HYBRID DYSGENESIS IN *DROSOPHILA MELANOGASTER*: THE GENETICS OF CYTOTYPE DETERMINATION IN A NEUTRAL STRAIN[1]

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ABSTRACT

The genetic determination of resistance to the sterility-producing genetic elements called *P* factors was studied in a strain characterized as neutral (*Q*) in the *P-M* system of hybrid dysgenesis. Sixteen lines were synthesized, representing all possible homozygous combinations of the three major chromosomes and differing maternal cytoplasms of an original resistant (*Q*) and susceptible (*M*) strain.——The results provide a detailed genetic analysis of the determination of cytotype (which mediates resistance or susceptibility to *P* factors) in the absence of the *P-M* dysgenic interaction. They extend the findings of ENGELS (1979) by providing specific information on both the location and relative magnitude of effect of cytotype-determining chromosomal factors and their interaction over time with maternally transmitted cytoplasm.——Cytotype was found to be primarily controlled by the genotype, but the maternal cytoplasm, under some circumstances, has an important short-term effect. Major cytotype-determining chromosomal factors are localized to the distal half of the *X* chromosome. However, there was also evidence for minor factors located on the major autosomes, particularly chromosome *3*. Under certain circumstances, cytotypic switches in either direction can be produced in a single generation by the substitution of an *X* chromosome carrying a major cytotype determinant. This may provide an explanation of why reciprocal differences have sometimes been interpreted as direct effects of *X*-chromosome suppressors. However, slow but systematic changes of *M* to *P* cytotype were observed in five synthesized lines of mixed origin over twenty generations with no chromosomal substitution. Alternative explanations of these changes in terms of delayed effects of minor autosomal factors or of the transposability of cytotype determinants are discussed.

IN *Drosophila melanogaster*, two nuclear-cytoplasmic interaction systems called hybrid dysgenesis, have been described (KIDWELL, KIDWELL and SVED 1977; SVED 1979; BREGLIANO *et al.* 1980). Hybrid dysgenesis is manifest as germ line defects and includes sterility (PICARD and L'HÉRITIER 1971; KIDWELL and KIDWELL 1975), chromosomal structural changes such as inversions and translocations (VOELKER 1974), transmission ratio distortion (HIRAIZUMI 1971) and high frequencies of both lethal and visible mutations (GREEN 1977; SIMMONS *et al.* 1980). Male crossing over, normally absent in this species, occurs at low but non-

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trivial frequencies (HIRAIZUMI 1971), and there is an analogous increase in female recombination frequencies (KIDWELL 1977; SINCLAIR and GREEN (1979). Three notable features of all of these traits are the marked inequality of frequencies of these events in reciprocal crosses between interacting strains, their usual absence in the progeny of intrastrain matings and their strong dependence on environmental factors such as temperature (KIDWELL, KIDWELL and SVED 1977).

Two distinct systems of hybrid dysgenesis have been recognized, the *I-R* system and the *P-M* system (PICARD *et al.* 1978; KIDWELL 1979). The two systems have many similarities, but also some significant differences, both in properties and distribution (reviewed by BREGLIANO *et al.* 1980; KIDWELL 1982).

Hybrid dysgenesis appears to result from the interaction of two genetic components: a chromosomal component, usually transmitted paternally, and a cytoplasmic component, transmitted maternally. In the *P-M* system, the chromosomal component consists of polygenic factors (*P* factors) linked to one or more of the major chromosomes of paternally contributing (*P*) strains (ENGELS 1979; M. G. KIDWELL, unpublished results). These factors have the potential for causing sterility (and other dysgenic traits) in an individual when inherited from either parent, providing the appropriate interacting maternal component is also inherited. Many lines of evidence suggest that the inheritance of *P* factors is not strictly Mendelian. Strong circumstantial evidence now indicates that *P* factors, like the inducer factors of the *I-R* system, may be mobile genetic elements (SLATKO 1978; YANNOPOULOS 1979; M. G. KIDWELL, unpublished results; J. A. SVED, personal communication; see also review by ENGELS 1980).

The maternal or regulatory component of the *P-M* system is complex and exhibits some unusual genetic characteristics. The first clue concerning the mode of regulation was provided by the observation of large reciprocal differences in the frequency of dysgenic traits (KIDWELL and KIDWELL 1975). Two types of hypothesis have been proposed to account for these differences. The suppression of male recombination and other dysgenic traits in at least one Texas line has been interpreted by SLATKO and HIRAIZUMI (1978) in terms of suppressors, with strictly Mendelian transmission, carried by the *X* chromosome. However, *X*-chromosome suppressors cannot account for the observation in many different laboratories of reciprocal differences in female dysgenic traits. Working with the Cranston strain, KIDWELL (1978) presented evidence for an alternative hypothesis of cytoplasm-chromosome interactions, and was also unable to detect an *X*-chromosome suppressor effect on male recombination.

ENGELS (1979), by means of repeated backcrossing, placed the chromosomes of a *P* strain in *M*-strain maternal cytoplasm and the chromosomes of an *M* strain in *P*-strain maternal cytoplasm. He was able to rule out both classical cytoplasmic transmission and simple Mendelian transmission as the mode of inheritance of the regulatory component of the *P-M* system. He described this component in terms of an entity called "cytotype." This can be considered as a property of the whole cell, being determined by both genotype *and* maternally transmitted cytoplasm. Individuals having *M* cytotype are apparently susceptible

to the action of *P* factors, but those with *P* cytotype are resistant. ENGELS found that the determination of cytotype involved chromsomal factors and limited cytoplasmic transmission. In the *P* strain with which he worked, cytotype-determining factors appeared to be located on all three major chromosomes, and the chromosomal distribution appeared to be closely correlated to that of *P* factors in the same strain. However, the design of this experiment left a number of questions unanswered. When *P* chromosomes exist in cells with *M* cytoplasm, the potential for dysgenic interaction exists, which may interfere with the study of the normal transmission of any *M* components. Study of the transmission of cytotype in the absence of interaction is therefore necessary. Also, the method of backcrossing allows only statements of probability to be made, and neither ensures complete chromosomal substitution nor provides information on the specific chromosomal locations and expression of cytotype determinants.

The purpose of this paper is to investigate the determination of cytotype using a so-called neutral (*Q*) strain, which does not carry active *P* factors for the gonadal sterility typical of the *P-M* system (KIDWELL and NOVY 1979), but does exhibit resistance to the action of such factors, *i.e.*, has *P* cytotype similar to that of the neutral strain of ENGELS and PRESTON (1981). The use of such a strain allows the study of cytotype without the disturbing effects of the *P-M* interaction. In particular, the chromosomal location and relative magnitude of the effect of cytotype-determining factors was studied, together with their time of action both directly and indirectly through the maternal cytoplasm. The monitoring of fixed genotypes over an extended period of time made it possible to follow the dynamics of this unusual regulatory system until cytotypic equilibrium was achieved.

MATERIALS AND METHODS

For descriptions of mutants and balancers, see LINDSLEY and GRELL (1968).

Stocks employed: Mount Carmel: an isofemale line collected in Mount Carmel, Illinois, in 1970 and obtained from L. H. THROCKMORTON in 1977 and designated as type *Q* in the *P-M* system and *I* in the *I-R* system (KIDWELL, NOVY and FEELEY 1981). Although this strain behaves as neutral with respect to gonadal sterility, *i.e.*, gives normal fertility in the progeny of matings with both *M* strain females and *P* strain males, it acts as a *P* strain with respect to male recombination (M. G. KIDWELL, unpublished results).

H-41: *Basc*; *In(2LR)* bw^{V1}, ds^{33K} *dp b* bw^{V1}/*SM1*; *Sb/TM2*; spa^{pol} (=*B/B*; *Pm/Cy*; *Sb/Ubx*). This strain is characterized as strong *M* in the *P-M* system and *R* in the *I-R* system.

Harwich: a strong *P* and *I* strain collected by M. L. TRACEY in Harwich, Massachusetts, in 1967.

y^2 *cv v f*: a laboratory strain designated *M* in the *P-M* system and *I* in the *I-R* system that carries multiple markers on the *X* chromosome: yellow, *y* (0.0); crossveinless, *cv* (13.7); vermilion, *v* (33.0); forked, *f* (56.7).

Basc: A strain characterized as *M* in the *P-M* system and neutral (*N*) in the *I-R* system that is homozygous for the *X* chromosome balancer *Basc*.

Method of cytotype determination: Following previously established procedures (ENGELS 1979; KIDWELL, NOVY and FEELEY 1981), cytotype was determined by measuring gonadal sterility (*GD* sterility) in the female progeny of crosses between males of a strong *P* strain (Harwich) and females of the strain under test. The temperature was maintained at 29° throughout the development of the F_1 generation. Females were aged for three to five days after eclosion and their ovaries were dissected in water (see SCHAEFER, KIDWELL and FAUSTO-STERLING 1979 for

more details). The frequency of gonadal sterility was estimated by the percentage of individual ovaries that appeared to be grossly undeveloped, no account being taken of concordance or discordance between the two members of a pair of ovaries of a single female. Such an estimate has been found to be highly correlated with the percentage of females laying no eggs in most dysgenic crosses (M. G. KIDWELL, unpublished results). In comparison with the method of sterility estimation based on absence of egg-laying (KIDWELL 1979), dissection has the advantage of providing increased information by identifying the occasional fertile individuals with unilateral dysgenic ovaries and, conversely, of ruling out other unrelated types of sterility.

Method of mapping X-linked cytotype determinants: The synthesized line A_8, which is homozygous for *X* chromosomes of *Q* origin and autosomes of *M* origin, was crossed to *y cv v f/Y* males. Single g_2 males, variously carrying nonrecombinant and all types of recombinant *X* chromosomes, were crossed to homozygous *Basc* females. These *X* chromosomes were made homozygous and tested for cytotype after both two and six generations by the methods described above.

A cornmeal-molasses-agar-Brewer's yeast medium, seeded with live yeast, was employed. All stocks were maintained at 20°, except where otherwise noted.

RESULTS

Synthesis of chromosomal substitution lines: In order to investigate the effects on cytotype of chromosome pairs, both singly and together, a number of lines were synthesized as indicated in Figure 1. The initial reciprocal crosses were between H-41, a strong *M* strain, and Mount Carmel, a *Q* strain that has *P* cytotype, but does not induce gonadal sterility in crosses with *M* strains and therefore apparently has no active *P* factors (KIDWELL, NOVY and FEELEY 1981). The aim was to investigate the genetics of cytotype in strains free from the complications of gonadal sterility. Two sets of substitution lines were synthesized (indicated as series A and B in Figure 1) in which the genotypes were identical, but the maternal cytoplasms that were transmitted to the initial generations differed. Within each series, lines with all 8 possible homozygous combinations of *Q* and *M* major chromosomes were constructed by selection of appropriate chromosome markers, using *Cy* and *Ubx* as balancers in heterozygous females and *Pm* and *Sb* in males. Lines homozygous for the Mount Carmel, wild-type *X* chromosome were obtained after two generations of chromosome selection, but those homozygous for the *Basc* chromosome took three generations to complete, because in females it it not possible to identify *Pm* in individuals homozygous for *Basc*. The genotype of the small chromosome *4* was not controlled.

When the synthesis of all 16 lines was complete (generation 4), each was maintained independently without artificial selection in half-pint bottles by mass transfer. Each was tested for its cytotype property in matings with Harwich males (see MATERIALS AND METHODS) for a number of successive generations. The results are summarized in Table 1. The data here are the percentages of all ovaries that were dysgenic in a sample of progeny from mass matings. The average size of the progeny sample dissected per line increased from about 30 females in generation 4 to about 200 in each of generations 10 and 20.

Examination of Table 1 reveals several general tendencies. First, with only a few exceptions, there is remarkably close general agreement in the results when pairs of lines of the same genotype within Series A and B are compared. This

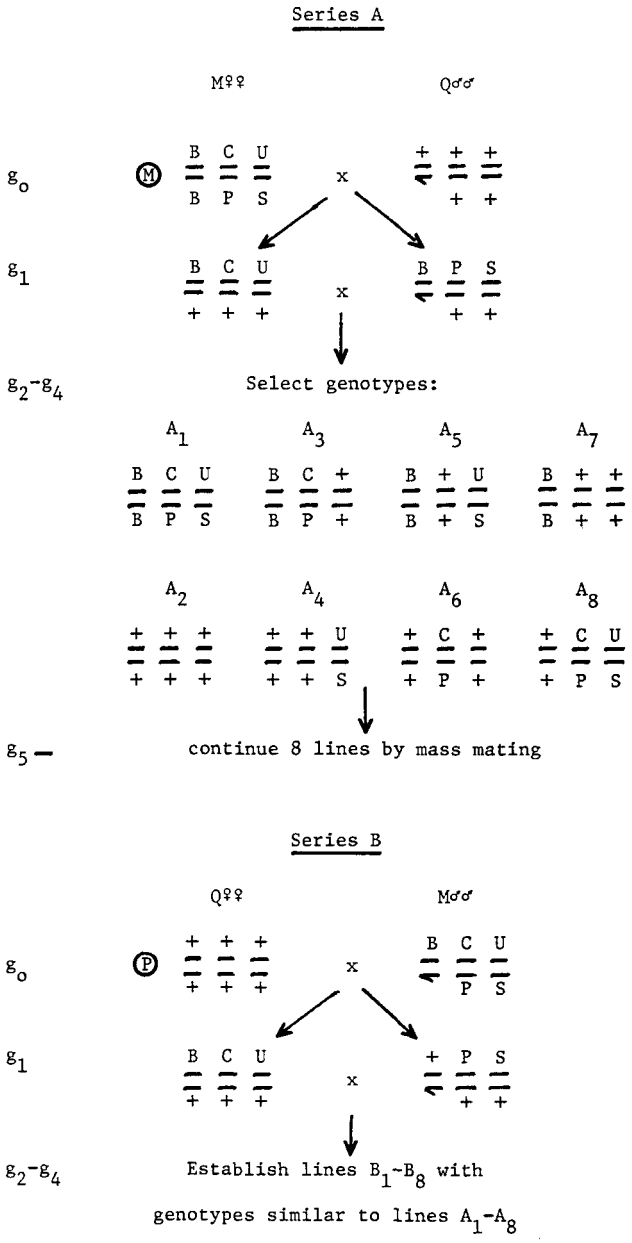

FIGURE 1.—Outline of mating scheme used to synthesize eight lines representing all possible combinations of the three major chromosomes of an *M* strain (H-41) and a *Q* strain (Mount Carmel). *M* strain chromosomes carried crossover suppressors and a dominant marker: *B* = Bar; *C* = Curly; *P* = Plum; *U* = Ultrabithorax; *S* = Stubble.

TABLE 1

Percentage of ovaries that were dysgenic in female progeny of crosses between synthetic line females and Harwich males

Line no.	Genotype	Generation number							
		4	5	6	7	8	9	10	20
A_1	*MMM*	26		90	99	100		99	99
B_1	*MMM*	41		96	100	99		96	97
A_3	*MMQ*	16	23	96	84	53	24	32	18
B_3	*MMQ*		97	62	28	35	7	37	9
A_5	*MQM*	65		93	92	96	90	91	85
B_5	*MQM*	21	66	87	89	43	69	64	12
A_7	*MQQ*	14	29	63	33	12	27	33	8
B_7	*MQQ*		83	33	43	35	19	29	12
A_2	*QQQ*	0		2	3	1			
B_2	*QQQ*	4		2	2	2			5
A_4	*QQM*	0	0	0	1	0			1
B_4	*QQM*	0	0	0	6	4			0
A_6	*QMQ*	14	0	5	1	1			8
B_6	*QMQ*	18	0	13	0	3			0
A_8	*QMM*	5	3	0	2	1			0
B_8	*QMM*	0	1	0	0	1			4

For details of line synthesis see Figure 1.

provides good evidence against any long-term effect of the original maternal cytoplasm *per se*.

In contrast to the overall similarity between the two mating series, when the results from lines of different homozygous genotypes are compared, there are some striking and consistent differences. The most clear-cut of these differences is between those lines homozygous *M* for the *X* chromosome that produced high sterility (Table 1) and those homozygous *Q* for the *X* chromosome that gave low sterility frequencies. This indicates a very strong, major effect of the *X* chromosome on cytotype. For those lines homozygous *Q* for the *X* chromosome (see bottom half of Table 1), the majority of females were of *P* cytotype, irrespective of the *Q* or *M* constitution of the major autosomes and the number of generations since the initiation of the lines.

On the other hand, in those lines homozygous *M* for the *X* chromosome (see top half of Table 1), high frequencies of sterility indicated the presence of females having *M* cytotype in the vast majority of tests. However, differences in the frequency of sterility were apparent when some individual lines were observed at different times and in tests of different lines in the same generation. The control lines A_1 and B_1 and the mixed line A_5 gave a consistent pattern of very high sterility in every generation subsequent to the first test in generation 4. In A_1 and B_1, this may be interpreted as the reconstitution of strong *M* stocks by reassembling together the original *M* strain chromosomes in the same genomes, with no detectable long-term effect of the original maternal cytoplasm. In line A_5, apparently the *Q* chromosome *2* had very little, if any effect on cytotype. In lines

A_3, B_3, B_5, A_7 and B_7, there was a general trend for the frequency of sterility (and, by inference, *M* cytotype) to rise quickly to a maximum after the initial synthesis of the lines and then to drop off slowly to reach relatively low frequencies by generation 20.

Cytotypic analysis of individual females: The data in Table 1 were obtained from the progeny of mass matings. In order to examine the distribution of cytotypes of individual females taken from each of the substitution lines, more extensive observations were made in generations 8 and 20. A sample of from 10 to 20 females was taken from each of the 16 lines, and each female was mated individually with two Harwich males. The progeny developed in 95 mm shell vials at 29° and approximaely 25 F_1 females were dissected per vial. Figure 2 provides a comparison of the frequency distributions of ovarian dysgenesis for individual females among the 16 lines for generations 8 and 20. As before, virtually all females clearly had *P* cytotype in lines homozygous *Q* for the *X* chromosome. Five of six mixed lines homozygous *M* for the *X* chromosome provided some evidence for bimodal distributions in generation 8, suggesting the presence of females of both *P* and *M* cytotype, as well as some with intermediate cytotype five generations after homozygosity was achieved. By generation 20, all five of these lines provided clear evidence of a shift in the distribution away from the *M* and towards the *P* cytotype end of the range. The sixth line (A_5) had a unimodal distribution similar to, but less extreme than, the distribution of the *MMM* controls A_1 and B_1. No marked qualitative shift occurred in A_5 between generations 8 and 20 as in the other five mixed populations described above.

Single generation effects of X-*chromosome changes*: Using the sixteen substitution lines described above, an experiment was performed to determine the effect on cytotype of heterozygous *M* and *Q X* chromosomes over a single generation in all combinations of genotypic and available cytoplasmic backgrounds. Between generations 4 and 6, each of the 16 lines was mated in reciprocal combinations with that line in the same series having an identical autosomal complement, but a different *X* chromosome. For example, line A_1 (*MMM*) was mated reciprocally with line A_8 (*QMM*) and line B_7 (*MQQ*) with line B_2 (*QQQ*). These reciprocal combinations are referred to as A_{18}, A_{81}, B_{72} and B_{27}, respectively; the female parental line is indicated first and the male parental line last in the subscript. Thus, in the first example, the same genotype *M/Q;M/M;M/M* was produced from mothers having *M* cytotype (A_{18}) and *P* cytotype (A_{81}).

Ten to 20 females heterozygous for the *X* chromosome were tested for cytotype by the usual matings with Harwich males for each of the sixteen pairwise combinations. The distributions of gonadal sterility for each combination are given in Figure 3. For the four combinations that were homozygous *M* for the two autosomes, the distributions appeared to be strongly influenced, but not completely determined, by the maternal cytotype. Thus, A_{18} and B_{18} frequencies tended to cluster towards the *M* cytotype side of the distribution and, conversely, A_{81} and B_{81} towards the *P* cytotype side of the distribution. When the remaining twelve combinations are considered, however, in most cases there is a tendency to cluster towards *P* cytotype, irrespective of the maternal state. Thus, in the

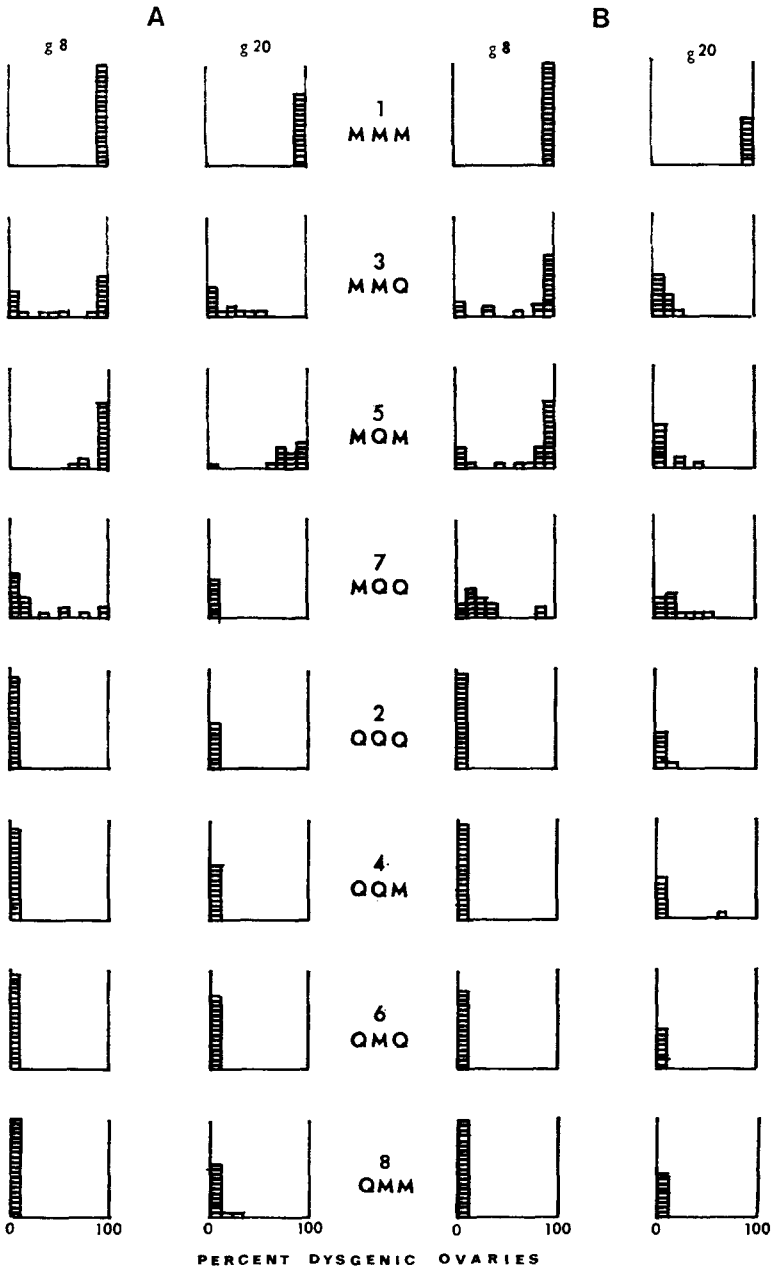

FIGURE 2.—Frequency distributions of ovarian dysgenesis at generations 8 and 20 when females of the 16 chromosomal substitution lines were tested for cytotype by mating with Harwich males. Each small rectangle represents the average sterility of a single female computed as the percentage of dysgenic ovaries in a sample of 25 of her daughters.

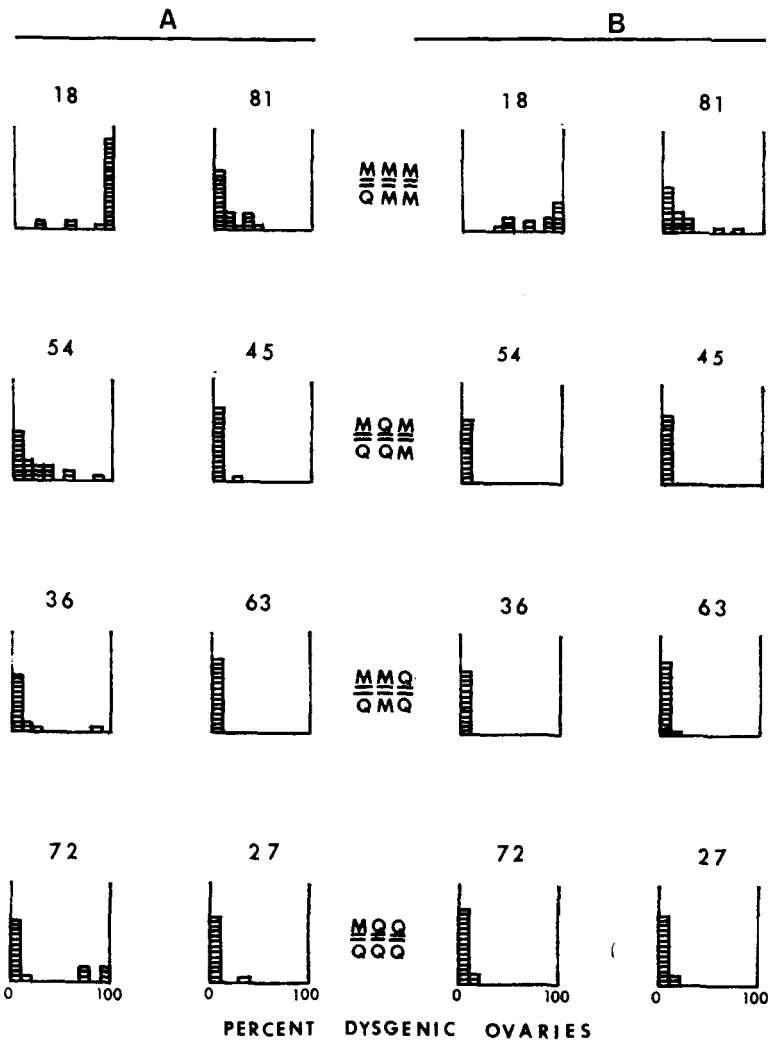

FIGURE 3.—Frequency distributions of ovarian dysgenesis in daughters of females whose *X* chromosomes were made heterozygous for a single generation in various genetic backgrounds. The cross made to produce the heterozygotes is shown above each distribution, the number of the maternal line first and that of the paternal line second within each series A and B. Each rectangle represents the dysgenic ovary percentage of a single female (mated with 2 Harwich males) determined by dissection of 25 of her daughters.

crosses involving females from odd-numbered lines, the presence of at least one homozygous pair of *Q* autosomes had the potential to counterbalance the effect of *M* maternal cytoplasm and shift the cytotype of the progeny from *M* to *P* in a single generation.

The ability of the cytotype to be switched in the reverse direction, *i.e.*, from *P* to *M*, by the substitution of a single *M X* chromosome in only one generation was demonstrated in the following way. Starting with the A_{54} F_1 females of Figure

3 (genotype *M/M;Q/Q;M/M*) mated with their brothers (genotype *M/Y;Q/Q; M/M*) at 20°, two types of female progeny were produced with *X* chromosome genotypes *M/M* and *M/Q*, respectively. Fifteen of each type of progeny were themselves in turn individually tested for cytotype after mating with Harwich males. The mating scheme and the gonadal sterility distributions of tested females are presented in Figure 4. The results indicate that, although the cytotype of the A_{54} mothers tended to be clustered towards the *P* cytotype end of the distribution, that of their daughters was in the majority of cases predominantly determined by their *X*-chromosome constitution. Nearly all the daughters with *X* chromosomes homozygous for *M* had apparently switched to *M* cytotype, while the *P* cytotype of the *X*-chromosome heterozygous daughters remained unchanged.

X-chromosome localization of major cytotype determinants: A mapping experiment was conducted to determine the location of cytotype-determining *X* chromosome elements in the Mount Carmel strain. The mating scheme described in MATERIALS AND METHODS was employed to produce a series of lines in which single recombinant chromosomes had been made homozygous for various intervals of the Mount Carmel *X* chromosome. By choosing line A_8 (*QMM*) as the female parental line for the experiment, it was ensured that the autosomal background was consistently *M* for all the derived lines.

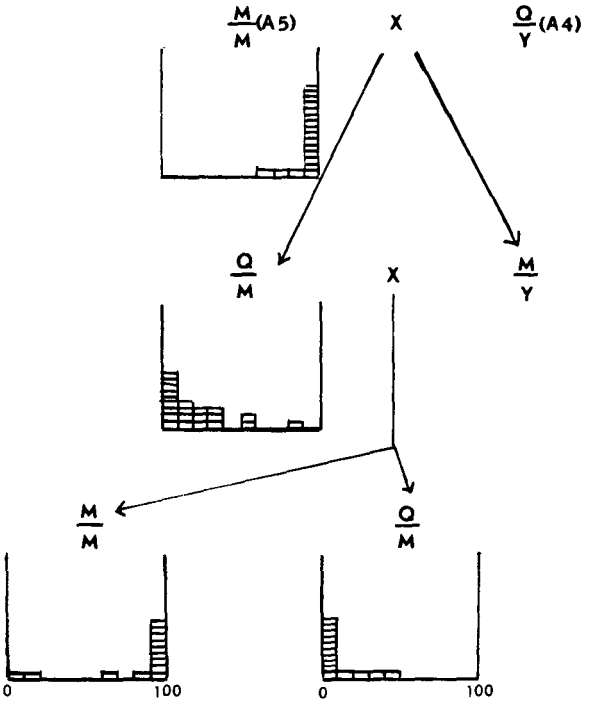

FIGURE 4.—Frequency distributions of ovarian dysgenesis in three generations from matings with Harwich males showing the effect on cytotype of substitution of a single *X* chromosome. Each rectangle represents the dysgenic ovary percentage of a single female (mated with 2 Harwich males) determined by dissection of 25 of her daughters.

The results of gonadal sterility tests of daughters of recombinant line females mated to Harwich males are presented in Table 2. Each entry in the body of the table is the dysgenic ovary percentage for an independently derived recombinant line. The results may be most economically interpreted in terms of two major cytotype-determining elements in the distal half of the *X* chromosome. Those lines homozygous *M* for the yellow (*y*) region fairly consistently showed *M* cytotype. Those lines homozygous *Q* for at least the distal half of the *X* chromosome consistently produced *P* cytotype. These results suggest that a minimum of two elements, one in the *y* region and one between *cv* and *v*, must be present to guarantee that the *P* cytotype will be manifested in an otherwise *M* genotypic background; however, the distal-most element can be sufficient.

Changes related to the I-R system: The Mount Carmel and H-41 strains used to initiate the experiment were previously characterized as inducer and reactive, respectively, in the *I-R* system of hybrid dysgenesis (M. G. KIDWELL, unpublished results). All of the synthesized lines were tested for their *I* or *R* characteristics at the completion of the experiment (approximately generation 20) by the method

TABLE 2

Percentage of ovaries that were dysgenic in progeny of female parents homozygous for various recombinant chromosomes and Harwich males

Genotype	g_6	g_{10}	Genotype	g_6	g_{10}
y cv v f	100	—	+ + + +	7	—
	100	—		0	—
	99	100		2	—
				4	0
y cv v +	92	97	+ + + f	0	0
	68	83		0	0
	94	—		6	4
	62	92		8	7
y cv + +	94	97	+ + v f	0	0
	80	71		68	74
	84	88		24	5
	84	82		85	49
	—	99		—	0
	—	92		—	6
	—	95		—	0
	—	88			
y + + +	100	95	+ cv v f	85	—
	94	95		87	46
	—	93		85	—
	—	43		—	9
	—	70		—	22
				—	12
				—	3
y + + f	65	100			

Each line represents an independent crossover event between Mount Carmel and *y cv v f X* chromosomes.

described by KIDWELL (1979). All 16 lines, including those with the complete H-41 chromosomal complement (lines A_1 and B_1), were unambiguously classified as *I* strains. This result is not unexpected, considering the very high frequencies of chromosomal contamination characteristic of the *I-R* system (BREGLIANO *et al.* 1980). It suggests, however, that the determination of cytotype in the *P-M* system is unrelated to *I-R* system designation and provides further evidence for the independence of the two systems.

DISCUSSION

In the experiments described above, the use of a neutral strain that lacks *P* factors active for gonadal sterility has made possible a detailed genetic study of the determination of the regulatory component of the *P-M* dysgenesis system because of the absence of disruptive interaction. The synthesis of chromosome lines, by whole-chromosome substitution rather than backcrossing, has not only made the mapping of chromosomal determinants of the regulatory system possible, but has provided detailed information on the relative magnitude of effect and mode of expression of different determinants. Moreover, this experimental design enabled us to monitor the interplay of both maternal cytoplasm and the various chromosomal determinants, together and separately, in their effect upon cytotype over several generations until the attainment of equilibrium. These results confirm the general pattern of cytotype determination described by ENGELS (1979), but they also provide new detailed information about the location, expression and complex interplay of the individual components of the system.

Several differences in details of transmission were observed in comparison with ENGEL's strain, π_2. Using the neutral (*Q*) Mount Carmel strain, it was found that, in any given generation, cytotype is usually determined by chromosomal factors together with the maternal cytoplasm. However, in some instances, the substitution of a single Mount Carmel *X* chromosome was able to induce a switch from *M* to *P* cytotype (or *vice versa*) in a single generation. With π_2, the cytotypic switch always required at least two generations (ENGELS 1979). However, it is uncertain whether the late generation A_5 line used in the Mount Carmel tests is completely equivalent to a pure *M* strain.

In the Mount Carmel strain, the major chromosomal determinants of cytotype are located on the *X* chromosome, with only minor factors on the major autosomes. This is in contrast to the π_2 strain studied by ENGELS, in which chromosome *2* and, to a lesser extent, the *X* chromosome are the most important in cytotype determination. Previous observations (M. G. KIDWELL, unpublished results) with another strain, Cranston, have also strongly suggested the importance of the *X* chromosome in cytotype determination. However, further extensive study of a variety of *P* and *Q* lines will be required before more general statements about the number and genomic distribution of cytotype-determining factors can be made. Our current knowledge of location of other hybrid dysgenesis determinants suggests that large interstrain variability is common.

Detailed mapping studies of hybrid dysgenesis determinants have previously been restricted to the chromosomal *P* and *I* factors. SLATKO and HIRAIZUMI

(1975) mapped a major male-recombination element to the centromeric region of chromosome *2* bounded by *pr* and *c*. Secondary elements with relatively minor effects were also suggested by their results. In a later experiment, SLATKO (1978) localized newly induced male-recombination activity to the centromeric region of chromosome *3* bounded by *st* and *cu*. In the *I-R* system, inducer factors have been mapped to single loci on the *X* and second chromosomes (approximate locations *1*–33 and *2*–61) by PELISSON and PICARD (1979). There was also evidence for two independent *I* factor loci on chromsome *3*. It should be pointed out that because of the apparent mobility of both *P* and *I* factors, their localization is difficult and has restricted general application; *P* and *I* sites are expected to differ with time in the same strain and between different strains.

Previous studies of the location of the regulatory components of hybrid dysgenesis have been restricted to the chromosomal level. The three major chromosomes are involved in the genotypic control of the reactive cytoplasmic state in the *I-R system* (BUCHETON and PICARD 1978). These chromosomal determinants appear to behave like a polygenic system, but with delayed expression of effects. In the *P-M* system, ENGELS (1979) found evidence for cytotype determinants on all three major chromosomes of the π_2 and ν_6 strains, but their map positions and relative magnitude of effect were not determined.

Although the presence of a single *X* chromosome appears to have a predominant effect on the determination of an individual female's cytotype, the data in Figure 3 indicate that the genotype or cytoplasm of the mother may, under some circumstances, strongly influence the probability of a cytotypic switch in her daughters. Daughters of mothers homozygous for all three *M* chromosomes tended not to switch from *M* to *P* cytotype when carrying a *Q X* chromosome for a single generation. However, when the mother was homozygous for at least one *Q* autosome in addition to a *Q X* chromosome, the results indicated that such a rapid switch could occur. Additional evidence for this statement is provided in Figure 4.

It is interesting to note that the observed change in cytotype over a number of generations in five out of six of the synthesized lines homozygous *M* for the *X* chromosome always occurred in the same direction, from *M* to *P*. This result is consistent with the temporal trend in the same direction observed in a number of mixed *P* and *M* and mixed *Q* and *M* populations by KIDWELL *et al.* (1981), in which chromosomes were not controlled and no artificial selection was applied.

The shift from *M* to *P* cytotype in the present experiment might be interpreted in two different ways. First, minor autosomal *P* cytotype determinants might require a number of generations to accumulate their effect. According to this explanation, the delayed expression of such determinants might be analogous to the time-lag involved in the transmission of reactivity in the *I-R* system (BUCHETON 1978; BREGLIANO *et al.* 1980). Second, *P* cytotype determinants may themselves be transposable elements. The acquisition of *P* cytotype by lines with *M X* chromosomes, but *Q* autosomes, might be explained by the transposition of *P* cytotypes determinants from the autosomes to the *X* chromosomes or to other autosomal locations.

The ability to produce *P* cytotype by transposition could be explained by either qualitative or quantitative changes. A variation in strength or function of cyto-

type determinants is possible according to the chromosomal location in which the element became integrated. Alternatively, the number of cytotype elements may be important in determining the potential for cytotypic switch. The number of elements might be increased by transposition of copies to new chromosomal locations, or it might be decreased by random genetic drift as proposed in the stochastic loss hypothesis (ENGELS 1980). An experiment is currently underway to attempt to distinguish between these alternatives: the *X* chromosomes of those mixed lines that acquired *P* cytotype without chromosomal substitution (*i.e.*, A_3, B_3, B_5, A_7, B_7) are being examined in an otherwise *M* genome to determine whether they have acquired *P* cytotype determinants by nonhomologous association over time with *Q* autosomes.

The finding of major cytotype-determining factors on the *X* chromosome raises the question of whether the *X*-chromosome suppressors of male recombination claimed by SLATKO and HIRAIZUMI (1978) are the simple Mendelian genes inferred from their report or whether they may also be cytotype determinants as defined here. The distinction between Mendelian suppressors and cytotype determinants, though not obvious, is important. The very nature of the concept of cytotype always implies a delay of at least one generation in the manifestation of chromosomal cytotype determinants compared to that of classical suppressors. Suppressor genes can be expected to act directly in the first generation to suppress *MR* factors or *P* factors in the same genome; if absent in the genome, their effect is expected to be immediately lost in the next generation. It is claimed that chromosomal cytotype-determinants are inherently different from the suppressors referred to above (a) because they appear to act *indirectly* through the maternal cytoplasm and, therefore, there is always at least a one-generation lag in their expression if initially present in the male parent, *i.e.*, at least two generations are required before suppression is manifested as the result of a switch in cytotype mediated by chromosomal determinants; and (b) although the effects of major chromosomal determinants may be strong enough to override those of other determinants in certain instances, they are nevertheless only one interacting component of a complex system; their presence or absence in any given generation does not necessarily guarantee presence or absence of effect in the next generation. A number of generations is often required for the components of the system to come to equilibrium with one another. Until more data are available, it is not possible to judge into which category, suppressor or cytotype determinant, SLATKO and HIRAIZUMI's example should most appropriately be placed. YANNOPOULOS (1978) demonstrated progressive resistance to the male recombination factor 31.1 MRF over 10 generations, suggesting a mode of cytotype determination similar to that for gonadal sterility.

As mentioned in the introduction, there is now strong circumstantial evidence that the *P* and *I* inducing factors of hybrid dysgenesis are mobile genetic sequences. If this interpretation is confirmed by detailed biochemical studies, then information on the regulation of these systems may have some broad implications for transposable element systems in other eukaryotic species in which knowledge of the regulatory systems is less far advanced (SHAPIRO 1981). An im-

portant aspect of *P* factor regulation not yet addressed is the apparent limitation of dysgenic changes to the DNA of the germ line; there is no evidence for increased frequencies of mutation or crossing over in somatic cells of *M/P* hybrids (THOMPSON, WOODRUFF and SCHAEFER 1978). It is possible that a mechanism analogous to zygotic induction in lambda may result in the repression of *P* factor excision and/or integration in somatic cells, but regulation in germ cells appears to be more complex.

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