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Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility*

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SUMMARY

Hybrid dysgenesis has been described as a syndrome of aberrant traits including sterility, male recombination, and mutation, which occurs in some inter-strain hybrids of Drosophila, but only from one of the two reciprocal crosses. In a series of experiments in which hybrids of various pedigrees were tested for sterility, it was found that a case of hybrid dysgenesis could be most easily interpreted as the interaction of two components. One component was found to be a polygenic Mendelian factor linked to each of the major chromosomes of π_2, the paternally contributing strain ('*P* strain'). These chromosomes were capable of causing sterility when inherited from either parent, provided the appropriate maternal component was also inherited. The ability to transmit this maternal component was designated 'cytotype' to indicate that it is a property of the entire cell. It was possible to classify nearly all hybrid females as either *P* or *M* cytotype on the basis of their ability to produce sterile daughters. All daughters of the *M*-cytotype mothers were susceptible to the sterilizing effects of the π_2 chromosome, whereas all, or nearly all daughters of *P*-cytotype mothers were immune. When more than one of the π_2 chromosomes were received by daughters of *M*-cytotype females, chromosomal interactions could be detected statistically, but the model of independent action remained a useful approximation. Cytotype was shown to be determined by chromosomal factors, but with limited cytoplasmic transmission. This unusual mode of inheritance can be compared with other cases of hybrid dysgenesis where the behaviour resembles that of self-replicating cytoplasmic particles which are dependent on certain chromosomes. The lack of sterility from intra-strain crosses can be explained by the fact that chromosomes capable of causing sterility also induce the *P* cytotype, and thus prevent sterility in the next generation.

1. INTRODUCTION

Inter-strain hybrids of Drosophila frequently display abnormal characteristics. Sterility, high mutation rates, male recombination, distortion of transmission ratios, non-disjunction, and chromosomal aberrations have all been observed in the syndrome of aberrant traits called hybrid dysgenesis (Kidwell, Kidwell & Sved, 1977; reviewed by Thompson & Woodruff, 1978). The various independent

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observations of hybrid dysgenesis employ a wide variety of Drosophila strains and techniques, but are united by two common characteristics. First, the traits (sterility, etc.) are never seen in established strains, but appear only in the hybrid offspring of certain pairs of strains. Secondly, the traits are manifest primarily in hybrids from just one of the two reciprocal crosses. This cross is usually between wild-derived males and laboratory females. The underlying nature of hybrid dysgenesis is unknown, but hypotheses have been formulated in terms of two transmissable but not necessarily Mendelian factors (Kidwell & Kidwell, 1976; Picard, 1976). One factor comes from the paternally contributing strain and one from the maternally contributing strain; their interaction leads to hybrid dysgenesis.

Picard & L'Heritier (1971) studied a case of hybrid dysgenesis in which the dysgenic females have reduced fertility. A large fraction of the eggs produced by these females failed to hatch regardless of which males they were mated with, provided the restrictive temperature (20°) was applied during oogenesis. Picard *et al.* (1976) found that most strains could be unambiguously classified as either the paternally contributing *inducer* type (*I*) or the maternally contributing *reactive* type (*R*). Very few strains were neutral. Dysgenic hybrids arise from the cross $R♀ \times I♂$, but not from the reciprocal or from $I \times I$ or $R \times R$ crosses. All *R* strains were long-established laboratory stocks, but *I* strains were found in natural as well as laboratory populations. Studies by Bucheton (1973), Picard (1976, 1978*a*, *b*) and Bucheton & Picard (1978) showed that the inheritance of this system was a complex mixture of chromosomal and cytoplasmic transmission.

Another kind of dysgenesis involving sterility in both sexes, male recombination and other traits occurs among the hybrids from crosses of laboratory females and π_2 males (a wild strain), but not from the reciprocal (Engels & Preston, 1979; Engels, 1979). Here the sterile females have only rudimentary ovaries and fail to produce eggs. The sterility is probably due to a failure in the early development of the germ line, and occurs when restrictive temperature ($> 27°$) is applied at late embryonic or early larval stages. A very similar case was described by Schaefer, Kidwell, & Fausto-Sterling (1979) and by Kidwell & Novy (1979). This type of hybrid dysgenesis was designated the *P–M* system, and was shown to be distinct from the *I–R* type (Kidwell, 1979). In fact some strains, such as the common laboratory stock *Canton S* contribute paternally in the *I–R* system but maternally in the *P–M* system.

In this paper an attempt is made to determine the rules of transmission within the *P–M* system. Female sterility was selected for use as an indication of hybrid dysgenesis because it is more amenable to study than any of the other traits. It can be observed in the dysgenic hybrids themselves rather than their offspring; it affects a larger proportion of the hybrids than any other trait; and its temperature-sensitivity permits experimental manipulation. The mode of inheritance of hybrid dysgenesis was determined by growing females of various pedigrees at restrictive temperatures (27–29°), then testing their ability to produce eggs. From these results there emerges a pattern of heredity in many ways similar to that of the *I–R* system with an unusual mixture of cytoplasmic and chromosomal transmission.

2. MATERIALS AND METHODS

Standard cornmeal-molasses medium was used for all experiments and stock maintenance.

(*i*) *Terminology and stocks*

Following the notation of Kidwell, Kidwell & Sved (1977), paternally contributing strains such as π_2 are *P strains* and the maternally contributing laboratory strains are *M strains*. The cross $M♀ \times P♂$ which produces dysgenic offspring is *cross A*, and the reciprocal is *cross B*.

The π_2 strain was derived from a Madison natural population in 1975 and inbred for 12 generations by full-sib mating. It is now maintained at room temperature (21°) by mass transfer.

The following laboratory strains, maintained at 25°, are all considered *M* strains since they produce at least 90 % sterile daughters when crossed to π_2 males as 29° (notation is from Lindsley & Grell, 1968).

bw; *st*: An isogenic stock which has been maintained by full-sibmating for approximately 300 generations. The second and third chromosomes carry eye-colour markers, *brown* (*bw*) and *scarlet* (*st*).

CS: The standard laboratory wild-type stock, *Canton S*.

bw'; *st'*: Chromosomal and cytoplasmic background are *Canton S*, but with the markers *bw* and *st* along with closely linked loci introduced from the isogenic stock by a six-generation backcross procedure.

CyO; *TM6* /*Xa*: Crossover suppressors with dominant visible and recessive lethal markers on the second and third chromosomes balanced by the translocation *apterous*^{Xa}.

M5: Crossover suppressor on the *X* chromosome marked by *white*^{a} and *Bar*.

(*ii*) *Sterility tests*

Females to be tested were raised at restrictive temperatures (27·5–29°), then transferred with their brothers to papered mating vials and kept for an additional 4 days. Each female was then placed in a cell of 96-cell tissue culture plate as described by Engels & Preston (1979). After 72 h at 25° each female was scored as either sterile if no eggs were produced or fertile if one or more were produced. This method was found to be a reliable test for sterility from the *P–M* system, and the *I–R* system does not interfere.

3. TRANSMISSION OF THE *P*-STRAIN CONTRIBUTION

In the following expriment we examine the ability of each of the three major chromosomes of the π_2 strain to cause sterility, both individually and in combinations. F_1 hybrid males from each of the two reciprocal crosses of π_2 and *bw'*; *st'* were mated to *bw*; *st* females at 27·5° and the offspring tested for sterility. Four eye-colour phenotypes are distinguishable from each of the two crosses with each

of the eight classes of offspring receiving one of the subsets of the π_2 chromosomes. The *bw'*; *st'* stock was used in the first generation to avoid deleterious homozygous effects in the second. As a control, the cross *bw*; *st*♀ × *bw'*; *st'*♂ was set up at the same temperature and the offspring tested for sterility

Results of the sterility tests are in Table 1. High levels of sterility occurred only in those classes receiving at least one of the major π_2 chromosomes, and combinations of π_2 chromosomes tend to be associated with more sterility than single chromosomes. The class receiving none of the major π_2 chromosomes had only 3% sterility. The difference between this class and the controls ($P = 0{\cdot}047$ by Fisher's

Table 1. *The ability of π_2 chromosomes to cause sterility*

Parental cross	Paternal chromosomes *X*	*II*	*III*	Number tested	Percent sterile Observed	Expected
bw; *st*♀ × *X'*; $\frac{bw'}{\pi_2}:\frac{st'}{\pi_2}$♂	0	0	0	113	3	3
	0	0	1	140	11	12
	0	1	0	126	53	64
	0	1	1	120	59	67
bw; *st*♀ × π_2; $\frac{\pi_2}{bw'}$; $\frac{\pi_2}{st'}$♂	1	0	0	124	24	40
	1	0	1	150	49	45
	1	1	0	128	91	78
	1	1	1	184	86	80
bw; *st*♀ × *bw'*; *st'*♂	(Control)			128	0	—

Results of sterility tests of females receiving the indicated paternal chromosomes: 0 = *M*-strain and 1 = *P*-strain chromosomes. Expected numbers refer to the hypothesis of independently acting chromosomes (see Appendix I).

exact test) maybe due to the fact that the small π_2 fourth chromosome was present in half the members of this class, or, since some male recombination is known to occur in the fathers of this class, parts of the π_2 major chromosomes may also be present. However, the low level of sterility in this class shows these two sources of π_2 genes are relatively unimportant and can be considered part of the background sterility.

The simplest model to explain these results is one in which each π_2 chromosome has a specific probability of causing sterility and acts independently of the others. The analysis of this model in Appendix I shows that the maximum likelihood estimates of these probabilities are $0{\cdot}38 \pm 0{\cdot}03$, $0{\cdot}63 \pm 0{\cdot}04$, and $0{\cdot}10 \pm 0{\cdot}01$ for the *X*, second and third chromosomes respectively, with $0{\cdot}025 \pm 0{\cdot}002$ the probability of sterility from all other sources. The assumption that each chromosome acts independently of the others was tested with a likelihood ratio test which showed that interactions between chromosomes were statistically significant ($\chi^2_4 = 43{\cdot}9$; $P < 0{\cdot}001$). However, this model may still be useful for prediction since the expected levels of sterility (Table 1) are close to those observed. The differences

between observed and expected sterility could reflect true interactions between sterility-causing factors, or they could be due to differences in genetic background or non-binomial variability.

A similar likelihood ratio test (Appendix I) shows that the sterility-causing ability of chromosome three, though much smaller than those of the X and second chromosomes, is significantly greater than zero ($\chi^2_4 = 26{\cdot}2$; $P < 0{\cdot}001$).

4. TRANSMISSION OF THE *M*-STRAIN CONTRIBUTION

In the previous section, the male contribution to hybrid dysgenesis was investigated by holding the female contribution constant and asking which kinds of sperm cause sterility in combination with *M*-strain eggs. It was found that the only requirement was that the sperm carry at least one π_2 chromosome. We now ask the complementary question: which eggs produce sterility in combination with π_2 sperm? The strategy will be to grow hybrid females at the permissive temperature, then mate them at the restrictive temperature to π_2 males and test the offspring for sterility.

(i) *Cytoplasmic transmission*

The first set of experiments was designed to test the possibility of cytoplasmic or maternal inheritance by gradually replacing *Canton S* by π_2 chromosomes in *Canton S* cytoplasm, and π_2 by *Canton S* chromosomes in π_2 cytoplasm. Mass matings with approximately 20 males and 20 females were set up at permissive temperatures (17°) for each of the two reciprocal crosses. When progeny emerged, virgin females were collected from each line. Approximately 20 of these were used to start the next generation at 17°, and the rest were individually test-crossed to π_2 males at 27·5°. From each of the test crosses, 16 female offspring (or as many as were available) were selected at random and tested for sterility. This procedure was repeated until the fourth generation.

The mating scheme to generate hybrid females is shown in Fig. 1, and the results of tests of these females are in Fig. 2. All of the 49 females designated A^1, most of which would have been sterile had they been raised at a restrictive temperature, produced mostly sterile daughters. However, the 50 B^1 females fell into two distinct categories, with 41 of them producing mostly fertile offspring and 9 producing mostly sterile offspring. Since the A^1 and B^1 females are genetically identical and differ only in the source of their cytoplasm, this result rules out maternal effect genes as the cause of the reciprocal cross effect and suggests cytoplasmic inheritance. However, the latter hypothesis is also clearly eliminated by tests of the subsequent generations. The A^2 females do not all produce sterile broods as would be expected. Instead, they fall into a bimodal distribution with half producing sterile and half fertile broods. The A^3 and A^4 females are also distributed bimodally, but with approximately $\frac{3}{4}$ and $\frac{7}{8}$ respectively producing fertile broods. The reverse trend can be seen in the B^2, B^3 and B^4 females of which approximately $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ respectively gave rise to fertile broods. Therefore, the

ability of females to produce sterile daughters is clearly influenced by paternally transmitted factors, but this inheritance follows a pattern distinctly different from Mendelian and cytoplasmic transmission.

The fact that the distributions are bimodal suggests that there are two qualitatively different categories of hybrid female. We may refer to those females producing fertile broods as '*P* cytotype' since they are similar to females of a *P* strain, and those with sterile broods as '*M* cytotype'. (I will use the word 'cytotype' rather than the more traditional 'plasmatype' since the latter implies independence from chromosomes.) The existence of these two classes suggests that any postulated sterility-causing factor is passed through the female either to all her offspring or to none of them regardless of the genotype of the offspring.

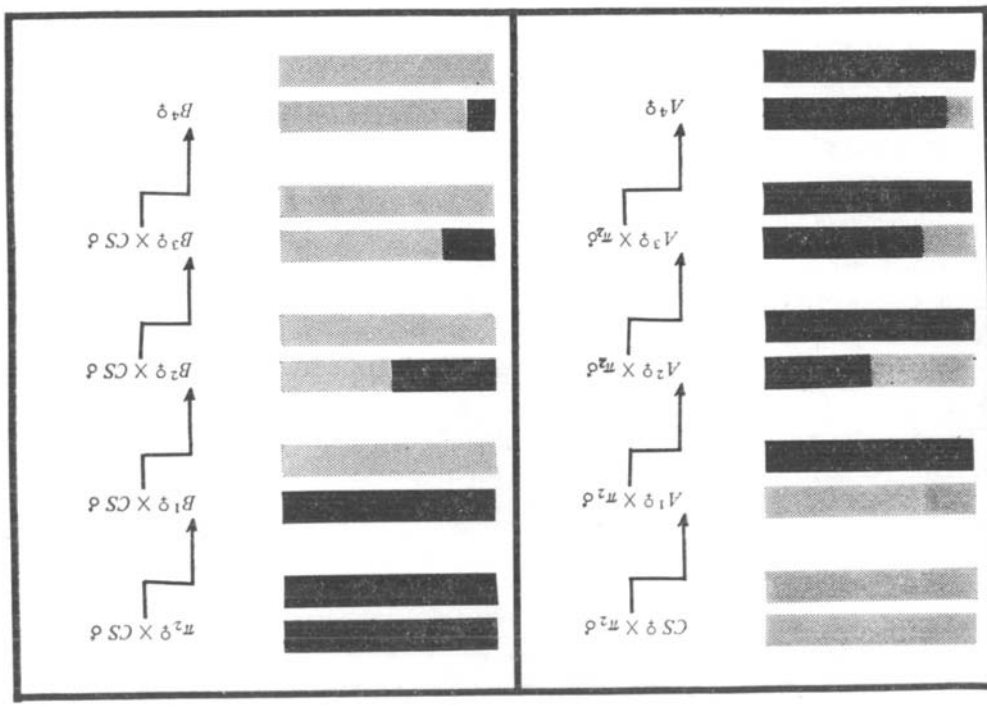

Fig. 1. Mating scheme used to produce hybrid females. All crosses were carried out at permissive temperatures. The solid bar represents the π_2 genome, and the shaded one represents the *Canton S* genome.

There might also be a few cases of intermediate cytotype. If each female belonged to one of two homogeneous classes, the histograms in Fig. 2 should resemble two *binomial* distributions. The B^2 data were used to test this hypothesis by the method in Appendix II. The analysis shows that the sterility frequencies among daughters of the B^2 females are more variable than would be expected from the double binomial distribution ($\chi^2_{13} = 52{\cdot}8$; $P < 0{\cdot}001$). Therefore, either some of the B^2 females have a third, intermediate cytotype, or there is variability within the two major classes.

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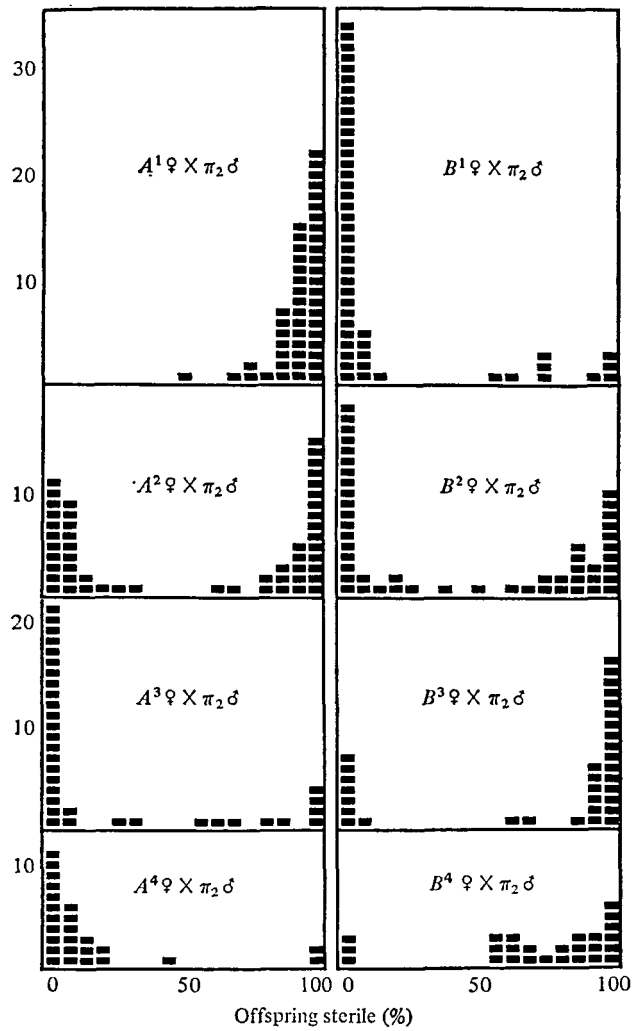

Fig. 2. Results of sterility tests at restrictive temperatures. Each block represents sterility tests of up to 16 daughters of a single female of the type indicated. These females were generated by the scheme in Fig. 1.

(ii) *Chromosomal transmission*

The following two experiments were designed to determine what role, if any, the chromosomes have in determining cytotype. For example, half of the B^2 females are of the *P* cytotype and half are *M*. Since their mothers were heterozygous for π_2 and *Canton S* chromosomes, could the difference be due to cytotype-determining alleles inherited maternally?

In the first experiment, B^2 females were produced by a scheme similar to that in Fig. 1 except that *M5* males were used instead of *CS* in the first generation. Since the *M5* *X* chromosome suppresses crossing over and carries a dominant marker, the B^2 females receiving the π_2 *X* chromosome could be distinguished from

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those receiving *M5*. Similarly, in another cross, males with marked crossover suppressors on the second and third chromosomes (*CyO*; *TM6/Xa*) were used in the first generation. The resulting B^2 females fell into four classes depending on their maternal genome. These are *CyO*; *TM6*, *CyO*; π_2, π_2; *TM6*, and π_2; π_2. As before, each B^2 female was tested for cytotype by mating her to π_2 males at restrictive temperatures and testing 16 of her daughters for sterility.

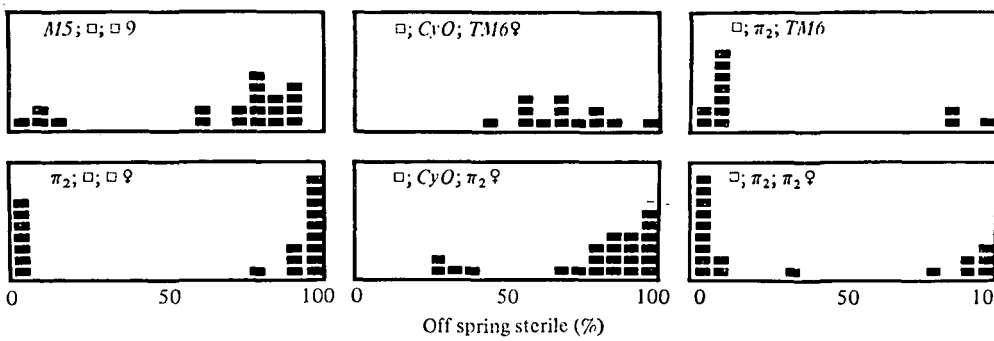

Fig. 3. Results of sterility tests. In each histogram, one block represents tests of up to 16 daughters of a single female of the indicated maternal genotype mated to π_2 males. The daughers were raised at restrictive temperatures, and the parental females were similar to the B^2 females of Fig. 1 except that *M5* or *CyO*; *TM6/Xa* males were used in the first generation rather than *Canton S*. □ = chromosome of unknown origin.

The results are in Fig. 3. It is clear that cytotype is strongly influenced by the π_2 chromosomes, especially the second chromosome. Those receiving more of the π_2 genome are more likely to be of the *P* cytotype. It is also clear that no single chromosome uniquely determines cytotype since all classes have at least one of each cytotype. One possibility is that each of the π_2 chromosomes has a characteristic probability of inducing the *P* cytotype, and acts independently of the other chromosomes. This model is similar to the one discussed previously in connexion with the determination of sterility rather than cytotype, and can be analysed by the method in Appendix I. If more than half of the daughters of a particular female were fertile, she was assumed to be *P* cytotype, and *M* otherwise. This analysis leads to the probabilities in Table 2, which are presented along with the probabilities obtained earlier that the same chromosomes will cause sterility. The similarity of these two sets of probabilities suggests that the ability to cause sterility is linked to the ability to induce the *P* cytotype, which *prevents* sterility, but not until the next generation.

The paternally inherited chromosomes can also determine cytotype, as shown by the following experiment. Males of the genotype CyO/π_2 were obtained from a cross of π_2 males with females of the *CyO* stock. These heterozygous males were then mated to π_2 females at permissive temperatures to produce daughters analogous to the B^1 females of Figs. 1 and 2. Fifteen of these females receiving the π_2 second chromosome and 22 receiving *CyO* were progeny-tested for cytotype as

in the previous experiments. Six of the *CyO* females produced at least 50 % sterile daughters and were therefore considered *M* cytotype, whereas none of those receiving the π_2 chromosome produced more than 17 % sterile daughters. The difference is significant at $P = 0{\cdot}032$ by Fisher's exact test. Therefore, as expected, the *P* cytotype is associated with the π_2 chromosome, and the *M* cytotype with *CyO*.

Table 2. *Probability of each of the* π_2 *chromosomes to cause sterility in* A^1 *females and the* P *cytotype in the* B^2 *females assuming independence of chromosome action*

	π_2 chromosome			
	X	*II*	*III*	Background
P (sterility) ± S.D.	0·38 ± 0·003	0·63 ± 0·04	0·10 ± 0·01	0·025 ± 0·002
P (*P* cytotype) ± S.D.	0·19 ± 0·15	0·67 ± 0·10	0·09 ± 0·08	*

* No background probability of *P* cytotype could be estimated since all classes could have received part of the π_2 genome.

(iii) *Total transmission*

So far it has been shown that neither cytoplasm nor chromosomes exclusively determine cytotype. Elements of both types of inheritance can be demonstrated. The first two generations of hybrid females will now be examined in greater detail to confirm the previous results and to obtain a fuller picture of the inheritance of cytotype.

Mass matings of the two reciprocal types between π_2 and the isogenic *M* strain *bw*; *st* were carried out at 21° (permissive). The female progeny were then mated individually to either π_2 or *bw*; *st* males at 21° to produce the second generation. After 4 days they were shaken into fresh vials and moved to 29° (restrictive). Up to 16 of the offspring from the second brood were tested for sterility to determine the cytotype of each of the first generation females. (As will be shown, F_1 females of the *M* cytotype give rise to some sterile daughters even when mated to *M* strain males. Therefore the cytotype of females mated to *bw*; *st* could also be determined.) This procedure reveals the cytotype of the mother of each of the second generation females. These second generation females were also progeny-tested for cytotype by mating them individually to π_2 males at 29° and testing up to 16 of the daughters for sterility.

A total of 347 females were tested for cytotype (5052 sterility tests). Each was classified as *M cytotype* if all or most of her daughters from 29° were sterile, and *P cytotype* if all or most were fertile. If an intermediate number were sterile such that the 80 % confidence interval for the fraction of sterile daughters included 0·5, the mother was classified as *undetermined*. In most cases, 16 daughters were tested so that those with 0–4 sterile daughters were classified as *P*, those with 5–11 were *undetermined*, and those with 12–16 were classified as *M*. Only 11 % of them fell into the *undetermined* category. (Those crossed to *bw*; *st* rather than π_2 males were classified as *undetermined* if the confidence interval included 0·15 rather than 0·5 since there is less sterility from this cross.)

From the mating scheme and results in Fig. 4 we make the following observations:

(1) A component of cytotype determination which is maternally inherited and independent of genotype is again demonstrated by comparing the class designated *A* with class *B*, class *F* with *I*, class *G* with *J*, and class *J* with *D*. The two classes in each pair are genetically identical, but differ in the cytotype of their mothers. In every case the proportions of the two cytotypes are markedly influenced by the cytotype of the mother.

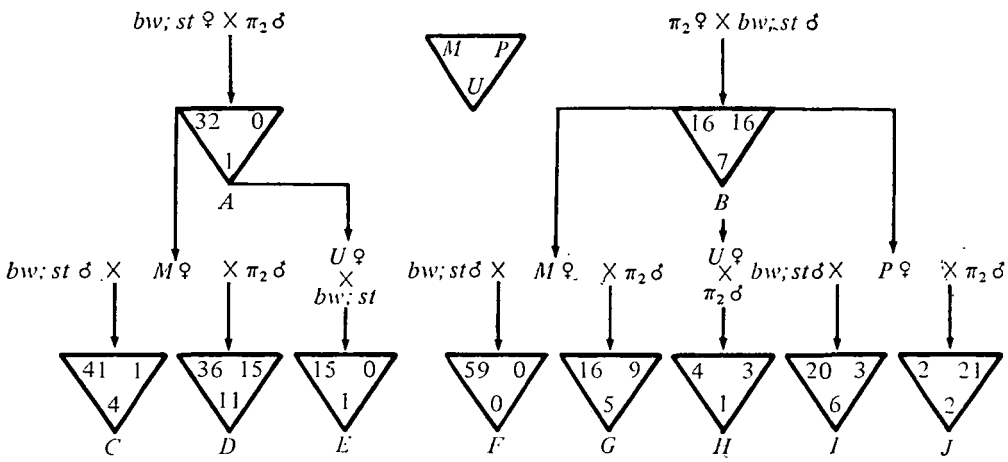

Fig. 4. Diagram showing the cytotypes of first and second generation backcross hybrids between *bw*; *st* and π_2. Each female was grown at permissive temperatures, and classified as either *M*, *P*, or *undetermined* (*U*) cytotype based on sterility tests of her offspring grown at restrictive temperatures. For all the second and about half the first generation females, the test cross was to π_2 males, and for the rest, it was to *bw*; *st* males. The triangles show the number of hybrid females to fall into each of the three categories.

(2) The fact that this maternal component can be transmitted for at least three generations can be seen by comparing class *C* with classes *F* and *I*, or class *D* with classes *G* and *J*. In each case the frequency of *P* cytotype in the class whose grandmother was *bw*; *st* is less than half the average frequency of the *P* cytotype of the two classes whose grandmother was π_2. Since cytotype was determined by testing sterility of the *progeny* of the females in question, we can say that the maternal effect from the initial cross was detectable in the great-granddaughters.

(3) The chromosomal component of the inheritance of cytotype is easily seen by comparing class *C* with *D*, class *F* with *G*, and class *I* with *J*. In each pair, the maternal contributions were identical, but the frequency of *P* cytotype was much greater among those whose father was π_2 rather than *bw*; *st*.

(4) Classes *A*, *B*, *D*, and the combined class of *F* and *I* in this experiment are analogous to classes A^1, B^1, A^2, and B^2 respectively in a previous experiment (Fig. 2) except that in the present experiment *bw*; *st* was used as the *M* strain rather than *Canton S*. Although the results are qualitatively similar in the cor-

responding classes of the two experiments, frequencies of the *M* cytotype tend to be much greater in the present experiment. Evidently, the efficacy of the *bw*; *st* chromosomes to bring about the *M* cytotype is greater than that of the *Canton S* chromosomes.

(5) The single *P* female of class *C* had only 1 sterile daughter of 16 tested, and the two *M* females of class *J* had 14 and 15 sterile daughters of 16 tested. These three individuals are of particular interest since they demonstrate that a female of a given cytotype backcrossed to a male of the corresponding strain can occasionally produce daughters of the opposite cytotype. No simple model involving only chromosomal and maternal inheritance can account for this result, and environmental or stochastic effects on the determination of cytotype are suggested.

5. INTERACTION OF *P*- AND *M*-STRAIN CONTRIBUTIONS

Hybrid females from each class tested for cytotype in Fig. 2 were also individually mated to *Canton S* males at the restrictive temperature, and 16 daughters from each were tested for sterility. As shown in Fig. 5, many of them produced large fractions of sterile daughters. In each category, the proportion producing only fertile daughters is about the same whether mated to π_2 males (Fig. 2) or *Canton S* males (Fig. 5), whereas the rest produce mostly sterile daughters when mated to π_2 males and approximate a uniform distribution when mated to *Canton S* males. All classes are consistent with the interpretation that the *P* cytotype females produce only fertile offspring when mated to either π_2 or *Canton S* males, and the *M* cytotype females, which give rise to mostly sterile offspring when mated to π_2 males, produce various mixtures of sterile and fertile offspring when mated to *Canton S*. Furthermore there is some tendency for the fraction of sterile daughters from the *M*-cytotype females to be greater when more of the π_2 genome is present. Among the eight classes, the rank correlation between the fraction of π_2 genome present and the percent of daughters sterile from those producing at least one sterile daugher (*M* cytotype) was 0·72; $P = 0{\cdot}03$. These results suggest that π_2 chromosomes can induce sterility in daughters of *M* cytotype females even when inherited maternally.

To test this hypothesis, females similar to the A^1 class, except that *M5* was used as the *M* strain rather than *Canton S*, were grown at permissive temperatures. Eighteen of these females, heterozygous for *M5* and the π_2 *X* chromosome were then mated to *Canton S* males at restrictive temperatures, and the two types of daughters were tested for sterility. As expected, in all 18 broods, the frequency of sterile daughters was higher among those receiving the π_2 chromosome (average = 86%) than those receiving *M5* (average = 53%). The difference is significant at $P = 2^{-18}$. A similar experiment using the *CyO* stock rather than *M5* gave a similar result for the second chromosome; those daughters receiving the π_2 second chromosome were more frequently sterile (average = 32%) than those receiving *CyO* (average = 6%). The difference is again significant ($P = 0{\cdot}035$ by the sign test). Therefore, the sterility-causing action of π_2 chromosomes appears

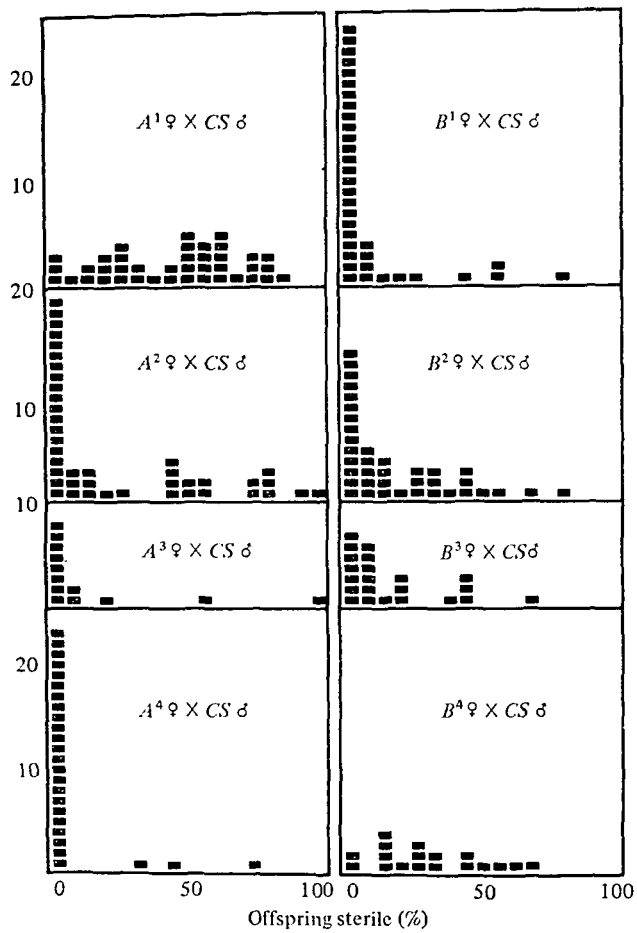

Fig. 5. Results of sterility tests at restrictive temperatures. Each block represents sterility tests of about 16 daughters of a single female of the kind indicated. These females are identical to those in Figs. 1 and 2, but the test cross was to *Canton S* rather than to π_2 males.

to be a true interaction with the maternal cytotype, rather than merely the result of paternal inheritance.

6. DISCUSSION

To sum up, hybrid dysgenesis appears to be made up of polygenic, Mendelian factors found on all major chromosomes of the *P* strains which act nearly, but not quite, independently of each other, and a non-Mendelian characteristic called 'cytotype'. Nearly all females of any pedigree can be classified as either *P* or *M* cytotype, operationally defined as follows. Females of the *P* cytotype produce few if any dysgenic offspring regardless of what male they are mated to. Hybrid dysgenesis occurs only among the offspring of *M* cytotype females, and only if these offspring possess at least one of the *P* strain chromosomes, regardless of

which parent contributed these chromosomes. The *M* cytotype can be thought of as either the ability to transmit a cytoplasmic factor causing susceptibility to the sterility-causing *P* chromosome, or the lack of a cytoplasmic suppressor of sterility. It is important to note that the *P* strain chromosomes do not necessarily cause sterility in the *M* cytotype females themselves – only in their offspring. For example, half the females from the cross π_2 female $\times$ *bw*; *st* male are of the *M* cytotype, yet virtually none of them are sterile, even when raised at restrictive temperatures.

The rules of inheritance of cytotype appear to be a complex mixture of cytoplasmic and chromosomal transmission. However, the following two generalizations are now firmly established: (1) assuming constant genotype, a female is more likely to be of a given cytotype if her mother was of that cytotype; (2) assuming constant maternal cytotype, a female is more likely to be a given cytotype if she has more of the corresponding chromosomes. It should be emphasized that these are merely statements about the *probability* of being one cytotype or the other. In general it does not seem possible to predict with certainty the cytotype of any particular female.

The lack of hybrid dysgenesis from intra-strain crosses can be explained by the fact that the π_2 chromosomes carrying sterility-causing factors are also capable of inducing the *P* cytotype, and thus *preventing* sterility in the next generation. It is tempting to suggest that the π_2 chromosomal factors responsible for causing sterility are also the source of the chromosomal side of cytotype determination. Although this possibility cannot be ruled out, the existence of *neutral* strains which are of the *P* cytotype but which produce no sterile hybrids when crossed to *M* strain females suggests that the two functions are separable. One such strain, designated ν_6 came from the same wild population as π_2, and others have been found by Kidwell *et al.* (1977). The chromosomes of the ν_6 strain were found to be similar to the π_2 chromosomes in their ability to bring about the *P* cytotype, but were unable to cause sterility (unpublished data). The complementary type of strain with the *M* cytotype and *P* chromosomes is not known. Of course such a stock could be maintained only at permissive temperatures.

These results can be compared with other cases of hybrid dysgenesis. In the I–R system, *inducer* strains have been shown to possess sterility-causing factors on more than one chromosome. Picard (1976), Sved (1976), and Kearsey *et al.* (1977) found that each of the major chromosomes is sufficient to cause some sterility of that type. The data of Sved are amenable to the analysis in Appendix I, and I find that they also show interactions between chromosomes ($\chi^2_4 = 22{\cdot}1$; $P = 0{\cdot}0002$), but that the hypothesis of independence is again a reasonable approximation in most cases. The multiplicity of dysgenesis-causing factors in the genomes of *P* and *I* strains suggests that they may be transposable or able to produce replicates at other sites. In fact, the finding of 'chromosomal contamination' by Picard (1976) implies exactly that. Matthews *et al.* (1978) and Slatko (1978) suggest that similar events may occur for male recombination. Although there was some initial evidence (Sochacka & Woodruff, 1976) that the *P* property could be transferred to

M strains by injection, large-scale attempts to repeat the finding with other strains have given negative results (Sved *et al.* 1978). All attempts to transfer the property by contact or feeding have been either negative (Picard, 1974; Waddle & Oster, 1974) or without statistical significance but interpreted as positive (Colgan & Angus, 1978; Hellack *et al.* 1978).

The rules of inheritance of cytotype, illustrated in Figs. 2, 4, and 5, are useful in interpreting some previous results on hybrid dysgenesis. For example Sved (1976) and Woodruff & Thompson (1977) studied male recombination in offspring from crosses analogous to $B^3 \times \pi_2$ and $B^4 \times \pi_2$ in Fig. 2. Although the former study was interpreted as proof of cytoplasmic heredity, and the latter as evidence against it, both are consistent with my results because B^3 and B^4 females are expected to include both *P* and *M* cytotypes. The results of Kidwell, Kidwell & Ives (1977) and Yannopoulos (1978) who studied crosses similar to $A^1 \times \pi_2$ and $B^1 \times \pi_2$ in Fig. 2, and $A^1 \times CS$ and $B^1 \times CS$ in Fig. 5 can also be interpreted in terms of the rules presented here.

The *M* cytotype might be considered analogous to the *reactive* state of the cytoplasm in the *I–R* system. Experiments on the inheritance of the level of reactivity by Bucheton (1973) and Bucheton & Picard (1978) involved hybrids between 'strong' and 'weak' reactive strains. The results showed that reactivity, like cytotype, is determined by polygenic chromosomal factors, but with limited cytoplasmic inheritance. However, reactivity does not seem to possess the dichotomous nature of cytotype. That is, bimodal distributions of reactivity analogous to those in Fig. 2 were not obtained by Bucheton & Picard. Instead, the level of reactivity varied continuously. Other important differences can be seen in the experiments by Picard (1978*a*, *b*). In contrast to the symmetrical results of the substitution experiments in Figs. 1 and 2, Picard found that substitution of *I* chromosomes into *R* cytoplasm was much more efficient than the reverse. He attributed this asymetry to the unidirectional chromosome contamination of the reactive chromosomes, which suggests that chromosomal contamination may be less important in the *P–M* system. He also found that when hybrid females were crossed with *I* males, they produced *more* fertile progeny than when crossed with *R* males. Exactly the opposite occurs in the *P–M* system, as can be seen by comparing Figs. 2 and 5.

I suggest that the key to understanding the differences between these two modes of inheritance can be found by assuming that the state of the cytoplasm can change during the development of the organism. In the early stages of development, including the temperature-sensitive period of gonadal dysgenesis of the *P–M* system, the cytoplasm resembles that of the mother. Then, depending on the chromosomal composition, it can change to the opposite type in time for *I–R* interactions to occur, but too late for *P–M* interactions to cause sterility. The otherwise puzzling finding that a certain proportion of the *cross B* females are of the *M* cytotype but are yet fully fertile is thus easily explained. Under this hypothesis, we would expect the inheritance of the *M* cytotype to resemble not the reactive cytoplasmic state, but rather sterility itself in the *I–R* system when I or

I-contaminated chromosomes are present. Comparison of the results in Fig. 4 with those of Picard (1978*a*) shows that this correspondence is exactly as expected. Furthermore, the bimodal distribution of *fertility* observed by Picard (1978*b*, fig. 3) would then agree well with the bimodal distribution for *cytotype* observed here. Finally, we can use this hypothesis to make predictions concerning the later-acting manifestations of P–M interactions such as male recombination and X–Y translocations. For example, the frequency of *cross B* males showing these traits should be the same as the frequency of the M cytotype in their sisters. Preliminary data (unpublished) are in good agreement with this expectation.

Any general theory of the underlying nature of hybrid dysgenesis must be able to explain (1) the multiplicity (and possibly transposability) of the chromosomally linked P property; (2) the dual nature of the inheritance of cytotype and the stochastic nature of its expression; and (3) the various dysgenic effects of the interaction of the P chromosomes and the M cytotype which are apparently restricted to the germ line. The first point is perhaps best handled by Green's (1977) suggestion that exogenous DNA elements similar to IS sequences of bacteria reside on the P strain chromsomes and are responsible for male recombination and high mutation rates. However, even if one adds to this hypothesis the idea that these sequences might be activated in a way analogous to zygotic induction in prokaryotes (Hayes, 1964, p. 463) to explain the reciprocal cross effect, the second and third points are still left largely untouched. There are two hypotheses which explain the second point equally well. Bucheton (1973) postulates a population of self-replicating cytoplasmic particles as determinants of cytotype whose equilibrium density is ultimately determined by the chromosomes. This equilibrium, however, is only reached gradually over several generations. Sved's (1976) model envisions a system of spatial organization of chromosomes inherited in a way much like Bucheton's particles, which can be incompatible with foreign chromosomes leading to hybrid dysgenesis. The third point, which encompasses the wide range of dysgenic effects and the variability of their occurrence in different crosses, has not been adequately dealt with by any model.

It has been suggested that hybrid dysgenesis may play a role in speciation (Kidwell *et al.* 1977; Engels & Preston, 1979). Notwithstanding the lack of specific knowledge of the nature of hybrid dysgenesis, inquiries into its inheritance such as those presented here, might help us assess its impact on natural populations and their evolution. The dynamics of a population in which P and M strains are allowed to interbreed is of primary importance. If we accept the model in which each chromosome acts to cause sterility independently of the others, and take it to its limit where each infinitesimal part of the genome is independent, then the probability that a daughter of an M cytotype female in such a population is sterile is

$$S = 1 - e^{-\theta\pi},$$

where π is the fraction of the daughter's genome originating from a P strain, and θ is a parameter which measures the intensity of the sterility-causing effect and increases with temperature. The probability that a particular individual is of the

M cytotype is a more complicated function of not only π, but the maternal cytotype as well. Knowledge of this function is needed before a complete population model can be constructed.

The excellent technical assistance of Christine Preston contributed greatly to this work.

APPENDIX I

Analysis of chromosomal effects

Suppose the X, second, and third chromosomes of a P strain can cause sterility with probabilities θ_1, θ_2, and θ_3 respectively, and let θ_0 be the probability of background sterility. If each chromosome is assumed to act independently of the others, then the probability that at least one of the chromosomes or the background will cause an individual of genotype j to be sterile is

$$S_j = 1-\phi_0\,\phi_1^{X_{j1}}\phi_2^{X_{j2}}\phi_3^{X_{j3}},$$

where X_{ji} is zero or one depending whether genotype j includes chromosome i, and $\phi_i = 1-\theta_i$. If N_j individuals of genotype j are tested, the number sterile, n_j, will be binomially distributed with parameter S_j. Then

$$L_0 = K\prod_{j=1}^{8} S_j^{n_j}(1-S_j)^{N_j-n_j} \tag{1}$$

is the likelihood of our observation of n_1, n_2,..., n_8 sterile females in the eight genotypic classes. K is a combinatorial constant. The maximum likelihood estimates of the θ_i are obtained by numerically solving the equations

$$\frac{\partial \ln L_0}{\partial\theta_i} = \frac{1}{1-\hat{\theta}_i}\sum_{j=1}^{8}\frac{X_{ji}(n_j-N_j\hat{S}_j)}{\hat{S}_j} = 0,$$

where the circumflex indicates the maximum likelihood estimates. The variance-covariance matrix of these estimates is gotten by inverting the information matrix whose elements are

$$-\frac{\partial^2 \ln L_0}{\partial\theta_i\,\partial\theta_j} = \frac{1}{(1-\hat{\theta}_i)(1-\hat{\theta}_j)}\sum_{k=1}^{8}\frac{X_{ki}X_{kj}n_k(1-\hat{S}_k)}{\hat{S}_k^2}.$$

The values of the $\hat{\theta}_i$ and their standard deviations are given in the text.

To test the hypothesis of independence of chromosome action, the test statistic

$$\chi_4^2 = -2\ln(\hat{L}_0/\hat{L}_1)$$

was used. $\hat{L}_0$ and $\hat{L}_1$ were obtained by replacing S_j in eqn (1) by $\hat{S}_j$ and n_j/N_j respectively.

The hypothesis that chromosome three causes no sterility was tested by a similar method. In this case

$$S_j = 1-\phi_0\,\phi_1^{X_{j1}}\phi_2^{X_{j2}}\phi_{12}^{X_{j1}X_{j2}},$$

with $1-\phi_{12}$ the probability of sterility due to interactions between the X and second chromosomes. Maximum likelihood estimates and a χ^2_4 statistic were obtained as in the previous case. Since both tests were significant at the 0·001 level, it was concluded that all chromosomes as well as chromosomal interactions can cause sterility.

APPENDIX II

A test for intermediate cytotypes

Let p and q be the average fractions of sterile daughters produced by P and M cytotype females when mated to π_2 males. If the frequency of P cytotype is c, then the probability that exactly k of the 16 daughters tested will be sterile is the average of two binomial probabilities;

$$P(k) = \binom{16}{k}\left[cp^k(1-p)^{16-k} + (1-c)\,q^k(1-q)^{16-k}\right].$$

An observation of $n_0, n_1, \ldots n_{16}$ females with zero, one, etc., sterile daughters is multinomially distributed with likelihood

$$L(p,q,c) = \binom{\Sigma n_i}{n_1, \ldots, n_{16}} \prod_{k=0}^{16} P(k)^{n_k}. \tag{2}$$

Values of p, q, and c were chosen to maximize the likelihood which was then compared with the general multinomial likelihood obtained by substituting $n_k/\Sigma n_i$ for $P(k)$ in equation (2). The resulting χ^2 statistic has $16-3 = 13$ degrees of freedom.

Since one of the 50 B^2 females produced only 13 rather than 16 daughters, only the other 49 were used. From them we obtain the maximum likelihood estimate $\hat{p} = 0{\cdot}052 \pm 0{\cdot}12$, $\hat{q} = 0.885 \pm 0.015$, $\hat{c} = 0{\cdot}51 \pm 0{\cdot}07$ and the test statistic

$$\chi^2_{13} = -2\sum_{k=0}^{16} n_k \ln\left(49\,\hat{P}(k)/\mathrm{n}_k\right) = 52{\cdot}8.$$

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