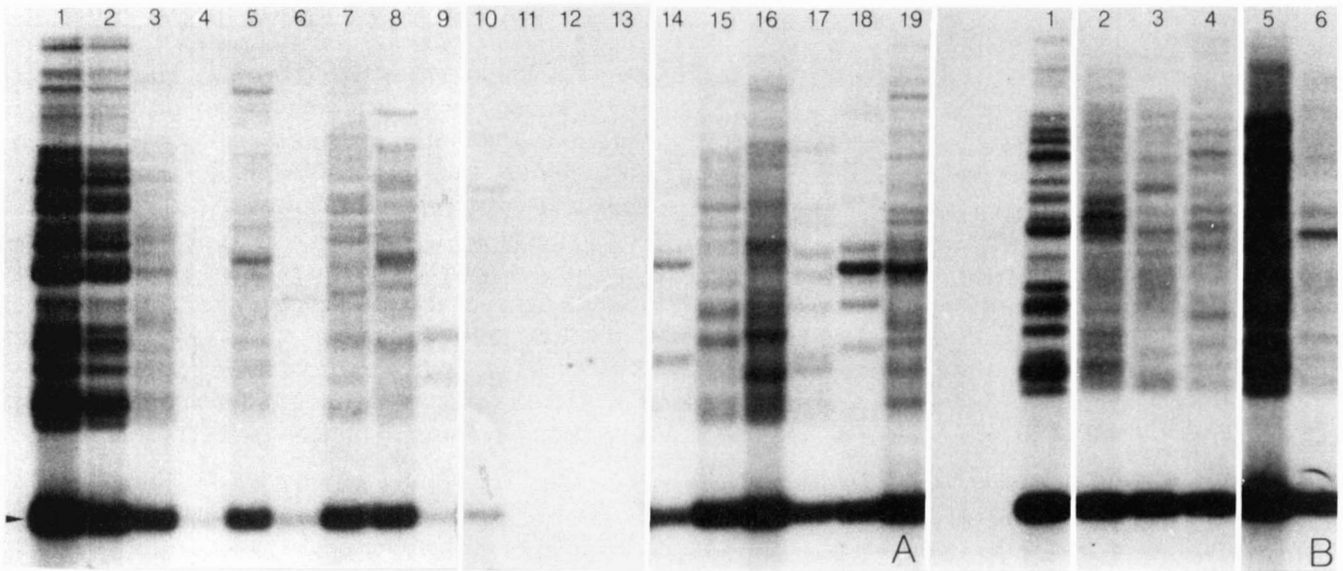


FIGURE 1.—Southern blots of *D. simulans* transformed lines at two points in time. Genomic DNA was digested with *Pvu*II and probed with the *Xho*I/*Sal*I fragment from pπ25.1. The arrowhead indicates the 0.9-kb internally derived *P* element fragment. A, Analysis of the 11 original transformed lines and six isopair sublines at month 12. Lanes 1 and 2, dilutions of DNA from a *D. melanogaster* P strain (Harwich-77) with, respectively, ½ and ¼ of the amount shown in the other lanes; lanes 3–13, the original tranformed lines in the order DsPπ-5, -9, -14, -22, -25, -42, -46, -1, -3, -4 and -15; lanes 14–19, single pair sublines derived from two of the original lines in the order DsPπ-5A, -5B, -5C, -25A, -25B and -25C. B, Reexamination, at month 16, of the five P-transformed lines selected for analysis of their P-M phenotypes. Samples are as follows: (1) *D. melanogaster* P strain, Harwich-77 (¼ the amount of DNA); (2) DsPπ-5; (3) DsPπ-25; (4) DsPπ-42; (5) DsPπ-5C; and (6) DsPπ-25C.

subcultures were made from each of the two lines with the highest *P* element copy number (DsPπ-5 and DsPπ-25). The molecular analysis of the original lines and single pair sublines during the first 8 months following transformation has been described by DANIELS, STRAUSBAUGH and ARMSTRONG (1985).

Four months after the completion of the initial molecular study, Southern blot analyses of the *D. simulans P* transformants were conducted in parallel with the initiation of a systematic phenotypic analysis that was undertaken to determine whether or not any of these lines manifested the properties characteristic of P-M hybrid dysgenesis in *D. melanogaster*. Figure 1A shows the *P* element profiles of the original transformed lines and single pair sublines 12 months following their transformation, compared with that of the *D. melanogaster* P strain, Harwich-77. Mass-extracted genomic DNA samples were digested with *Pvu*II and probed with the *Xho*I/*Sal*I P fragment from pπ25.1. With this combination, each complete *P* element yields a 0.9-kb internally derived band, as well as a unique band corresponding to the 3′ portion of the element and a piece of the flanking genomic DNA. The unique band is an identifying feature of a particular element at a given location.

Of the original eleven transformed lines, only four (DsPπ-, 5, -14, -25, and -42) showed appreciable numbers of *P* elements at month 12, based on the intensity of the 0.9-kb band. Four others (DsPπ-1, -9, -22 and -46) showed only weak indications of *P* elements within their populations, and three (DSPπ-3, -4 and -15) appeared to have completely lost all of their elements since the original molecular analysis done by DANIELS, STRAUSBAUGH and ARMSTRONG (1985). Among the single-pair sublines, *P* element copy number was also seen to vary. The line containing the highest number of *P* elements after 1 yr was DsPπ-5C. This line was subsequently selected for an extensive time course analysis, which is described later.

The five lines containing the highest number of *P* elements at month 12 (DsPπ-5, -25, -42, -5C and -25C) were reexamined at month 16 (Figure 1B). At this time DsPπ-5C continued to harbor the largest number of *P* elements. An extensive analysis of the P-M phenotypes of lines shown in Figure 1B was begun at month 26 and will be described in a later section.

**Intrastrain sterility due to *atrophie gonadique* in *D. simulans*:** The accurate identification of *P* element-related gonadal dysgenesis in *D. simulans* may be complicated by the temperature-dependent condition of intrastrain sterility, known as "atrophie gonadique" (*ag*), previously described in *D. simulans* by PÉRIQUET (1981). The *ag* condition is observed at low frequencies in many *D. simulans* strains and appears to be completely unrelated to the presence of *P* elements. As a precaution, therefore, all of the nontransformed *D. simulans* strains used in this study have been screened for the occurrence of *ag*, including 8DS, the *D. simulans* M strain from which the *P*-transformed lines were generated. Tests were conducted at 29°,

**TABLE 1**

**Percent gonadal sterility in the $F_1$ female progeny of crosses between DsP$\pi$ 5C-27 males and females from different nontransformed (M) *D. simulans* strains**

| M strain | Cross | | | |
|---|---|---|---|---|
| | Intrastrain (*ag*)[a] | Control[b] | A | B |
| 025.2 | 0 (±0)[c] | 1 (±1) | 94 (±2) | 2 (±1) |
| 025.3 | 0 (±0) | 4 (±2) | 84 | 6 |
| 025.5 | 7 (±1) | 0 (±0) | 78 (±8) | 0 |
| 025.8 | 4 (±1) | 1 (±1) | 54 (±4) | 0 |
| DsTuc 2 | 0 (±0) | 0 | 54 (±9) | 5 |
| DsTuc 4 | 1 (±1) | 0 | 60 (±10) | 6 |
| DsTuc 6 | 1 (±1) | 0 (±0) | 60 (±8) | 2 |
| DsTuc 8 | 0 (±0) | 0 | 66 (±5) | 2 |
| 8DS | 1 (±1) | | 77 (±9) | 4 (±1) |

[a] *atrophie gonadique*.
[b] M♀♀ × 8DS♂♂.
[c] Numbers in parentheses represent standard errors, which were computed when two or more tests were performed.

the temperature at which our GD sterility tests were routinely performed (see MATERIALS AND METHODS). Only trivial levels of *ag* sterility, ranging from 0 to 7%, were observed (see Table 1, column 2), thus effectively eliminating *ag* from consideration in the analysis of the GD sterility results.

**The development of P strain properties in a transformed line of *D. simulans*:** Following the Southern blot analysis at month 12 (Figure 1A), we chose the line containing the highest number of *P* elements, DsP$\pi$-5C, for an extended phenotypic analysis, which was carried out between months 14 and 30. The purpose of this analysis was to monitor the P-M properties of a transformed line during the critical period when *P* elements were potentially increasing within the population.

P activity potential and *P* element regulation in the DsP$\pi$-5C subline were measured at various points during the 16 month test period (see MATERIALS AND METHODS for details). To assess P activity potential, levels of GD sterility were measured in A-type crosses between DsP$\pi$-5C males and females from two nontransformed lines, 8DS and 0251.2. The control cross was 0251.2 ♀ ×8DS ♂. The results are shown in Figure 2A. At the beginning of the test period (month 14), GD sterility frequencies ranged from 12 to 27%; by its conclusion (month 30), frequencies approached 75%, with the largest increase occurring between months 14 and 18. The control frequencies remained at less than 10% during the entire test period. The time of rapid increase in P activity potential coincides with the period in which a sharp increase in the number of *P* elements within the DsP$\pi$-5C subline was observed (compare lane 16 in Figure 1A with lane 5 in Figure 1B). It is also apparent from the results in Figure 2A that higher frequencies of GD sterility were obtained using 0251.2 as the M strain than with 8DS.

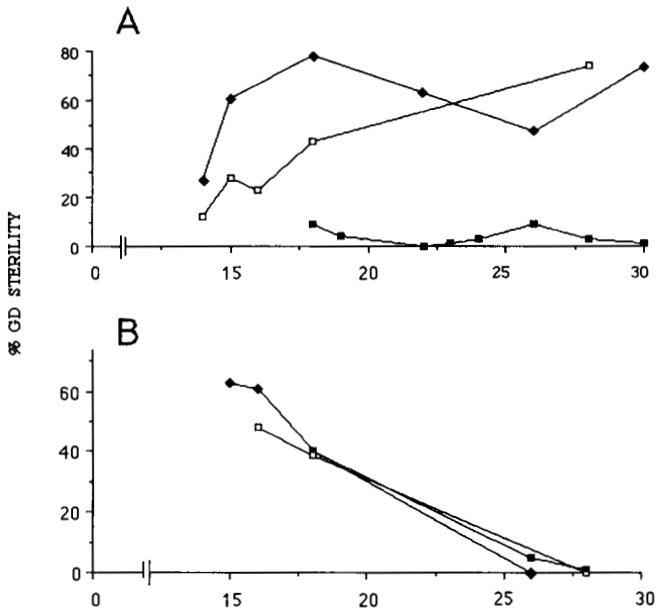


FIGURE 2.—Frequencies of GD sterility in the $F_1$ progeny of various DsP$\pi$-5C crosses measured over a 16-month period. A, DsP$\pi$-5C ♂♂ × ♀♀ from two nontransformed strains, 8DS (□——□) and 0251.2 (♦——♦). These crosses measure the potential for P activity and are referred to as crosses of the A type in *D. melanogaster*. The control cross was 0251.2 ♀♀ × 8DS ♂♂ (■——■). B, DsP$\pi$-5C ♀♀ × ♂♂ from 8DS (□——□) and 0251.2 (♦——♦). These are referred to as crosses of the B type in *D. melanogaster* and are the reciprocals of the A crosses. The intrastrain mating was between DsP$\pi$-5C ♀♀ and ♂♂ (□——□). Both the B crosses and the intrastrain mating provide a measure of *P* element regulation. All crosses were performed at 29° (see MATERIALS AND METHODS for details).

The influence of the choice of M strain on the expression of gonadal dysgenesis will be addressed later.

To assess *P* element regulation, we followed the rationale described in MATERIALS AND METHODS. Levels of GD sterility were measured in DsP$\pi$-5C intrastrain matings and in crosses of the B type between DsP$\pi$-5C females and males from the 8DS and 0251.2 strains at several points during the test period. The results are shown in Figure 2B. In all three sets of tests, it is seen that GD sterility frequencies declined rapidly from levels between 50 and 65% at month 14 to near zero after month 25. This is interpreted as a rapid increase in the capacity for *P* element regulation during this period. The overall results of the time course analysis suggest that the DsP$\pi$-5C subline has developed the major features characteristic of a *D. melanogaster* P strain, *i.e.*, P activity potential and the ability to regulate *P* element expression.

**Establishment and testing of DsP$\pi$-5C sublines:** At month 16, 34 single pair subcultures were established from DsP$\pi$-5C in order to assess whether or not members of its population were uniform with respect to their molecular and phenotypic properties. A Southern blot analysis of all 34 sublines was performed during the second generation following their

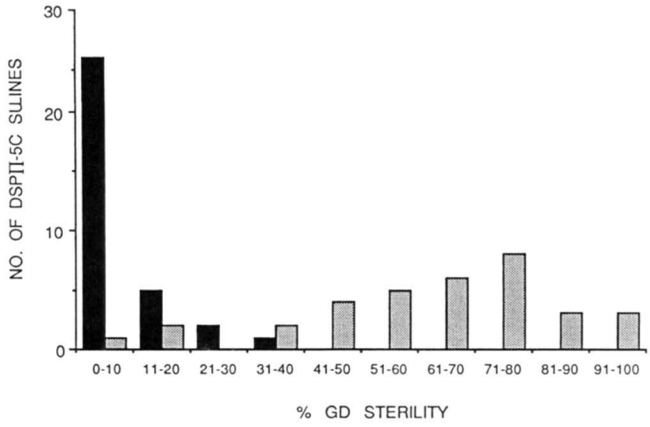


FIGURE 3.—Histogram depicting the distribution of GD sterility frequencies in the female progeny of cross A (stippled bars) and intrastrain (solid bars) tests of the 34 DsP$\pi$-5C sublines. Cross A matings were between 0251.2 ♀♀ and ♂♂ from the DsP$\pi$-5C sublines.

establishment using genomic DNA, extracted from 10 flies (5 males and 5 females per subline). Although all of the sublines contained roughly similar numbers of *P* elements, their hybridization patterns were highly variable, indicating considerable *P* element heterogeneity in the parental DsP$\pi$-5C population (data not shown).

The phenotypic analysis of the 34 sublines was begun 6 months after their establishment (at month 22 following transformation). P activity potential was measured by calculating the frequency of GD sterility in the progeny of cross A matings between transformed males and 0251.2 females. Assessments of *P* element regulation were made by calculating GD sterility frequencies in the progeny of intrastrain matings. There results are presented in Figure 3. Although a high degree of variability is seen in the cross A sterility frequencies, which ranged from 9 to 98%, the modal frequency is consistent with the level of cross A sterility observed in the DsP$\pi$-5C parental line at month 22 (Figure 2A). The range in intrastrain sterility frequencies is considerably smaller than that of the cross A frequencies, suggesting that all of the DsP$\pi$-5C sublines had developed a high level of *P* element regulation. Thus, it is apparent that, at the time of the analysis, members of the DsP$\pi$-5C population were heterogeneous with respect to their *P* element profiles and P activity potential, but homogeneous with respect to their regulatory capabilities.

**The selection and molecular analysis of a strong *D. simulans* P strain:** Since some of the DsP$\pi$-5C sublines appeared to have the phenotypic properties characteristic of strong P strains of *D. melanogaster*, such as Harwich, it seemed possible that one or a few of these sublines could be employed as *D. simulans* reference strains in crosses of the A* type. In *D. melanogaster*, cross A* is used as the most direct measure of *P* element regultion (*i.e.* P or M cytotype) and requires a strong P reference strain (see MATERIALS AND METHODS). Accordingly, the DsP$\pi$-5C subline with the highest demonstrated P activity potential in the previous analysis, DsP$\pi$-5C-27, was selected for testing as a *D. simulans* reference P strain.

Prior to the phenotypic tests, further investigation of the *P* element properties of the DsP$\pi$-5C-27 line was undertaken. The salient features are as follows (data not shown):

1. Individuals from the DsP$\pi$-5C-27 line carry as many as 30 *P* elements in their genomes. This estimate was obtained by *in situ* hybridization analysis of salivary gland polytene chromosomes, using BWC DNA as the probe. The sites of hybridization were distributed along all the major chromosome arms.

2. The DsP$\pi$-5C-27 line has nearly as many potentially complete *P* elements as the *D. melanogaster P* strain, Harwich-77. This estimate was obtained from an analysis similar to the one described by DANIELS *et al.*(1987b). Briefly, samples containing equal amounts of DNA were digested with *Acc*I, which produces a large (2.4 kb) internal fragment for each complete *P* element. A dilution series was then made for each of the digested samples. The hybridization signals of the 2.4-kb *Acc*I fragment in the DsP$\pi$-5C-27 series were nearly identical in intensity to those of the Harwich-77 series. It has been previously shown that Harwich-77 has about 20–25 potentially complete elements per haploid chromosome complement (DANIELS *et al.* 1987b).

3. DsP$\pi$-5C-27 contains defective *P* elements. It was previously shown that defective elements arose in at least one of the original *D. simulans* lines during the first 12 generations following transformation (DANIELS, STRAUSBAUGH and ARMSTRONG 1985). In similar experiments, we have determined that defective elements are also present in the DsP$\pi$-5C-27 line.

**Phenotypic analysis of *D. simulans P* element transformants:** An extensive phenotypic analysis of 13 *P*-transformed lines and derived sublines was begun at month 26 and completed at month 31. The following were chosen for testing: three of the original transformed lines, DsP$\pi$-5, -25 and -42; two of the derived sublines, DsP$\pi$-5C and -25C; and eight of the DsP$\pi$-5C sublines. Tests designed to measure P activity and its regulation were initiated by making the appropriate reference crosses and scoring GD sterility in $F_1$ females. In crosses A and B, three different nontransformed strains, 0251.2, 8DS and DsTuc2, were used as M parents; DsP$\pi$-5C-27 was employed as the *D. simulans* reference P strain in crosses A* and B*. The nontransformed 8DS parental strain was included in the analysis as a control. The results of the phenotypic tests are presented in Table 2. The points to be made from the data are as follows:

1. In cross A, all of the transformants exhibited

**TABLE 2**

**Percent $F_1$ gonadal sterility in various crosses using *D. simulans P*-transformed lines[a]**

| Strain (U) | A M♀ × U♂ 0251.2 | A M♀ × U♂ 8DS | A M♀ × U♂ DsTuc2 | B U♀ × M♂ 0251.2 | B U♀ × M♂ 8DS | B U♀ × M♂ DsTuc2 | A*[b] U♀ × P♂ | B*[b] P♀ × U♂ | Intrastrain U♀ × U♂ |
|---|---|---|---|---|---|---|---|---|---|
| DsPπ-5 | 56 (±31)[c] | 49 (±16) | 36 | 12 (±2) | 7 | ND[d] | 22 (±7) | 4 (±1) | 28 |
| DsPπ-25 | 57 | 84 | 59 | 70 | 34 | 44 | 61 (±8) | 4 (±1) | 64 (±12) |
| DsPπ-42 | 77 | 80 | 63 | 82 | 19 (±1) | 19 | 38 (±13) | 4 | 17 |
| DsPπ-5C | 64 (±1) | 74 | 58 | 0 | 0 | 0 | 4 (±4) | 3 (±1) | 3 (±2) |
| DsPπ-25C | 33 | 17 (±2) | 19 (±8) | 21 | 7 (±1) | 15 | 4 (±2) | 4 (±1) | 1 (±1) |
| *DsPπ-5C sublines* | | | | | | | | | |
| −4 | 71 (±13) | 25 | 20 | 0 | 3 | 4 | 0 | 6 | 3 (±1) |
| −8 | 77 (±10) | 50 | 62 | 0 (±0) | 0 | 0 | 1 (±1) | 0 (±0) | 4 (±2) |
| −16 | 53 (±8) | 34 | 23 | 0 | 3 | 2 | 0 | 12 (±1) | 1 (±1) |
| −17 | 72 (±7) | 73 | 47 | 1 (±1) | 4 | 3 | 2 (±2) | 0 (±0) | 3 (±2) |
| −18 | 82 | 37 | 34 | 8 | 1 | 4 | 8 | 3 | 7 (±5) |
| −27 | 94 (±2) | 77 (±9) | 54 (±9) | 2 (±1) | 4 (±1) | 5 | | | 3 (±1) |
| −29 | 71 | 43 | 28 | 1 | 5 | ND | 0 | 0 | 27 (±8) |
| −32 | 26 (±7) | 20 | 12 | 2 (±1) | 5 | 8 | 6 (±3) | 0 | 2 (±2) |
| *Control* | | | | | | | | | |
| 8DS | 2 | | 0 | 0 | | ND | 4 | 5 | 1 |

[a] Tests were conducted between months 26 and 31.
[b] DsPπ-5C-27 was used as the reference P strain.
[c] Numbers in parentheses represent standard errors, which were computed when two or more tests were performed.
[d] ND = not determined.

significant levels of P activity relative to the 8DS control, but the amount of sterility varied considerably among lines (ranging from 12 to 94%). This phenotypic variability presumably reflects variability at the molecular level in numbers of autonomous and nonautonomous *P* elements and their genomic locations, but data were not available to allow such detailed correlations to be made. In addition, the choice of M strain appeared to have some effect on sterility frequencies (see below).

2. The cross A* results indicate that only the original (unselected) transformed lines, DsPπ-5, -25 and -42, continued to show susceptibility to P activity; all the derived sublines appeared to have achieved complete or nearly complete regulation, although the DsPπ-25C cross B results and the DsPπ-5C-29 intrastrain results suggest that regulation was perhaps less well established in these lines than in the others.

3. As mentioned earlier, cross B* served as a control for factors other than P that might produce a type of sterility similar to that of P-M dysgenesis. The low sterility frequencies observed in cross B* progeny are consistent with the conclusion that the sterility observed in the test crosses was, in fact, caused by the expression of *P* elements.

4. The DsPπ-5C-27 line appeared to function successfully as a *D. simulans* reference P strain, since the cross B and intrastrain results tended to corroborate those of the cross A*.

Following the phenotypic analysis, further tests were initiated to examine the influence of different M strains on the GD sterility potential of DsPπ-5C-27. Accordingly, males from this line were crossed to females from nine nontransformed *D. simulans* M lines, all of which had been previously tested for the *ag* trait. As a control, a parallel set of crosses was made using males from the nontransformed 8DS parental strain. The regulatory capacity of DsPπ-5C-27 was tested with each of the nine M strains by making a cross of the B type. The results are presented in Table 1 (columns 3 and 4). Although all of the cross A tests showed substantial levels of GD sterility, there was nevertheless a wide range in the P activity potential of DsPπ-5C-27 among the different M strains. Similar results were obtained with a *D. melanogaster* P strain in crosses with an array of true M strains (DANIELS *et al.* 1987a). Only trivial levels of sterility were observed in the female progeny of the cross B and control matings.

Figure 4, A–C provides a comparison of pairs of normal *D. simulans* ovaries with typical samples of unilateral and bilateral ones from $F_1$ females produced by a *D. simulans* dysgenic cross. It is seen that dysgenic ovaries in *D. simulans* are morphologically indistinguishable from those of *D. melanogaster* (see Figure 1 in SCHAEFER, KIDWELL and FAUSTO-STERLING 1979 and Figure 2 in ENGELS and PRESTON 1979), although there is one additional feature that warrants mention. At 29°, the developmental temperature at which GD sterility tests are routinely conducted, the ovaries of $F_1$ females from dysgenic crosses between *D. melanogaster* strains are usually easily scored as either normal or dysgenic. However, the ovaries of $F_1$ females from dysgenic crosses between *D. simulans* strains often

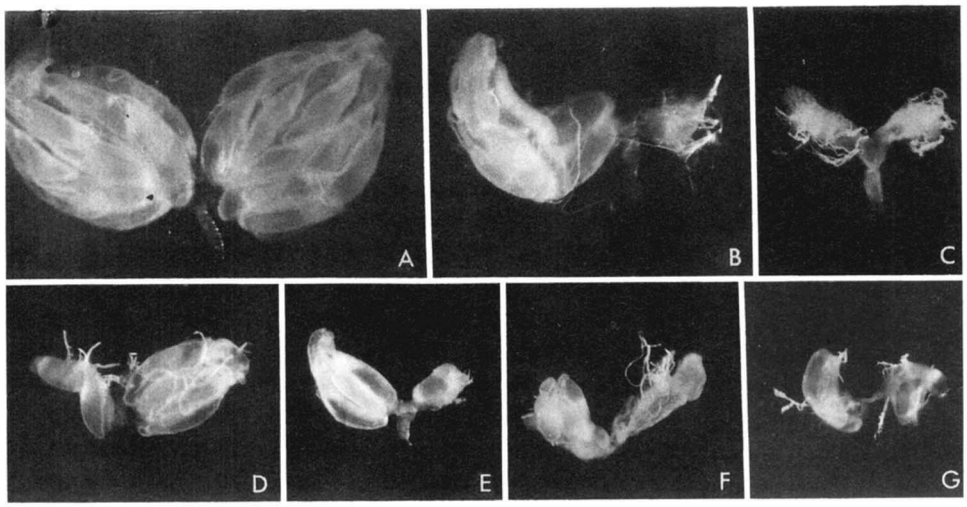


FIGURE 4.—Photomicrographs of $F_1$ ovaries from dysgenic *D. simulans* crosses (× 60). A, Normal ovaries. B, Unilaterally dysgenic ovaries. C, Bilaterally dysgenic ovaries. D–G, Ovaries showing intermediate phenotypes. D and E, The ovary on the left in (D) and on the right in (E) contains only a single developing ovariole. F and G, Ovaries with abnormal morphology and egg development.

show a continuum of phenotypes between the two extremes. Intermediate phenotypes include ovaries with only one or a few developed ovarioles and ovaries with obviously abnormal morphology and egg development (Figure 4, D–G). Such intermediate phenotypes are occasionally seen in *D. melanogaster*, but their occurrence is usually restricted to crosses performed at temperatures below 25°.

**The effect of developmental temperature on gonadal dysgenesis in *D. simulans*:** A comparative analysis of the effect of developmental temperature on gonadal sterility in *D. simulans* and *D. melanogaster* was carried out using the *D. simulans* P strain, DsPπ-5C-27, and two *D. melanogaster* P strains, Harwich-77 and Agana. Males from Harwich-77 and Agana were crossed to females from two different true M strains, Canton S and $ry^{506}$, males from DsPπ-5C-27 were crossed with females from three different nontransformed strains, 0251.2, DsTuc2 and 8DS. All crosses were made at four different temperatures, 23°, 25°, 27° and 29°, and the frequency of GD sterility in $F_1$ females calculated. The results clearly indicate that the level of GD sterility in the *D. simulans* dysgenic crosses increases with ascending temperature (Figure 5), indicating that P-induced gonadal sterility in *D. simulans* is a temperature-dependent phenomenon as it is in *D. melanogaster*.

**Dysgenesis-induced male recombination in *D. simulans*:** Male recombination was measured under dysgenic, nondysgenic and control conditions as described in MATERIALS AND METHODS. The results are presented in Table 3. $F_1$ males from both the A and B crosses between DsPπ-5C and DsPπ-5C-27 and the *f*; *pm net*; *st e* marker stock exhibited recombination frequencies that were significantly higher than those from the control matings. However, male recombination in the A crosses was two to three times higher than in the B crosses. The levels of male recombination in these *D. simulans* crosses are very similar to those that were observed in similar reciprocal crosses in *D. melanogaster* (KIDWELL and KIDWELL 1976). The discrepancies between the frequencies of total recombinants and the minimum frequencies of recombinant events imply that crossing over is occurring in mitotic divisions of the germ cells prior to meiosis. Similar observations have been made in *D. melanogaster* (*e.g.*, HIRAIZUMI *et al.* 1973; KIDWELL and KIDWELL 1976).

## DISCUSSION

At the outset of this study, the formal possibility existed that some, or all, of the phenotypic manifestations of hybrid dysgenesis might be mediated by host functions that are present in *D. melanogaster*, but absent in *D. simulans*. However, the results of the extended analysis of DsPπ-5C demonstrate that the potential for the expression of the major phenotypic features of P-M hybrid dysgenesis has developed in at least one transformed line of *D. simulans* and its derivatives. This represents a clearly documented instance of P-M hybrid dysgenesis in a species other than *D. melanogaster*, albeit in an artificially treated and manipulated system. Moreover, *P* elements appear to behave in *D. simulans* essentially as they do in *D. melanogaster*, although some relatively minor differences in the degree of expression may exist between the two sibling species.

Some preliminary information about the phenotypic manifestations of *P* elements in species other than *D. melanogaster* has been reported earlier, but it has been sketchy and not always consistent. In the initial phenotypic analysis of the *D. simulans P* transformants described herein, DANIELS, STRAUSBAUGH and ARMSTRONG (1985) detected up to 10% gonadal dysgenesis in outcrosses between *P*-transformed males and nontransformed females during the first 12 generations following transformation. Low levels of intrastrain GD sterility were also occasionally observed. In contrast, *D. simulans* lines that were simultaneously

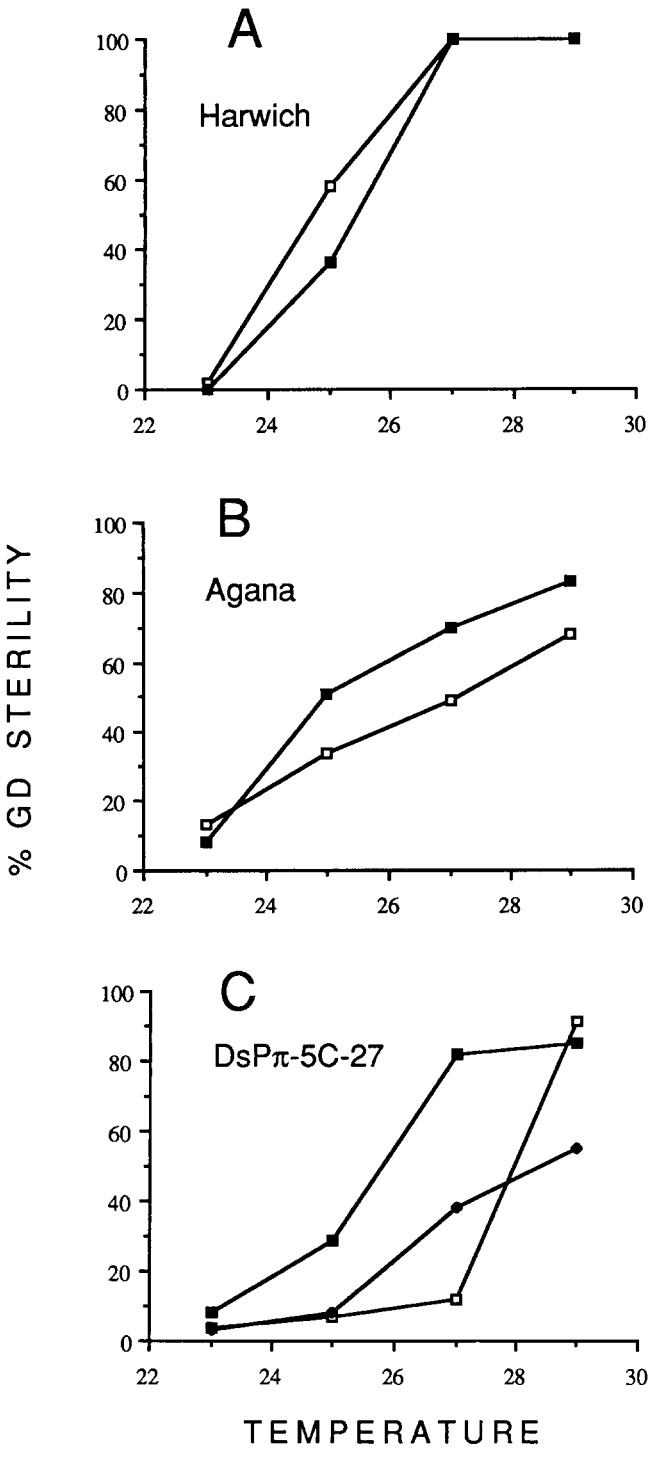


FIGURE 5.—A comparison of the effect of $F_1$ developmental temperature on the expression of GD sterility in *D. melanogaster* and *D. simulans* dysgenic crosses. A, Males from the *D. melanogaster* P strain, Harwich, crossed to females from two different M strains, Canton-S (□——□) and $ry^{506}$ (■——■). B, Males from the *D. melanogaster* P strain, Agana, crossed to females from Canton-S (□——□) and $ry^{506}$ (■——■). C, Males from the *D. simulans* strain, DsPπ-5C-27, crossed to females from three different nontransformed strains, 0251.2 (□——□), DsTuc2 (◆——◆) and 8DS (■——■).

co-transformed with a $ry^+$ transposon and an autonomous *P* element (SCAVARDA and HARTL 1984; 1987) provided no indications of gonadal dysgenesis over the course of 40 generations. There were some indications of another type of temperature-dependent sterility in these transformed lines, but there was no clear evidence that this was related to transposition of *P* elements. The absence of *P* element-related sterility was not too surprising since the estimated number of *P* elements in these lines remained very low. On the other hand, BRENNAN, ROWAN and DICKINSON (1984) reported female sterility in their *P*-transformed lines of *D. hawaiiensis* that carried the highest numbers of *P* elements. This sterility was described as being phenotypically similar to gonadal dysgenesis in *D. melanogaster*, but no further details were provided.

**TABLE 3**

**Analysis of male recombination in DsPπ-5C and its derivative subline DsPπ-5C-27**

| Strain | Cross | No. $F_1$♂ | Percent male recombination in chromosome 2 | Percent male recombination in chromosome 3 | Total progeny scored |
|---|---|---|---|---|---|
| DsPπ-5C | A | 34 | 0.96 (0.60)[a] | 0.16 (0.11) | 3,648 |
| | B | 32 | 0.26 (0.26) | 0.09 (0.09) | 3,465 |
| DsPπ-5C-27 | A | 50 | 1.09 (0.36) | 0.17 (0.14) | 11,302 |
| | B | 47 | 0.35 (0.21) | 0.08 (0.07) | 8,264 |
| 8DS (control) | A | 46 | 0.06 (0.06) | 0.01 (0.01) | 7,158 |

[a] Figures in parentheses are minimum percent of recombination events.

The characteristic features of hybrid dysgenesis which have been demonstrated in the present study include the associated induction of gonadal sterility and male recombination and the demonstration of a strong reciprocal cross effect. Also, as in *D. melanogaster*, the emergence of P activity in *D. simulans* was later followed by the development of *P* element regulation and consequent stabilization of the system. This stabilization is observed as an absence of gonadal dysgenesis in A* and B crosses and in P × P and intrastrain matings. However, the observation of significant frequencies of male recombination in both A and B crosses suggests that *P* element regulation may be incompletely developed with respect to this trait. At the molecular level, a rapid increase of *P* element copy number accompanied the appearance of P activity. Thus, overall, the observed sequence of events resembles very closely that previously seen in the development of *P* genomic complements of transformed and contaminated lines of *D. melanogaster* (*e.g.*, DANIELS *et al.* 1987b; KIDWELL, KIMURA and BLACK 1988).

This study illustrates the difficulties inherent in any attempt to demonstrate the existence of hybrid dysgenesis when elements are newly introduced into a species. At the outset of such an endeavor, mature P strains (such as Harwich in *D. melanogaster*) are not available as reference strains to measure regulation of the element system. However, previous work with

newly evolving P lines of *D. melanogaster* strongly suggested that genomic *P* elements behave differently before and after regulation has become established (DANIELS *et al.* 1987b; KIDWELL, KIMURA and BLACK 1988). This knowledge enabled us to use other information from the *D. simulans* transformed lines to estimate the level of regulation until the stage when stabilized P strains were available for this purpose.

At several points during our analysis, we have compared the *D. simulans* transformants with P lines of *D. melanogaster* in an effort to determine whether the behavior of *P* elements differs in the two sibling species. Three sets of comparisons are of particular interest in this regard. First, both the highly selected *D. simulans* transformed line, DsP$\pi$-5C-27, and the strong *D. melanogaster* line, Harwich-77, have roughly similar numbers of complete *P* elements. Harwich-77 invariably produces 100% GD sterility in cross A at 29°, irrespective of the M reference strain used. However, DsP$\pi$-5C-27 frequently does not produce complete cross A $F_1$ sterility, and there is a wide range in sterility frequencies, depending on the M strain used in the cross (Table 1). Second, in dysgenic $F_1$ *D. melanogaster* females, only rarely are ovaries seen that are intermediate in size and morphology between the dysgenic and normal structures; there is usually a clear distinction between the two types. In contrast, ovaries that were intermediate in morphology were frequently observed in dysgenic *D. simulans* females, even when they were raised at 29° (Figure 4). Third, in the developmental temperature experiments (Figure 5), a comparison between DsP$\pi$-5C-27 and two *D. melanogaster* P strains suggests that the expression of sterility in *D. simulans* tends to be more depressed at lower temperatures than it is, under similar conditions in *D. melanogaster*.

From these observations it can be concluded that any differences between the two species tend to be of a fairly minor, quantitative nature; they do not suggest any major qualitative dichotomy. It is possible that *D. simulans* may be somewhat more "resistant" to the disruptive effects of *P* element mobilization than is *D. melanogaster*. However, no firm, general conclusions can be made at present because of the very limited sample of *D. simulans* lines employed in this study and the fact that the lines which were subjected to the most intensive examination were highly selected.

The main evolutionary significance of the results of the present study is that host properties allowing the integration, transposition, excision and regulation of *P* elements and their phenotypic manifestations in P-M hybrid dysgenesis have been essentially conserved since the divergence of the two lineages that produced the present day sibling species of *D. melanogaster* and *D. simulans*. From the results of *P* element excision assays in a broad range of species, both from within and without the genus *Drosophila* (RIO *et al.* 1988; O'BROCHTA and HANDLER, 1988) it might have been expected that the molecular mechanisms of excision and transposition would not differ markedly between lineages as closely related as sibling species. However, almost no previous work has been reported which would guide our expectations with respect to the effect of genetic relatedness on host-mediated phenotypic manifestations of *P* element expression. In this regard it will be of considerable interest to find out the extent of phylogenetic conservation of host functions in the genus *Drosophila* which support the expression of hybrid dysgenesis.

If *D. melanogaster* has harbored *P* element sequences for less than 50 yr, as postulated by the recent invasion hypothesis (KIDWELL 1979; 1983), then both *D. melanogaster* and *P*-transformed lines of *D. simulans* can be considered as being similar with respect to the recent introduction of *P* elements into their genomes. Thus, both are expected to be naive in their recent evolutionary experience of *P* elements. It seems possible that hybrid dysgenesis expression in these species may show interesting differences in this regard compared with that in species such as *Drosophila willistoni*, which has apparently harbored active *P* elements for relatively long periods of time. This possibility is currently under active investigation.

We thank KIYOSHI KIMURA for technical assistance and ATANU DUTTA ROY for providing slides for the *in situ* analysis. We are also indebted to IAN BOUSSY, BILL ENGELS, KIYOSHI KIMURA and KEN PETERSON for valuable discussions and for reading an earlier version of the manuscript. This investigation was supported by U.S. Public Health Service grants GM-37241 and GM-09886 to A. C. and GM-25399 and GM-36715 to M. G. K.

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Communicating editor: W. M. GELBART