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HYBRID DYSGENESIS IN *DROSOPHILA MELANOGASTER*: FACTORS AFFECTING CHROMOSOMAL CONTAMINATION IN THE *P-M* SYSTEM

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Manuscript received September 30, 1982
Revised copy accepted January 28, 1983

ABSTRACT

The two interacting components of the *P-M* system of hybrid dysgenesis are chromosomally associated elements called *P* factors and a susceptible cytoplasmic state referred to as *M* cytotype. Previous experiments have indicated that *P* factors are a family of multiple-copy transposable genetic elements dispersed throughout the genome of *P* strains but absent in long-established *M* strains.——Evidence is presented that the sterility and male recombination-inducing potential of *P* elements may be acquired by X chromosomes, derived from *M* strains, through nonhomologous association with *P* strain autosomes, a process referred to as "chromosomal contamination." The frequencies of chromosomal contamination of X chromosomes by *P* strain autosomes were highly variable and depended on a number of factors. *M* cytotype (as opposed to *P* cytotype) was essential for high frequencies of *P* factor contamination. There were large differences in contamination potential among individual female families, and a weak negative correlation existed between family size and contamination frequency. Chromosomal contamination in the *P-M* system was shown to be independent of that in the *I-R* system.——Frequency distributions suggested that the relationship between sterility production and *P* factor insertion is complex. The majority of *P* element transpositions, identified by *in situ* hybridization in one X chromosome, were not associated with gonadal sterility. However, high sterility potential was found to be associated with the presence of at least one *P* element inserted into the X chromosome. This potential was lost at a rate of about one-sixth per generation in *M* cytotype but was stabilized in *P* cytotype. Various hypotheses concerning the relationship between transposition and chromosomal contamination are discussed.

HYBRID dysgenesis is a phenomenon resulting from cytoplasm-chromosome interactions between various types of strains. It is manifest in a number of aberrant traits, such as mutation, sterility and male recombination, resulting from genetic changes in the hybrid germ line. Two notable features of all of these traits are the marked inequality of their frequencies in the progeny of the two possible reciprocal crosses between pairs of interacting strains and, with a few exceptions, their absence in the progeny of intrastrain matings (for reviews see KIDWELL, KIDWELL and SVED 1977; SVED 1979; BREGLIANO *et al.* 1980; ENGELS 1981a; BREGLIANO and KIDWELL 1983; KIDWELL 1983a).

One system of hybrid dysgenesis, the *P-M* system, results from interactions between chromosomally linked factors called *P* factors and a particular type of cytoplasm referred to as the *M* type (KIDWELL, KIDWELL and SVED 1977). *P*

factors are members of a dispersed family of mobile genetic elements called *P* elements (RUBIN, KIDWELL and BINGHAM 1982; BINGHAM, KIDWELL and RUBIN 1982) which may be located on any or all of the major chromosomes. The cytoplasmic component of the *P-M* interaction may be considered as a susceptibility or lack of resistance to the action of chromosomally linked *P* factors, a characteristic referred to as "cytotype" by ENGELS (1979a). Individuals having *P* cytotype are seemingly resistant to the action of *P* factors, and those with *M* cytotype are susceptible. The *M* or *P* state of the cytoplasm can be considered as a self-replicating property of the cytoplasm over one or a few generations, but it is dependent for its long-term maintenance on chromosomally linked factors (ENGELS 1979a; KIDWELL 1981).

The characterization of the *P-M* system of hybrid dysgenesis was largely achieved using the dysgenic trait of gonadal (*GD*) sterility. In crosses between females of an *M* strain with males of a *P* strain, gonadal sterility may occur in both male and female hybrids and is usually dependent on high developmental temperatures. The gonads may fail to develop either unilaterally or bilaterally (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979; SCHAEFER, KIDWELL and FAUSTO-STERLING 1979; YANNOPOULOS 1978). A third type of strain, the *Q* type, has also been distinguished in the *P-M* system. *Q* strains appear to have most of the properties of *P* strains except for their potential to produce gonadal sterility (KIDWELL 1979; ENGELS and PRESTON 1981; SIMMONS *et al.* 1980).

Studies of the molecular basis of hybrid dysgenesis (RUBIN, KIDWELL and BINGHAM 1982; BINGHAM, KIDWELL and RUBIN 1982; RUBIN 1983) have shown that *P* elements belong to a distinct family of moderately repetitive DNA whose members are heterogeneous in size (0.5–3.0 kb) but which share substantial sequence homology. *In situ* hybridization experiments showed that *P* elements were present in 30–50 copies per genome of all *P* and *Q* strains studied but not in the majority of long-established *M* strains. *P* element insertion mutations at the white locus reverted to wild type at a high rate in *M* cytotype but not in *P* cytotype. The rate of transposition of *P* elements in males, from the autosomes of a *P* strain to an *M* strain X-chromosome, was estimated to be 0.8 elements per fly generation. Although transposition of *P* elements has thus been demonstrated, very little is known about the functional potential of these elements, after transposition, for the production of dysgenic traits such as gonadal sterility.

A second system of hybrid dysgenesis, the *I-R* system, has also been described in considerable detail (reviewed by BREGLIANO *et al.* 1980; BREGLIANO and KIDWELL 1983). In this system, *I* (inducer) strains are analogous to *P* strains and, likewise, *R* (reactive) strains to *M* strains. A third type, *N* (neutral) strains behave functionally as very weak *R* strains (BREGLIANO *et al.* 1980). Note that the type of sterility associated with the *I-R* system is quite different from the *GD* sterility associated with the *P-M* system. Although the *P-M* and *I-R* systems have many striking similarities, they also possess some important differences (KIDWELL 1979; BREGLIANO and KIDWELL 1983) which provide evidence for at least partial independence. In the *I-R* system, a phenomenon called "chromosomal contamination" has been observed and extensively studied (PICARD 1976; BREGLIANO and KIDWELL 1983). Under certain well-defined condi-

tions, chromosomes from *R* strains can acquire the inducer properties peculiar to *I* strain chromosomes. The necessary conditions include the presence in females of reactive cytoplasm and at least one chromosome bearing an *I* factor. The most compelling hypothesis is that chromosomal contamination, observed at the phenotypic level, is a result of transposition at the molecular level.

In the *P-M* system, although there have been fairly strong indications from studies of male recombination of a phenomenon analogous to *I-R* system chromosomal contamination (SLATKO 1978; MATTHEWS *et al.* 1978; YANNOPOULOS 1979; SVED and COLGAN 1982) and extensive observations consistent with the occurrence of insertion mutations (GREEN 1977; SIMMONS and LIM 1980; ENGELS 1981b; RUBIN, KIDWELL and BINGHAM 1982), evidence for chromosomal contamination resulting in gonadal sterility has not been available. Despite the advantages of gonadal sterility for the study of chromosomal contamination, due to the specificity of this trait to the *P-M* system and its ease of scoring, several preliminary experiments (ENGELS 1979a; W. R. ENGELS, unpublished results; M. G. KIDWELL, unpublished results) produced completely negative results. This suggested there might be some important differences between the *P-M* and *I-R* systems with respect to the detection of sterility after transposition.

The purpose of this study is to determine whether gonadal sterility potential can be acquired by *M* strain chromosomes under conditions favoring the mobility of *P* elements, to determine whether genetic and environmental factors influence the rate of chromosomal contamination and to examine the relationship between *P* element transposition and the induction of gonadal sterility.

MATERIALS AND METHODS

Strains employed: The designation of each strain in both the *I-R* and *P-M* systems, respectively, is given in parentheses, immediately after the name. For description of mutants and balancers, see LINDSLEY and GRELL (1968).

1. Cranston (*IP*): A strain descended from several flies caught in Cranston, Rhode Island, in 1964 by the author.

2. Harwich (*IP*): A strain descended from two females collected by DR. M. L. TRACEY, JR., at Harwich, Massachusetts, in 1967.

3. Oxford (*IP*): An isofemale line descended from a female collected by DR. ALICE CHABORA in Oxford, North Carolina, in 1966.

4. Canton-S (*IM*): A long-established, standard, laboratory strain.

5. H-41 (*RM*): *In(1) Basc* sc^{S1}*B In* w^{a} sc^{8}; *In (2)SMl,* al^{2} *Cy* sp^{2}/*In(2) Pm, dp b Pm* ds^{33K}; *C Sb/* $Ubx^{130}e^{s}$; spa^{pol}, a multiple chromosome balancer strain.

6. H-40 (*NM*): A strain identical with H-41, except that wild-type X chromosomes from the Canton-S stock were substituted for *Basc* X chromosomes.

7. B-7 (*IQ*): A strain synthesized by KIDWELL (1981). The X chromosomes were derived from the H-41 strain, and the autosomes were from the Mount Carmel (*IQ*) strain.

8. A-8 (*IQ*): A strain synthesized by KIDWELL (1981). The X chromosomes were derived from the wild-type Mount Carmel (*IQ*) strain and the autosomes from the H-41 strain.

9. *al b sp; ve st ca (IM):* A laboratory stock carrying the following markers: aristaless, *al*(2-0.01); black, *b*(2-48.5); speck-2, sp^{2}(2-107.0); veinlet, *ve*(3-0.2); scarlet, *st*(3-44.0); claret, *ca*(3-100.7). Note that the terminal markers bound most of the active genetic material of both major autosomes. Thus, male recombination frequencies for most of the total genome may be estimated.

10. C(1)DX, *y f/v f* Bx^{r49k}*car (IM):* An attached-X stock obtained from the Bowling Green Stock Center.

11. *Cy/Pm; DcxF/Sb (IM)*: A strain synthesized by BUCHETON and BREGLIANO (1982) and referred to by them as "B". The wild-type X chromosome is noninducer (i^0). The autosomes are inducer and have the genotype *In(2L+2R] Cy/Pm; In(3LR) DcxF/Sb*. Obtained from the Université de Clermont-Ferrand II in November 1980.

12. *Basc (NM)*: An X chromosome balancer stock: $In(1)sc^{S1L}sc^{8R}$+ S, sc^{S1} sc^8 w^a *B*.

13. y^2 *ec cv v f car (IM)*: A multiple-marked X chromosome stock; yellow, *y* (0.0); echinus, *ec* (5.5); crossveinless, *cv* (13.7); vermillion, *v* (33.0); forked, *f* (56.7); carnation, *car* (62.5).

Estimation of the frequency of GD *sterility*: The presence of unilateral and bilateral dysgenic gonads is an indicator of gonadal sterility in the *P-M* system (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979). In the present investigation, the frequency of dysgenic ovaries was measured directly by dissection according to the criteria of SCHAEFER, KIDWELL and FAUSTO-STERLING (1979). Direct dissection has two advantages over other methods of measuring gonadal sterility such as absence of egg production; it identifies this type of sterility specifically, and it allows the observation of unilateral dysgenesis, a condition not necessarily resulting in complete sterility of the individual concerned.

RESULTS

Experiment I: The first experiment was designed to determine whether chromosomal contamination, as defined by PICARD (1976) for the *I-R* system, could be detected in the *P-M* system by observation of gonadal sterility frequencies. It was also designed to determine whether differences among major chromosomes existed in both the efficiency of *P* autosomes as donors in the contamination process and in the efficiency of *M* chromosomes as recipients in the contamination process.

The mating scheme is shown in Figure 1. All matings were made *en masse* with 20 parents of each sex per bottle. In g_1 males, the *M* strain-derived X chromosome was nonhomologously associated with *P* strain autosomes. In the second generation, females were selected, on the basis of genotype, to represent all four possible combinations of potential donor chromosomes from the *P* strain: (a) *2* and *3*, (b) *2*, (c) *3* and (d) none. These *P* autosomes were removed by selection in g_3. Eight different combinations of potentially contaminated *M* chromosomes were represented by appropriate genotypes for each of the four donor classes, a through d, in g_4 females.

The g_4 gonadal sterility frequencies are presented in Table 1. There was considerable variation in frequencies among subclasses, but several trends were apparent. Clear evidence for chromosomal contamination in g_2 females was provided by substantial sterility frequencies when two donor chromosomes were present (line a of Figure 1) compared to the consistently low values in the absence of donor chromosomes (line d). In the presence of one donor chromosome (lines b and c), intermediate sterility frequencies were usually observed, but, on average, chromosome *2* appeared to be a more efficient contamination donor than chromosome *3*. An indication of the relative efficiencies of different chromosomes as contamination recipients may be obtained by comparing different columns in Table 1. One rather consistent trend was for sterility frequencies to be higher when the X chromosome had had the opportunity for contamination in both g_1 males and in g_2 females (columns 2, 4, 6 and 8) than when X chromosome contamination was possible only in g_2 females (columns 1, 3, 5 and 7). However, sterility frequencies were zero or negligible in those

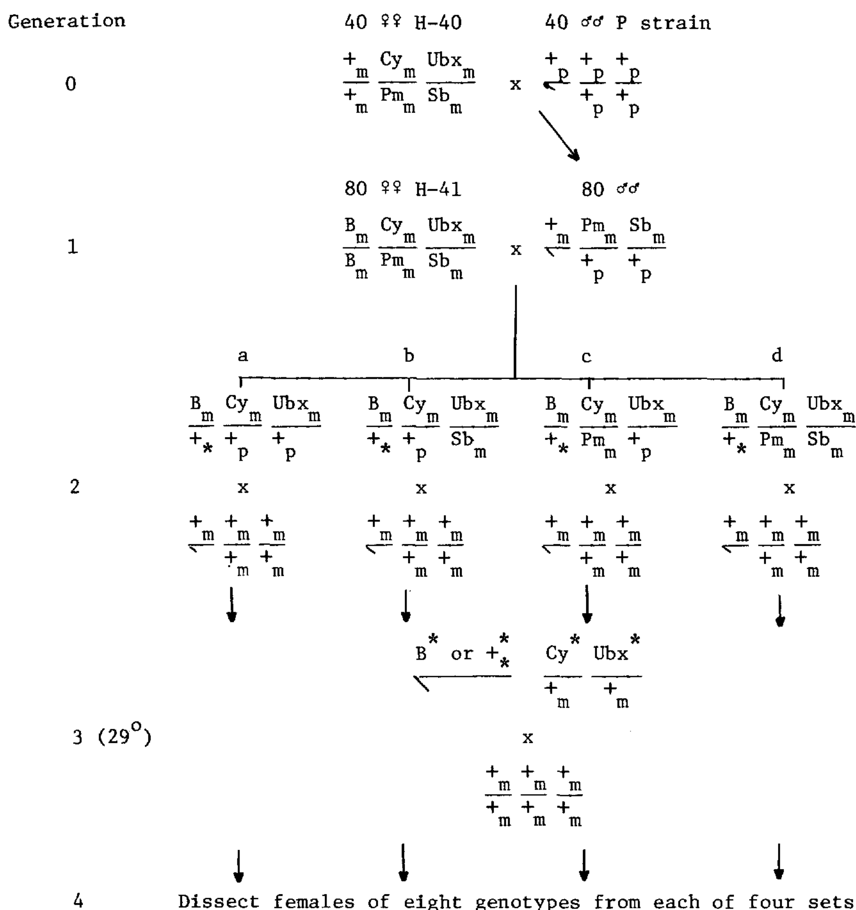

FIGURE 1.—Mating scheme for experiments I and II and partial mating scheme for experiments II and IV. The subscripts m and p indicate chromosomes of *M* and *P* strain origin, respectively. Asterisk subscripts and superscripts denote chromosomes that had the possibility of chromosomal contamination in g_1 males and g_2 females, respectively. The *B* (*Basc*), *Cy* and *Ubx* balancer chromosomes carried dominant markers and multiple inversions which allowed their transmission and selection as intact units. In g_2, 40 females of each of the four genotypes, a through d, were mass mated with 40 Canton-S males. In g_3, 40 males of each of the eight subclasses were mass mated with 40 Canton-S females.

subgroups in which only X-chromosome contamination in g_1 males was possible (line d, columns 2, 4, 6 and 8). This suggests that contamination in males alone may be considerably less efficient than that in females and that the effects of contamination in two successive generations may be greater than the sum of the effects of exposure to contamination in each generation separately.

A second interesting trend is apparent when certain columns of Table 1 are compared in pairwise combinations. For all those comparisons in which chromosomal contamination was possible in g_1 males and g_2 females (columns 2 and 4, 2 and 6, 2 and 8, 4 and 8, 6 and 8), the presence of recipient autosomes consistently produced only sterility that was explainable by the presence of the

TABLE 1

Frequencies of chromosomal contamination in experiment 1 as measured by the percentage of dysgenic ovaries, using Cranston as the donor strain

	1	2	3	4	5	6	7	8
	Potential recipient chromosomes in $g_2^{♀♀}$							
P donor chromosomes	X	X_*	X 2	X_*2	X 3	X_*3	X 2 3	X_*2 3
a. 2 and 3	0	21	2	20	8	24	15	19
b. 2	1	20	3	18	1	14	5	11
c. 3	3	6	3	4	3	1	0	5
d. None	0	0	1	0	1	0	0	1

Approximately 50 g_4 females were dissected for each of the 32 subclasses. See Figure 1 for mating scheme.

Asterisk subscripts denote the possibility of contamination by autosomes derived directly from the *P* strain in g_1 males. For all recipient chromosomes shown, contamination was possible in g_2 females in the presence of *P* donor chromosomes.

recipient X chromosome alone. In the remaining combinations, in which contamination was not possible in g_1 males, but only in g_2 females (columns 1 and 3, 1 and 5, 1 and 7, 3 and 7, 5 and 7), there was some evidence that recipient autosomes produced some sterility in addition to that produced by the X chromosome alone. One possible explanation of these observations is that, if *Basc* chromosomes are, in fact, relatively inefficient contamination recipients, low level contamination of *Cy* and *Ubx* chromosomes may be more apparent than when more highly efficient wild-type X-chromosome recipients are present.

Experiment II: Using the same basic mating scheme as in experiment I, we sought to determine whether the potential for inducing male recombination could be transferred from autosomes to X chromosomes in a similar way to that of gonadal sterility. Again, using Cranston as the *P* strain for the initial generation matings, we followed the scheme of Figure 1 as far as the production of g_2 females. The subsequent modified mating scheme, partially shown in Figure 2, was designed to measure male recombination in chromosomes *2* and *3*. In this experiment, the measurement of recombination was restricted to males in which only the X chromosomes were exposed to contamination, *i.e.*, no autosomes were directly exposed. Table 2 indicates that when contamination of the X chromosome was restricted to g_2 females, male recombination frequencies were no higher than in the control group in which only male contamination was possible (bottom line of Table 2). All three groups in which contamination was possible in both g_1 males and g_2 females gave nontrivial frequencies of male recombination. There was no evidence from this experiment for significant differences between single autosomes in donor potency. Otherwise, the overall pattern of contamination in this male recombination experiment was very similar to that in which gonadal sterility was the method of assay (experiment I).

Experiment III: This experiment was designed to examine the effect of temperature on contamination in two different *P* strains using gonadal sterility

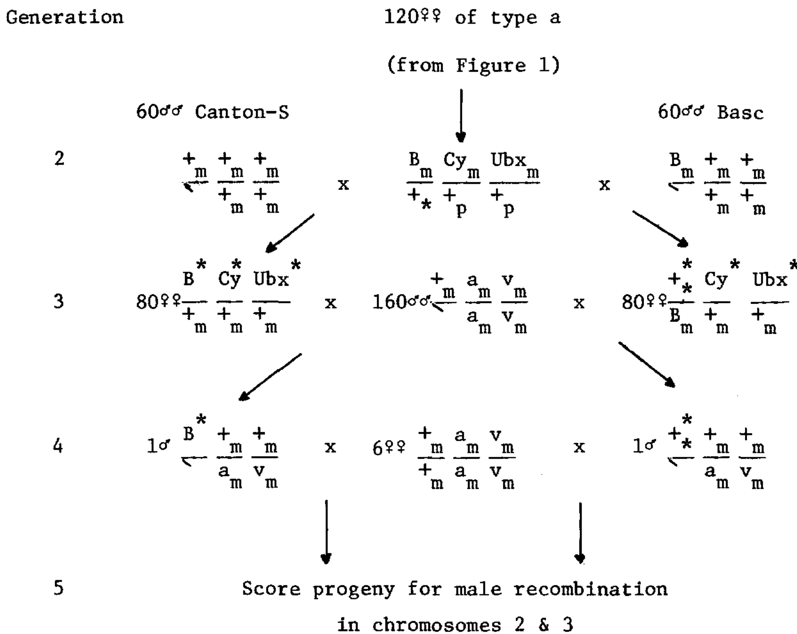

FIGURE 2.—Partial mating scheme for experiment II in which male recombination was measured as an indicator of chromosomal contamination. See Figure 1 for the mating scheme of the first two generations. g_2 females of types b, c and d (Fig. 1) were also mated in a similar way to the g_2, type a, females shown. B = *Basc*; a_m = *al b sp*; v_m = *ve st ca*. Asterisk subscripts and superscripts denote chromosomes that had the possibility of chromosomal contamination in g_1 males and g_2 females, respectively.

TABLE 2

Male recombination frequencies in chromosomes 2 and 3 in experiment II, using Cranston as the donor strain.

	X chromosome exposed to contamination					
	$g_2^{♀♀}$			$g_1^{♂♂}$ and $g_2^{♀♀}$		
g_2 donor chromosomes	Mean % male recombination	Mean minimum % events[a]	N[b]	Mean % male recombination	Mean minimum % events[a]	N[b]
2 and 3	0.08	0.08	2547	0.85	0.53	2447
2	0.04	0.04	2668	0.23	0.23	3465
3	0.03	0.03	2916	0.26	0.19	2621
None	0.12	0.08	2664	0.04	0.04	2286

See Figures 1 and 2 for mating schemes.

[a] An estimate of male recombination frequency taking into account premeiotic clusters (KIDWELL and KIDWELL 1976).

[b] N is the total number of progeny counted.

as the method of assay. The same basic mating scheme was employed as in Figure 1, substituting the Harwich and Oxford P strains for Cranston in experiments III A and III B, respectively. In each experiment, the g_2 females were raised at two different temperatures, 20° and 25°. Flies of generations 1

and 3 were raised at 20°. The results are presented in Table 3. Similar to the Cranston strain (experiment I), the Harwich strain provided no evidence for chromosomal contamination in males, but the Oxford results suggest the possibility of a low contamination frequency in males. In both experiments, the frequencies of contamination tended to be considerably higher at 25° than at 20°. Although the presence of the recipient autosomes, *Cy* and *Ubx*, did not consistently increase the frequency of contamination over that produced by contaminated X chromosomes alone, there was evidence for higher frequencies of autosomal contamination, particularly of the second chromosome, than observed in experiment I, suggesting possible strain differences in the ability to contaminate.

Experiment IV: Although the results of experiments I and III consistently suggested that the autosomal contamination target chromosomes (*Cy* and *Ubx*) did not contribute to the sterility of g_4 females at as high a frequency as did the target X chromosome, the design of these experiments did not allow a direct estimation of the degree of contamination of these autosomes in the complete absence of a target X chromosome. By a small modification of the Figure 1 mating scheme (see Figure 3), g_4 females were produced which carried only target chromosomes *2* and *3*. The gonadal sterility of these females was compared with that of g_4 females carrying X, *2* and *3* target chromosomes. The modification of Figure 1 involved mating individual g_3 males with both Canton-S virgin females (as before) and, also, with females from an attached-X stock (strain 10 in MATERIALS AND METHODS). The *M* characteristics of this stock were

TABLE 3

Effect of developmental temperature on the frequencies of chromosomal contamination in experiment III as measured by the percentage of dysgenic ovaries

			1	2	3	4	5	6	7	8
			Potential recipient chromosomes in g_2 ♀♀							
g_2 temperature	Experiment no.	*P* donor chromosomes	X	X_*	X *2*	X_**2*	X *3*	X_**3*	X *2 3*	X_**2 3*
20°	III A	a. *2* and *3*	1	3	3	6	7	3	7	6
		d. None	0	1	2	0	3	0	0	0
	III B	a. *2* and *3*	6	4	7	0	3	6	8	4
		d. None	1	6	0	2	0	4	1	5
25°	III A	a. *2* and *3*	23	9	11	30	19	15	25	13
		d. None	1	0	0	1	0	1	2	1
	III B	a. *2* and *3*	12	19	12	37	4	14	3	21
		d. None	0	0	0	6	1	0	0	9

HARWICH and OXFORD were the donor strains in experiments III A and III B, respectively, and approximately 50 g_4 females were dissected per group. See Figure 1 for mating scheme.

Asterisk subscripts denote the possibility of contamination by autosomes derived directly from the *P* strain in g_1 males. For all recipient chromosomes shown, contamination was possible in g_2 females in the presence of *P* donor chromosomes.

even stronger than those of Canton-S. As shown in Figure 3, the mean frequency of ovarian dysgenesis in females carrying potentially contaminated X chromosomes was of the order of three to four times higher than that in females carrying noncontaminated X chromosomes, inherited matroclinously. This result is consistent with those of the earlier experiments and confirms that the second and third chromosomes are relatively resistant to chromosomal contamination compared with the X chromosome. Even though previous experiments (ENGELS 1979b) have shown that *P* autosomes can cause *GD* sterility, it is not possible to say, from the limited observations reported here, whether the resistance to contamination of autosomes relative to the X chromosome is a general property of these chromosomes or whether it is peculiar to the balancers, (*Cy* and *Ubx*), used in these experiments.

Experiment V: Two questions were raised by the design of the experiments I–IV based on the Figure 1 mating scheme. The first was whether association of an *M*, X chromosome with *P* strain autosomes in g_1 males increases susceptibility to chromosomal contamination in females of the following generation (g_2). Such a possibility might explain the higher frequencies of sterility in the presence of wild type, compared to *Basc* X chromosomes (*e.g.*, Table 1). The second question concerned the distribution of contamination among individual g_2 females and whether the lack of consistency observed in some of these experiments could be explained by the use of mass rather than individual matings in g_2 and g_3. A modification of the Figure 1 mating scheme was made to attempt to answer these questions. In experiment V A, the types of females used in g_0 and g_1 were simply reversed in comparison with the standard Figure 1 mating scheme (experiment V B). Only females of type a (Figure 1) were selected in g_2. Females of all genotypes, as available, were dissected in g_4. Harwich was used as the *P* strain, and the g_2 females were raised at 25°. To examine the distribution of chromosomal contamination among families, g_2 females and g_3 males were individually mated, and the sterility of a group of about 30 g_4 females from each g_3 male family was estimated.

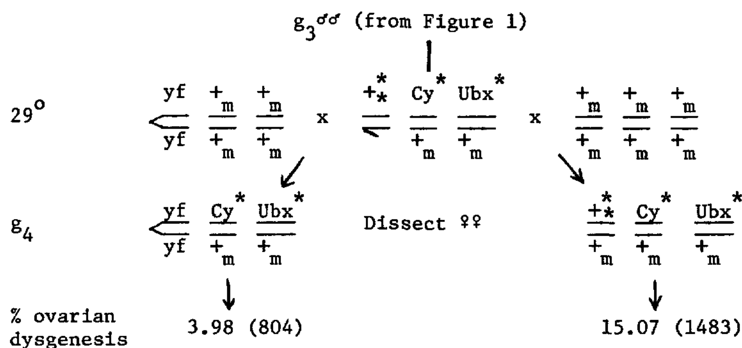

FIGURE 3.—Mating scheme for the third and fourth generations of experiment IV. Individual g_3 males from the Figure 1 mating scheme were mated in turn with harems of three attached-X females and three Canton-S females. Asterisk subscripts and superscripts denote chromosomes that had the possibility of chromosomal contamination in g_1 males and g_2 females, respectively.

Figure 4 shows the distribution of sterility frequencies in the granddaughters of individual g_2 females. The sterility of each family was estimated by dissection of as many groups of granddaughters as were available, but the number of fertile sons of a single g_2 female was often low. Including only those g_2 females having five or more sons, significant heterogeneity was demonstrated in both parts of the experiment ($P = 0.01$, in experiment V A; $P < 0.05$ in experiment V B). One likely reason for this heterogeneity is the premeiotic occurrence of transposition resulting in clusters of contaminated chromosomes in some g_2 females, but not in others.

In Figure 5, g_2 female family size (number of fertile sons) is plotted against the percentage of dysgenic ovaries in the daughters of each g_3 male (based on the dissection of 30 daughters per male). As before, the frequency of dysgenic ovaries is considered to be a measure of chromosomal contamination. Computation of Kendall's tau, a nonparametric correlation coefficient, indicated a tendency for chromosomal contamination frequency and family size to be inversely related ($\tau = -0.22$, $P < 0.05$).

In Figure 6 (A through D) are presented the means and frequency distributions of gonadal sterility for the daughters of individual g_3 males for the *Basc* and wild-type X chromosomes within experiments V A and V B, respectively. Despite the variation among the means for each of the four groups, the general shape of the distributions was similar, and no obvious bimodality could be discerned.

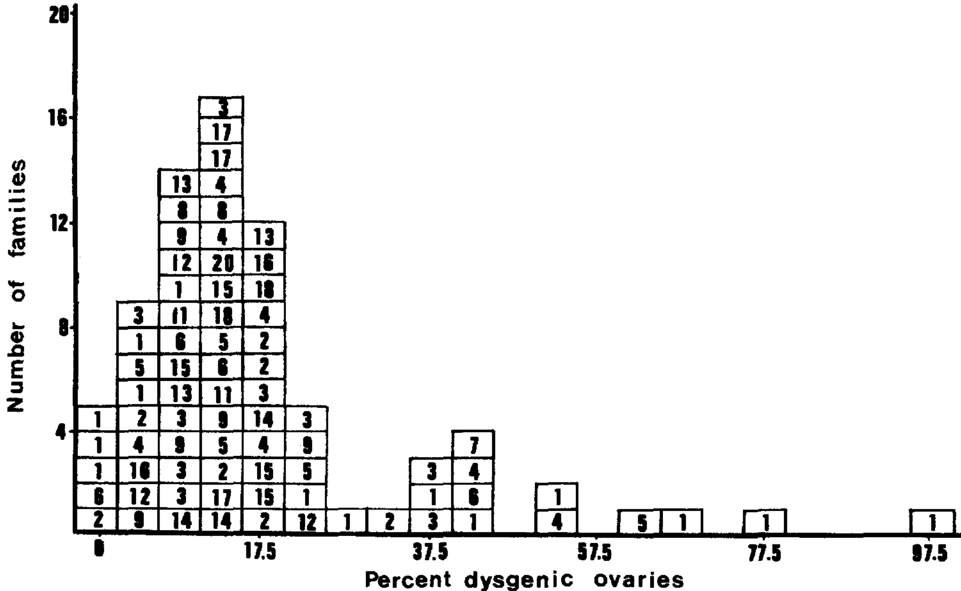

FIGURE 4.—Frequency distribution of individual g_2 female families according to the mean percentage of ovarian dysgenesis measured in their g_4 granddaughters in experiment V A and B. Each small rectangle represents a g_2 female family, and the number inside the rectangle indicates the family size (number of fertile g_3 sons).

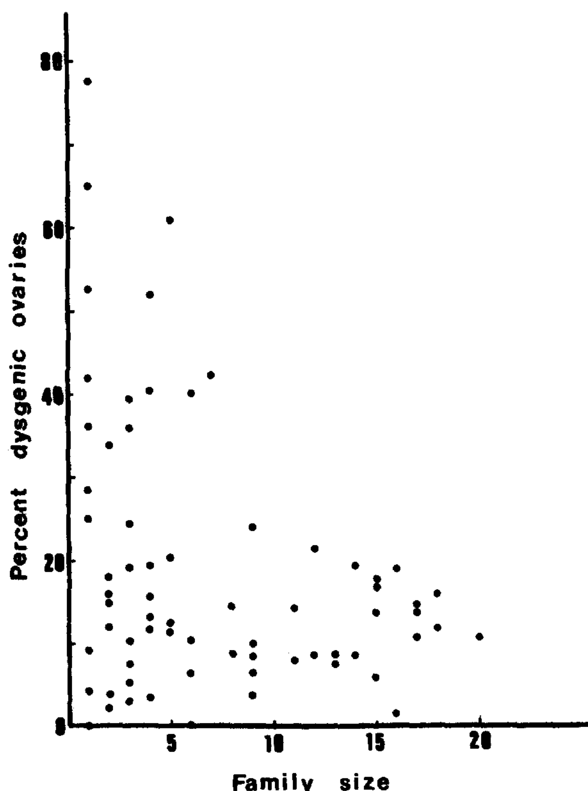

FIGURE 5.—Relationship of g_2 female family size (number of fertile sons per female) and frequency of chromosomal contamination as measured by mean percent ovarian dysgenesis in g_4 granddaughters of experiment V A and B.

Experiment VI: This experiment was designed to determine the effect of cytotype on chromosomal contamination. In the *I-R* system, it is known that chromosomal contamination is dependent on reactive cytoplasm in *I/R* chromosomal hybrids (see BREGLIANO and KIDWELL 1983 for review). In experiments I through V, the maternally transmitted cytoplasm of both male and female hybrids was always of the *M* type. It was, therefore, important to determine whether, analogous to the *I-R* system, the *M* cytotype was necessary for chromosomal contamination by *P* factors or, conversely, whether the transposition process was independent of cytotype.

The mating scheme is given in Figure 7. Hybrid g_1 males were mated to virgin females of two strains, H-40 possessing *M* cytotype and a synthesized *IQ* strain having *P* cytotype but the same autosome balancers as H-40 (strain 8 in MATERIALS AND METHODS). In the third generation, males carrying a wild-type X chromosome were selected. Thus, exposure to chromosomal contamination was allowed only in g_2 females. The results are presented in Figure 8. They indicate that high frequencies of chromosomal contamination are dependent on *M* cytotype. The low, but nonzero, values in *P* cytotype may be explained as a low

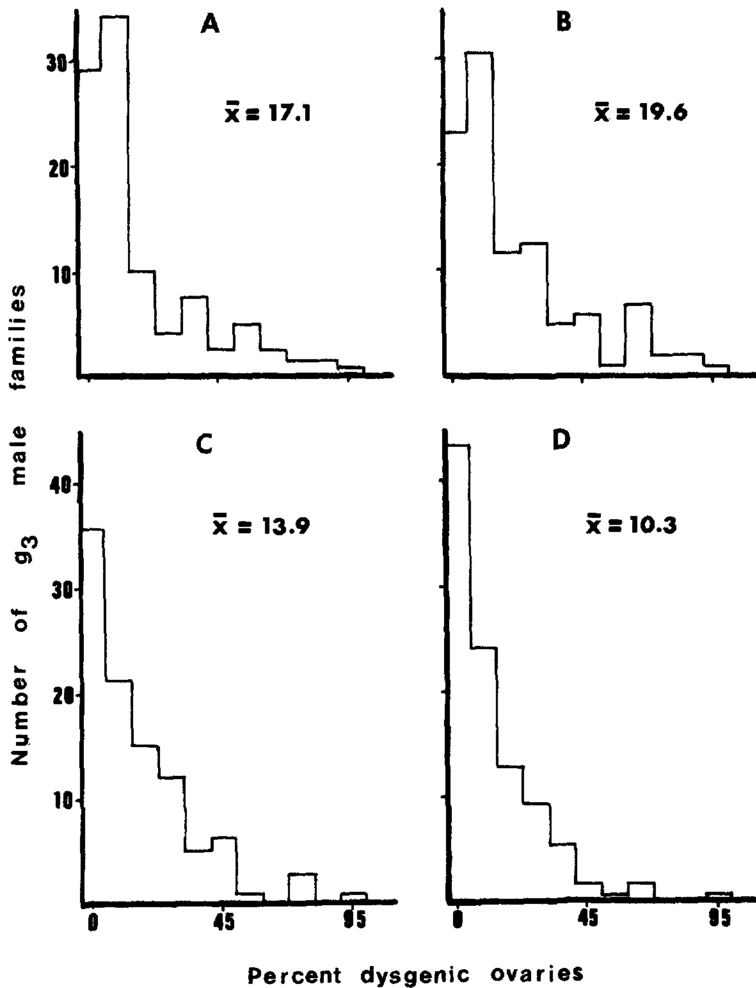

FIGURE 6.—Means and frequency distributions of g_3 male families with respect to ovarian dysgenesis in their g_4 daughters. A, Experiment VA, g_3 males with *Basc*, X chromosome. B, Experiment VA, g_3 males with +, X chromosome. C, Experiment VB, g_3 males with *Basc*, X chromosome. D, Experiment VB, g_3 males with +, X chromosome.

background frequency of ovarian dysgenesis encountered previously in both nondysgenic hybrids as well as nonhybrids.

Experiment VII: The purpose of this experiment was to determine whether *P-M* chromosomal contamination was independent of chromosomal contamination in the *I-R* system. In previous experiments, the *M* strains used in the first two generations were either *R* (H-41) or *N* (H-40) in the *I-R* system of hybrid dysgenesis. Because all *P* stocks used as male parents were also *I*, the possibility existed that *P* factors might be mobilized as a result of *I* factor transposition rather than autonomously. Experiment VII was designed to test this hypothesis (Figure 9). H-41 (*RM*) females were used in the g_0 generation for all groups. The

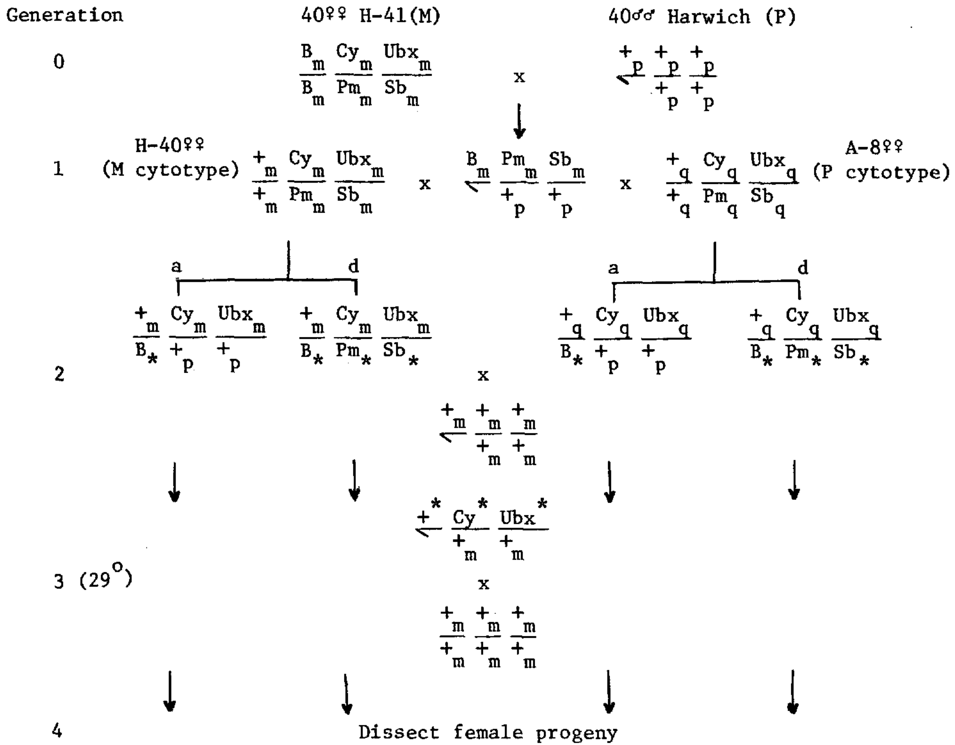

FIGURE 7.—Mating scheme for experiment VI designed to compare the effects of *M* and *P* cytotype on the frequency of transposition. Matings were made *en masse* in g_0 and g_1 and individually in g_2 and g_3. Asterisk subscripts and superscripts denote chromosomes that had the possibility of chromosomal contamination in g_1 males and g_2 females, respectively.

NM and *IM* strains used in g_1 are those numbered 6 and 11, respectively, in MATERIALS AND METHODS. The Canton-S wild-type strain was used in both g_2 and g_3. *I-R* chromosomal contamination in g_1 males can be ruled out because it never occurs in this sex (PICARD 1976). It was possible to produce three types of g_2 females by varying the types of females used in g_1 in relation to the males used in g_0. In series E, both the *P-M* and *I-R* interactions were possible. In series F and G, only one type of interaction was allowed (*P-M*, in series F; *I-R*, in series G). The numbers of g_2 females individually mated were 24, 21 and 32 for series E, F and G, respectively. The number of g_3 males tested in each series varied between 37 and 59. The frequency distributions of ovarian dysgenesis in g_4 females are given in Figure 10. The mean frequency of dysgenic ovaries was nearly equal in the two series with *P-M* interaction (E and F) and at least an order of magnitude higher than that in the *I-R* series alone (G). Although a low percentage of dysgenic ovaries was observed in series G, this may be interpreted as low background levels due to causes other than dysgenesis and similar in frequency to that observed in other experiments. This experiment, therefore, provides strong evidence that the *I-R* system is not involved in *P-M* chromosomal contamination.

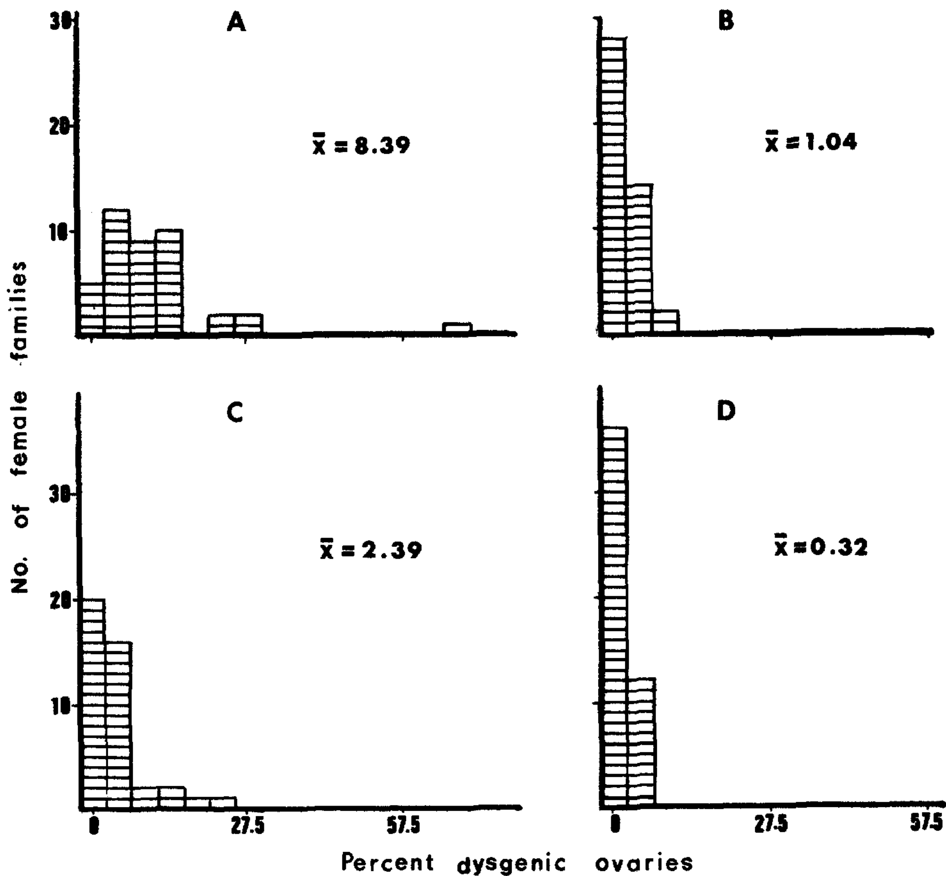

FIGURE 8.—Means and frequency distributions of individual g_2 female families with respect to ovarian dysgenesis in experiment VI. A, Cytotype *M* and chromosomes X, *2* and *3* exposed to contamination. B, Cytotype *P* and chromosomes X, *2* and *3* exposed to contamination. C, Cytotype *M* and no chromosomes directly exposed to contamination. D, Cytotype *P* and no chromosomes directly exposed to contamination. The number of males tested varied between 122 and 196 among the four groups. Approximately 25 g_4 daughters were dissected per male.

Experiment VIII: Due to the limitations of the methodology involved, the study of chromosomal contamination yields information only on that subset of transposition events that provide defined phenotypic changes (e.g., gonadal sterility and male recombination). It is expected that a fraction of transposition events will have no such phenotypic effects, and many questions arise as to the relationship between *P* element insertion at the molecular and chromosomal levels and the detection of chromosomal contamination by gonadal sterility at the phenotypic level.

Some information concerning the relationship between ovarian dysgenesis induction and *P* element insertion was obtained from the further study of an X chromosome carrying a hybrid dysgenesis-induced insertion mutation at the white locus (RUBIN, KIDWELL and BINGHAM 1982). The X chromosome, of *M* strain origin, carrying this newly induced white mutant allele, $w^{hd80k17}$, and

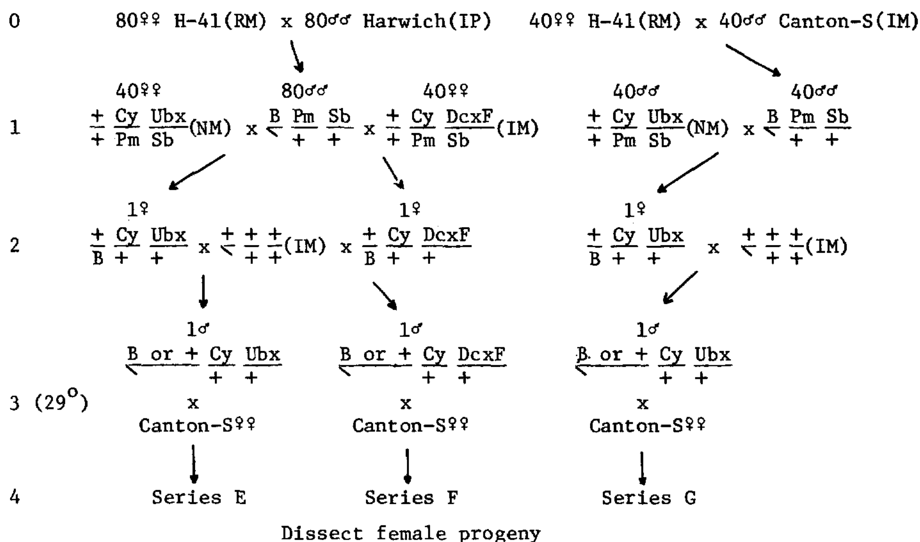

FIGURE 9.—Mating scheme for experiment VII. Matings were made *en masse* in g_0 and g_1 and individually in g_2 and g_3. Series E allows both *P-M* and *I-R* interactions; series F allows only the *P-M* interaction; series G allows only the *I-R* interaction. The *NM* and *IM* strains used in g_1 were the strains numbered 6 and 11, respectively, in MATERIALS AND METHODS.

additional insertions of the *P* factor at other chromosomal sites, was monitored for its sterility potential for 20 generations. $w^{hd80k17}$ males, carrying all autosomes of *M* strain origin, were continuously backcrossed *en masse* for 20 generations to females of an *M* cytotype attached-X stock (no. 10 in MATERIALS AND METHODS). The sterility potential of the $w^{hd80k17}$ X chromosomes was estimated at intervals of several generations by crossing males carrying these X chromosomes with Canton-S (*M*) females at 29°.

The results are summarized in Figure 11. The original male carrying the newly induced $w^{hd80k17}$ mutation had an unusually high *GD* sterility potential. All of his approximately 200 F_1 daughters raised at 20° were sterile, and many of his daughters raised at 20° had unilateral or bilateral dysgenic ovaries. However, the average sterility potential of this chromosome in *M* cytotype decreased in subsequent generations. The conclusion that this sterility was specifically associated with the X chromosome was confirmed by the absence of dysgenesis, at 29°, in attached-X daughters of the tested males which did not carry the $w^{hd80k17}$ X chromosome.

It was further observed that the distribution of *GD* sterility potential among individual $w^{hd80k17}$ males mated with Canton-S females was markedly bimodal. This is illustrated in Figure 12A which shows the distribution of sterility for the 22 males that were tested individually at generation 8 (see Figure 11). Before the routine sterility test mating, each of these 22 males had been individually mated with four females of an attached-X strain (no. 10 in MATERIALS AND METHODS) in order to maintain the X chromosome lines patroclinously. Eighteen $w^{hd80k17}$ sons of one of the two high sterility males, which had been identified in generation

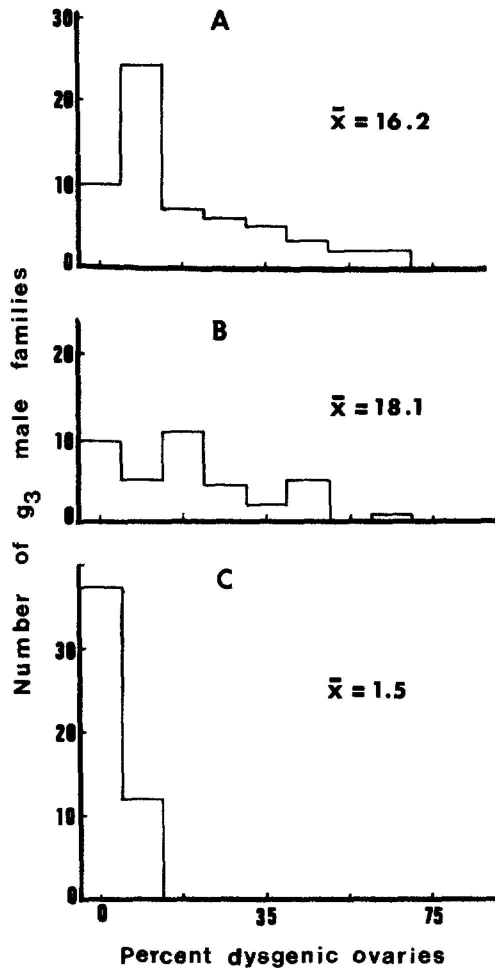

FIGURE 10.—Means and frequency distributions of individual g_3 male families in experiment VII with respect to ovarian dysgenesis. A, Series E, both *P-M* and *I-R* interactions. B, Series F, *P-M* interaction. C, Series G, *I-R* interaction.

8, were themselves tested for *GD* sterility potential. The results are given in Figure 12B. Strong bimodality of the distribution was again observed. Fifteen of eighteen sons maintained the high potential of their father and the remaining three sons lost virtually all their sterility potential in a single generation. From this result, the probability of loss of sterility function by individual X chromosomes was estimated to be one of six per generation. When this expected rate of loss is plotted in Figure 11, there is remarkably good agreement with the observed mean sterility frequencies. The observation of bimodality of the distribution strongly suggests that the sterility switch is associated with a change at a single chromosomal site.

To determine whether the instability of the sterility function of the $w^{hd80k17}$

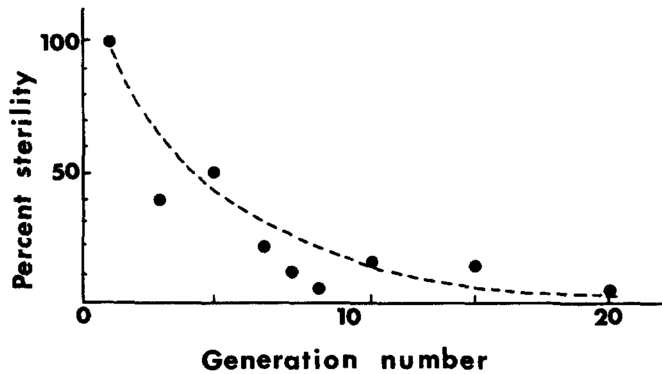

FIGURE 11.—Mean *GD* sterility potential of $w^{hd80k17}$ X chromosome maintained in *M* cytotype over a period of 20 generations. The sterility of each male carrying this chromosome was estimated by dissection of 20 of his F_1 daughters from matings with Canton-S females at 29°. Except in the first generation, at least 15 males were tested for each generation examined. The curve of expected values was computed assuming a loss of sterility function of one of six per generation (see text).

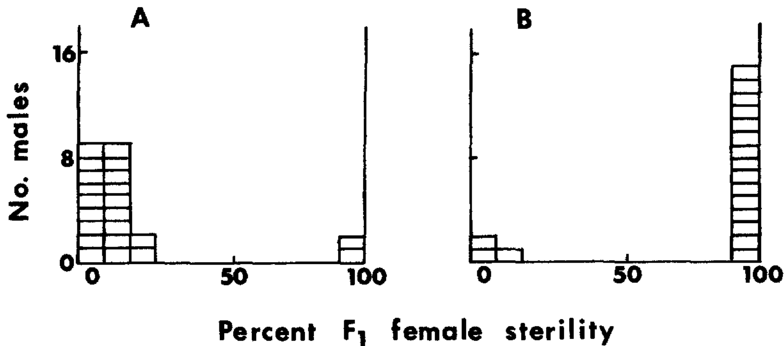

FIGURE 12.—Distribution of the sterility potential of $w^{hd80k17}$ chromosomes. A, Twenty-two unselected males, after backcrossing to attached-X, *M* cytotype females for eight generations (see Figure 11); B, 18 sons of a single selected g_8 high sterility male. The sterility frequency for each male (represented by a rectangle) was computed after dissecting 20 of his F_1 daughters.

chromosome was regulated by cytotype, high sterility males carrying this chromosome were crossed with females of the regular (*M* cytotype) *Basc* stock (strain 12, MATERIALS AND METHODS) and with *Basc* females from the synthesized line B7 (strain 7, MATERIALS AND METHODS) which was functionally a *Q* strain having *P* cytotype. Female *Basc*/*w* heterozygotes were backcrossed to their respective *M* cytotype and *P* cytotype parental lines for five generations, and then, by selection, homozygous $w^{hd80k17}$ stocks in both *M* and *P* cytotype were synthesized. Subsequent sterility tests indicated that X chromosomes from the *M* cytotype stock lost their sterility potential at a predictable rate, as before, but the high sterility potential of X chromosomes of the same origin in the *P* cytotype stock was stabilized.

Localization of the sterility factor(s) on the $w^{hd80k17}$ chromosome was attempted by mapping experiments. Preliminary experiments using the y^2 *cv v f*

marker stock suggested that the sterility potential was located in the distal 13.7-cM map region between *y* and *cv*. It was, therefore, clearly not associated with the $w^{hd80k17}$ *P* element insertion. Tests with a second marker stock y^2 *ec cv v f car* were carried out. $w^{hd80k17}$, *P* cytotype females (derived by backcrossing to the B7, Q strain as described before) were crossed with marker males. F_1 males and females were mated in pairs. A number of crossover males in each of the *w-ec* and *ec-cv* intervals were selected and crossed with Canton-S females at 29°. Approximately 20 female progeny were dissected for each crossover male.

The frequency distributions of ovarian dysgenesis in the daughters of individual crossover males are presented in Figure 13. The data clearly indicate that the sterility factor is located in the *ec-cv* interval. However, the absence of bimodality in the distribution of + *w* + *cv v f car* crossover males (Figure 13D) suggests that a second chromosomal site may be necessary for maximum sterility production. *In situ* hybridization assays, using a cloned *P* element as probe (P. M. BINGHAM, personal communication) indicated a labeling site at 4C which is within the *ec-cv* interval. In addition to the known labeling site within the white locus (RUBIN, KIDWELL and BINGHAM 1982) there were at least three more sites distal to the 4C site.

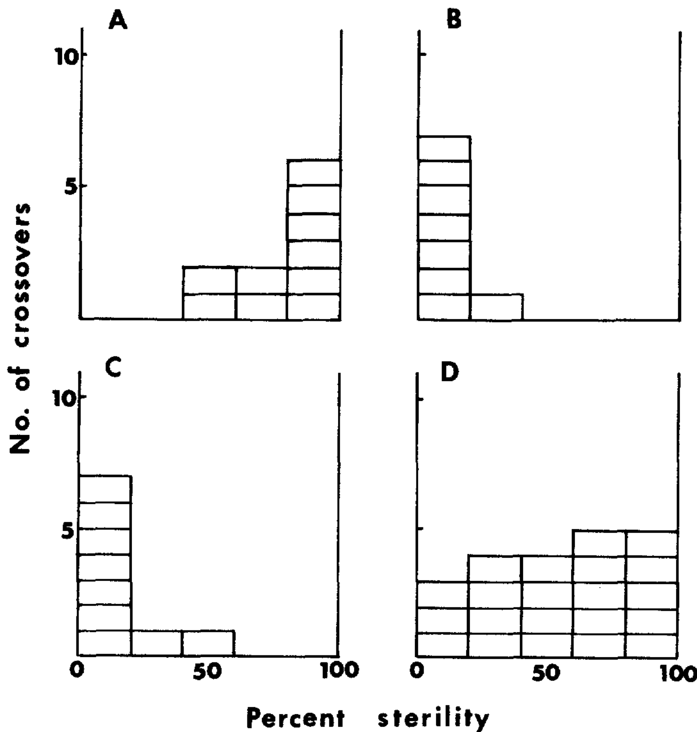

FIGURE 13.—Localization of the sterility potential of the $w^{hd80k17}$ X chromosome. The distribution of sterility potential of crossover males: A, *y* + + + + + +; B, + *w ec cv v f car*; C, *y* + *ec* + + +; D, + *w* + *cv v f car*. Each rectangle represents the percent ovarian dysgenesis of 20 F_1 daughters of a single male from a mating with Canton-S females at 29°.

DISCUSSION

Taken together, the various independent experiments reported do provide strong evidence that the gonadal sterility potential of *P* strain autosomes can, under certain conditions, be transferred to *M* strain X chromosomes during their nonhomologous association in the same genome. This transfer has many similarities but also some differences compared with chromosomal contamination in the *I-R* system (PICARD 1976), as will be discussed, and these processes in the two systems are independent of one another. In the *P-M* system, contamination is most readily interpreted in terms of the transposition of a subset of *P* factors from one genomic location to another as previously demonstrated for *P* elements by RUBIN, KIDWELL and BINGHAM (1982) and BINGHAM, KIDWELL and RUBIN (1982) using a mating scheme which was almost identical with that of Figure 1 in the present study.

As strictly defined in terms of the ability to induce gonadal sterility, chromosomal contamination frequencies are dependent on a number of genetic and environmental factors. *M* strain X chromosomes were consistently more efficient as recipients in the contamination process than were the autosomes, but there appeared to be strain differences in ability for autosomal contamination. Both major *P* strain autosomes were active as donors. Consistent with previous findings, the presence of *M* cytotype was necessary for chromosomal contamination. Higher frequencies of contamination were observed at developmental temperatures of 25°, compared with 20°, and in hybrid females, compared with hybrid males. Individual hybrid females differed in their frequencies of chromosomal contamination, and there was a negative correlation between these frequencies and family size.

The finding that *M* cytotype is necessary for high frequencies of transposition was expected because of the previously known dependence on *M* cytotype for the production of dysgenic traits (KIDWELL and KIDWELL 1975; ENGELS 1979a; SIMMONS *et al.* 1980) and release of instability at the singed locus (ENGELS 1979b, 1981b). The dependence of chromosomal contamination on cytotype is also directly analogous to the situation in the *I-R* system in which the frequency of chromosomal contamination increases with the reactivity level of the maternal strain (PICARD 1978).

The increase of chromosomal contamination frequencies with temperature is consistent with the previously observed tendency for the induction of dysgenic traits to be temperature dependent. However, various dysgenic traits vary in their temperature dependence. At one extreme, the manifestation of *GD* sterility is usually completely dependent on high developmental temperature (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979). At the other extreme, ENGELS (1981b) reported that reversion of the highly mutable allele, singed-weak, at the singed locus was only weakly temperature dependent.

The results of the present experiments strongly suggest that the rate of contamination of the X chromosome was significantly higher than that of the autosomes. However, because the autosomes measured were balancers with multiple inversions, it is possible that these produced results not typical of

homosequential autosomes. Further investigation will be required to settle this point. In the *I-R* system, PICARD (1978) observed that various chromosomes of reactive origin differed in their ability to be contaminated by the *I* factor. In his experiments, the X chromosome was systematically contaminated with lower probability than the second chromosome.

With respect to the Cranston *P* strain, the frequency of chromosomal contamination resulting from the presence of the third chromosome appeared to be small relative to that from the second chromosome (Table 1). This result parallels the situation in the *I-R* system in which the *I* factor may be linked to any of the four chromosomes, but a single chromosome of a strong inducer strain may be sufficient to produce maximum sterility (PICARD 1976; PICARD and PELISSON 1979).

In the Figure 1 mating scheme, a comparison can be made between groups in which contamination was possible in both g_1 males and g_2 females (group a, with wild-type X chromosome) and in g_1 males only (group d, with wild-type X chromosome). In certain experiments, group d(+) individuals provided some evidence for a low frequency of contamination in males, but, on average, the frequency in group a(+) was approximately an order of magnitude higher.

The difference between groups carrying the wild-type X chromosome and those carrying the B X chromosome in Figure 1 provides a comparison between chromosomes subjected to contamination in both g_1 males and g_2 females and those subjected in g_2 females only (e.g., columns 1 *vs.* 2, 3 *vs.* 4, 5 *vs.* 6 and 7 *vs.* 8 in Table 1). The difference was often larger than that which could be accounted for by male contamination alone (e.g., line d of Table 1). However, exposure differences were confounded with differences in chromosome source (B *vs.* +) in the first three experiments, and there was a possibility that different X chromosomes vary in their susceptibility to contamination. Another possible interpretation was that passage of an X chromosome through hybrid males, for one generation, might increase the probability of contamination in their daughters (over and above any increase due to contamination in the males themselves). However, the results of experiment V did not provide confirmation of this interpretation; in fact, they were inconsistent with those of experiment I in that g_4 females, whose wild-type X chromosomes were exposed to contamination only in g_2 females, had a considerably higher frequency of sterility than g_4 females whose wild-type X chromosomes were exposed in both g_1 males and g_2 females. No conclusive answers can, therefore, be given to the questions raised by the variable contamination frequencies of the X chromosome in experiment 1.

The overall conclusion concerning the effect of sex on chromosomal contamination in the *P-M* system is that, in contrast to the *I-R* system, it can occur in males, albeit at a lower frequency than in females under these experimental conditions. Compelling evidence for the occurrence of *P* element transpositions in males was obtained by BINGHAM, KIDWELL and RUBIN (1982), using the method of *in situ* hybridization. However, comparable transposition rates were not measured in female hybrids. Also, SIMMONS *et al.* (1980) and RAYMOND and SIMMONS (1981) have provided abundant evidence for mutation in *P-M* dysgenic

males. We must, therefore, conclude that *P* element transposition does occur in males, but the vast majority of these transpositions are defective in *GD* sterility potential.

In the early chromosomal contamination experiments, the repeatability of results between experiments tended to be low when females were mated *en masse* in the second generation (see Figure 1). When females were mated individually at this stage, although there were large family differences, the overall mean frequencies from different experiments, run under the same conditions, were more consistent. The frequency of chromosomal contamination tended to be inversely proportional to family size. This finding is consistent with previous evidence that *P* factor-induced traits often result in a disruption of normal germ line development and lowered reproductive fitness of their carriers. Thus, the consistently higher frequencies of chromosomal contamination observed from individual as opposed to mass matings might most readily be interpreted in terms of a higher rate of recovery, under relatively uncrowded conditions, of individuals carrying newly induced insertions. Alternatively, despite the large numbers of parents used, accidents of sampling under mass mating might result in disproportionate representation of some individuals and might account for the inconsistent results between experiments. Because of the possibility of this random variation, less weight should perhaps be placed on the results of those experiments in which mass matings were used throughout than on those in which individual matings were employed in g_2 and g_3. For example, the results on the effect of temperature should be considered somewhat tentatively until similar experiments have been carried out using single individuals.

One deficiency of the experimental design was the lack of control of secondary contamination in both g_2 females (X chromosome donor) and g_3 males (all three chromosomes are possible donors). This difficulty only arises if contamination occurs in males. It seems unlikely to be an important source of error, given the demonstrated low frequency of chromosomal contamination in that sex. The experimental design also theoretically underestimates chromosomal contamination frequencies in general. Any insertions that result in a hemizygous inviable X chromosome are automatically eliminated. The association of insertions with large deletions and other gross anomalies has been well documented.

In addition to the similarities between the *I-R* and *P-M* systems in their patterns of chromosomal contamination, discussed before, there are several striking differences. One of these is the possibility of contamination in both sexes in the *P-M* system compared to the limitation to females in the *I-R* system. This difference is not surprising in view of the strict restriction of dysgenesis to females in the *I-R* system. A second major difference is, however, surprising. This is the absence of discernible bimodality in the *P-M* sterility distributions (see Figure 6) compared to the very marked bimodality of *SF* sterility frequencies resulting from chromosomal contamination in the *I-R* system (see, for example, Figure 5 of PICARD 1976). The most likely interpretation of the bimodality in PICARD'S results is that *R* chromosomes receiving one or more *I* factors by chromosomal contamination can be readily distinguished, by means of *SF*

sterility tests, from those not carrying *I* factors. One explanation of the absence of clear bimodality in distributions associated with the *P-M* system is the relative small effect on *GD* sterility production of an average single transposed *P* element. Nevertheless, the marked bimodality observed in experiment VIII demonstrates the existence, albeit rare, of *P* elements that do have very strong sterility potential.

The observations reported in relation to the $w^{hd80k17}$ chromosome are consistent with the hypothesis that the observed sterility potential is due to the insertion of a *P* element within the *ec-cv* interval with the degree of sterility being modified by a second *P* labeling site, distal to the first. It is suggested that the complete loss of sterility in *M* cytotype which occurs with a one in six probability is due to internal deletion of a sequence or sequences of the *P* element insertion in the *ec-cv* interval. *P* elements are known to be highly unstable in *M* cytotype (ENGELS 1981b; K. O'HARE and G. M. RUBIN, personal communication). The frequency of sterility switching observed here is less than the approximately 50% rate observed for the mutability of singed-weak (ENGELS 1981b) but considerably greater than the frequency of 0.004 for the reversion of w to w^+ by complete excision of the *P* element (RUBIN, KIDWELL and BINGHAM 1982).

An important conceptual clarification which is emerging from recent studies of the *P-M* system of hybrid dysgenesis is that not every *P* element transposition event is necessarily accompanied by the acquisition of sterility potential by recipient chromosomes. More precisely, chromosomal contamination events, detectable by the transfer of the potential for specific dysgenic traits at the phenotypic level, represent only subsets of the totality of *P* element transpositions. This conclusion follows, not only from the experiments reported here, but also from the results of SIMMONS *et al.* (1980) who reported sex-linked lethal mutations, resulting from putative *P* element insertions in $M \times Q$ dysgenic crosses. Although *Q* strains lack *GD* sterility potential (KIDWELL 1979; ENGELS and PRESTON 1979), BINGHAM, KIDWELL and RUBIN (1982) showed that they do carry *P* elements. Further, K. O'HARE and G. M. RUBIN (personal communication), after sequencing some of the small *P* element insertions responsible for mutation induction at the white locus (RUBIN, KIDWELL and BINGHAM 1982), found that each of these sequences are related to a 3-kb "ancestral" *P* element by single, relatively large, internal deletions. These results, taken together with those of recent natural population studies (KIDWELL 1983b), strongly suggest that internal deletion and loss of function of *P* elements may be quite common.

The question, therefore, arises as to the quantitative and qualitative relationships between chromosomal contamination, as defined here, and the totality of *P* element transposition events. The limited evidence described in experiment VIII suggests that only a minority of *P* element transpositions may result in chromosomal contamination that can be detected by *GD* sterility assays and that the sterility potential of these transposed elements is highly variable.

One of the most interesting outstanding questions concerning hybrid dysgenesis concerns the role of *P* elements in sterility production and the nature of the genetic mechanisms involved. Although in the present experiments the produc-

tion of sterility is most commonly associated with contamination of the X chromosome, clearly it can, in some instances, be due to autosomal contamination. One possibility is that sterility production is dependent on the insertion of a *P* element into specific "sterility" loci. According to this idea, sterility production would be the consequence of a mutated locus and contamination would be dependent on the site specificity of a transposed *P* element. An alternative and perhaps more likely possibility is that the precise site of *P* element insertion is of only minor importance in sterility production and that the internal structure of the *P* element itself determines its sterility potential. Elements with the functional potential for sterility production might exert their effect in many chromosomal locations when destabilized. Such inserted *P* elements might be particularly sensitive to temperature-dependent destabilization in early stages of germ line development, resulting in massive chromosome fragmentation, development arrest of the germ line and consequent gonadal sterility. *P* elements deficient for sterility production (perhaps due to internal deletion of a specific DNA sequence) could, however, produce certain other dysgenic traits such as visible or lethal mutations merely by insertion into functional genes.

The author thanks J. B. NOVY for excellent technical assistance, DR. W. R. ENGELS for helpful suggestions and discussion throughout the course of this work and MRS. BARBARA SCHMITT for secretarial and other contributions of a rare variety. This work was supported by Public Health Service research grant GM-25399.

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Corresponding editor: W. W. ANDERSON