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Germ line aberrations associated with a case of hybrid dysgenesis in *Drosophila melanogaster* males*

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SUMMARY

Male sterility, male recombination, and transmission ratio distortion – all examples of a syndrome known as hybrid dysgenesis in *Drosophila melanogaster* – were found to involve chromosome–cytoplasm interactions. The latter two have temperature optima near 25° and involve pre-meiotic events. In addition, sex ratio distortion, and induction of certain translocations of the *X* and *Y* chromosomes (but not the autosomes) were found to be part of hybrid dysgenesis. Both are caused by chromosome–cytoplasm interactions with pre-meiotic events playing a crucial role. The results agree with previous data on female sterility in hybrid dysgenesis, which also has cytoplasmic components and premeiotic origins.

1. INTRODUCTION

The biology and genetics of a case of hybrid female sterility in *Drosophila melanogaster* have been studied in detail and reported elsewhere (Engels & Preston, 1979; Engels, 1979). The sterility was found to be due to the rudimentary condition of one or both ovaries, and is manifested as the inability of the affected females to produce eggs. There is a temperature-sensitive period in the late embryonic and early larval stages, with 27° restrictive and 21° permissive. The morphological details of the sterility suggest that it is the result of failure in the development of the germ line. Sterility occurs in the hybrid offspring of males from a wild-derived stock known as π_2 and females from laboratory strains. It does not occur in non-hybrids and occurs only very rarely in hybrids from the reciprocal cross. The rules of transmission of this sterility indicate that it is the result of interactions between factors which display an unusual mixture of cytoplasmic and chromosomal inheritance.

However, female sterility is just one aspect of a syndrome of aberrant traits known as *hybrid dysgenesis* (Kidwell, Kidwell & Sved, 1977; reviewed by Thompson & Woodruff, 1978), which has been found in a number of different inter-strain crosses. These traits include male sterility, male recombination, segregation distortion, non-disjunction, altered female recombination, meiotic bridge and fragment formation, and mutagenesis. There is evidence of at least

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two separate systems of hybrid dysgenesis (Kidwell, 1979). The details of the female sterility in the present case resemble those of the P–M system, and I will use that notation. Those strains, such as π_2, which contribute paternally are designated 'P strains', and are typically found in natural populations. The maternally contributing 'M strains' are always established laboratory stocks.

In this report, I show that male sterility, male recombination and segregation distortion occur in π_2 dysgenic hybrids, and that the latter two are temperature-sensitive. I also tested π_2 hybrids for two new traits not previously studied. These are sex ratio distortion, and the induction of certain types of translocations. The results confirm that they are both associated with hybrid dysgenesis.

To show that a trait is associated with hybrid dysgenesis, it is important to demonstrate that it appears primarily in hybrids from one of the two reciprocal P × M crosses. In the case of female sterility, the reciprocal difference is due to interactions between the π_2 chromosomes and a cytoplasmic factor contributed by females of a specific 'cytotype' (Engels, 1979). However, when dealing with hybrid effects in *males*, one must keep in mind that the two reciprocal types of hybrids are not genetically identical; they differ in the source of their sex chromosomes. Therefore, the possibility exists that reciprocal differences are due to genetic background rather than cytoplasm–chromosome interactions. If, however, one of two parental strains carries an attached-*X* chromosome, and the other does not, then sons from the two reciprocal crosses (but not the daughters) are genetically identical. Therefore I used attached-*X* M strains in these experiments to show that for each of the various traits studied in males, reciprocal differences are due to cytoplasm–chromosome interactions.

2. MATERIALS AND METHODS

Flies were grown on standard cornmeal–molasses medium. The temperature was 25° unless specified. π_2, an inbred, wild-type strain derived in 1975 from a natural population near Madison, was used as the P strain. The following laboratory stocks were used as M strains (notation is from Lindsley & Grell, 1968).

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

bw'; *st'*: Chromosomal and cytoplasmic background are from the standard laboratory stock, *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.

cn bw: An isogenic stock which has been inbred by full- or half-sib mating for approximately 300 generations. It carries two second chromosome eye colour markers, *cinnabar* (*cn*) and *brown* (*bw*) separated by approximately 47 map units on the right arm.

yf: = A standard laboratory stock, carrying the attached-*X* C(1)DX homozygous for *yellow body* (*y*) and *forked bristles* (*f*).

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yf: = ; *cn bw*; *e* Homozygous for *cn*, *bw*, and *ebony body* (*e*) on the third chromosome. Females carry attached-X C(1)DX. This stock was obtained by several generations of backcrossing *yf*: = females to males from a laboratory stock with *cn bw*; *e*.

yf: = ; *bw*; *st* Homozygous for *bw* and *st*. Females carry C(1)DX. This stock was obtained after several generations of backcrossing *yf*: = females to males from the isogenic *bw*; *st* stock.

All crosses of the form, M♀ × π_2♂ are designated 'cross A', and the reciprocal, 'cross B'.

3. RESULTS

(i) *Male recombination and segregation distortion*

Both reciprocal crosses between *yf*: = ; *cn bw*; *e* and π_2 were carried out at 25°, and the two reciprocal crosses between π_2 and *cn bw* were carried out at 17, 25 and 29°. Hybrid males from each of the eight sets were then individually mated to *cn bw* females at 25°, then transferred after 5 days to fresh vials and kept at the same temperature to produce a second brood. Counts of the four types of progeny (*cn bw*, + *bw*, *cn* +, and + +) from these crosses provided estimates of male recombination and segregation distortion.

The results (summarized in Table 1) showed that some male recombination occurred in each class of males, especially those from cross A raised at 25°. However, the level of male recombination was not homogeneous among males within a class; i.e. recombinants were clustered. For example, a standard likelihood ratio test for homogeneity among males from cross A with *cn bw* raised at 25° gave $\chi^2_{26} = 63{\cdot}2$; $P < 0{\cdot}001$. The largest cluster in this class was one with nine recombinants (eight + *bw*, and one *cn* +) and 122 non-recombinants. An even larger cluster with 26 recombinants (all + *bw*) and 166 non-recombinants occurred in another experiment. The latter case demonstrates that some clusters include only one of the two possible recombinant types.

A similar test for homogeneity was conducted for segregation distortion as measured by k = (number of + + offspring)/(total non-recombinants). Again, high values of the χ^2 statistics indicated that for all but two of the classes (cross B with *cn bw* at 17 and 25°), the k values of individual males within a class were heterogeneous.

Since neither k nor recombination frequency can be considered binomially distributed, pre-meiotic events are probably involved, and comparisons between classes can only be made with distribution-free statistical procedures. For this reason, the progeny count from each male was taken as a separate observation of either male recombination or segregation distortion, and Mann–Whitney–Wilcoxon tests (Lehmann, 1975) were used to check for differences between classes. Exact probabilities were computed in cases where large numbers of ties precluded use of the normal approximation. Results of such tests comparing each class with the reciprocal cross at the same temperature, and with the corresponding crosses at different temperatures are shown in Fig. 1. There was an

interesting parallel between tests of male recombination and those of segregation distortion. Each significant difference in male recombination corresponded to a significant difference in segregation distortion such that high male recombination goes with low transmission frequency of the π_2 chromosome. At 25°, there is a clear difference between reciprocal crosses for both male recombination and segregation distortion, but not at the two extreme temperatures. This reciprocal effect persists even when *yf*: = ; *cn bw*; *e* was used as the M strain, showing that it is not due to a difference in sex chromosomes. The temperature effect is also clear, but only among cross A classes. Both male recombination and segregation distortion are maximized at 25°.

Table 1. *Male recombination and segregation ratio distortion in π_2 hybrids*

M strain	T† (°)	Cross	Number of males tested	Total progeny	Average frequency of male recombination (%)	Average k‡	Kendall's τ§
cn bw	17	A	29	4198	0·262	0·592	0·400**
	17	B	14	2336	0·129	0·595	0·147
	25	A	30	4409	0·794	0·463	−0·238
	25	B	10	1460	0·069	0·605	−0·149
	29	A	27	5286	0·095	0·573	−0·281*
	29	B	10	1305	0·613	0·575	−0·600**
yf: = ; *cn bw*; *e*	25	A	10	1537	0·846	0·463	−0·368
	25	B	40	9281	0·043	0·522	−0·290**

* Significant at $0{\cdot}01 < P < 0{\cdot}05$. ** Significant at $P < 0{\cdot}01$.
† Temperature at which parental males were raised.
‡ k = (+ +)/(total non-recombinants).
§ Measure of correlation between male recombination and segregation distortion.

Since classes with high male recombination tend to have low k values, one might expect there to be a negative correlation between these two variables among males within each class. In fact, Hiraizumi (1971) observed such a correlation in dysgenic hybrids from other strains. To test for this possibility, Kendall's τ, a distribution-free measure of correlation (Kendall, 1955), was calculated for each class, and presented in Table 1. As expected, there were strong negative correlations at the high temperature, but the correlations were weaker at the intermediate temperature, and actually became positive at the low temperature. The source of variability underlying this correlation is somewhat enigmatic since all males in a class have identical genotype and cytoplasm, and have similar environments.

(ii) *Male sterility*

The strain *yf*: = ; *bw*; *st* was selected as the M strain for this experiment since its autosomes and *Y* chromosome are derived from the strain used most extensively for studies of female sterility. Both reciprocal crosses between *yf*: = ; *bw*; *st* and

π_2 were performed at 29°. Forty of the male offspring from cross A and ten from cross B were individually test-mated at 25° to two fertile females from a laboratory stock. At 4-day intervals, each male was moved to a fresh vial containing two new females, and the old females were left in the vial. After three transfers, all males were discarded. Nineteen of the cross A males, but only one of the cross B males were sterile as defined by the failure to produce even a single larva from any of the matings. The difference was significant at $P = 0{\cdot}03$ by Fisher's exact test. It was concluded that male sterility follows the usual rules of hybrid dysgenesis, with a reciprocal cross effect.

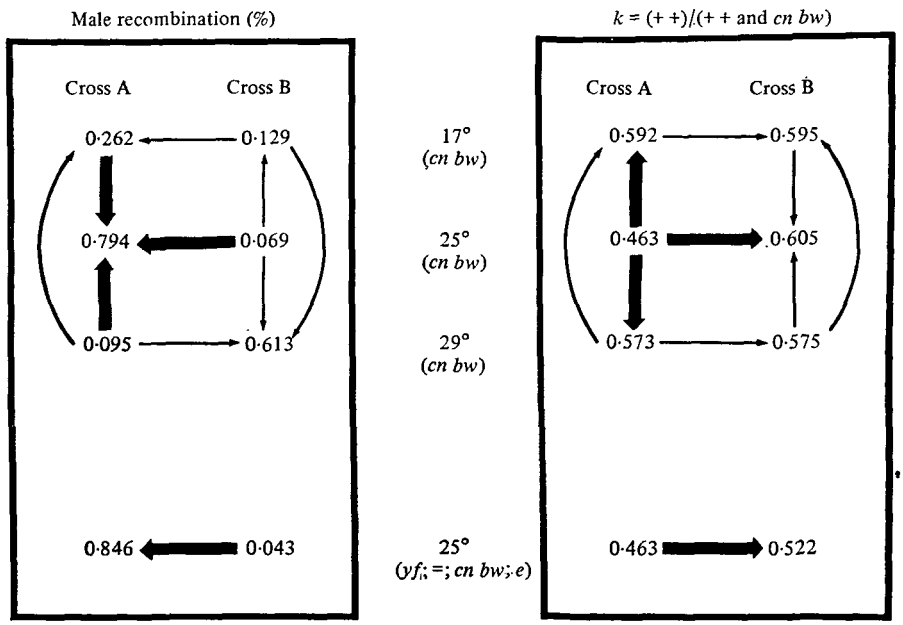

Fig. 1. Results of Mann–Whitney–Wilcoxon rank sum tests for differences in either male recombination or segregation distortion. Each arrow represents one such test. Significant differences ($P < 0{\cdot}05$, one tail test) are represented by heavy arrows.

(iii) *Translocations other than* T(X, Y)

Fig. 2 shows the mating scheme used to detect translocations among the progeny of males and females of either dysgenic or control types. It is a modification of the standard method in which a potentially translocation-bearing male of the genotype *bw*/+ ; *st*/+ is crossed to homozygous *bw*; *st* females, and the progeny examined for the absence of certain sex and eye-color combinations. Three classes of males were tested in this way. *Class A* males came from pair matings in which both parents were dysgenic hybrids suspected of producing translocation-bearing gametes at enhanced frequencies. *Class B* came from pair matings of hybrid parents genetically similar to those of *Class A*, but derived from crosses of the B type, and therefore not expected to produce high frequencies

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of translocation-bearing gametes. *Class C* came from matings in which both parents were non-hybrids, again not expected to produce translocations beyond normal spontaneous levels. To test for clustering of translocation events, and to control for the possibility of pre-existing translocations in the stocks, 12 males (or as many as were fertile) were tested from each pair mating.

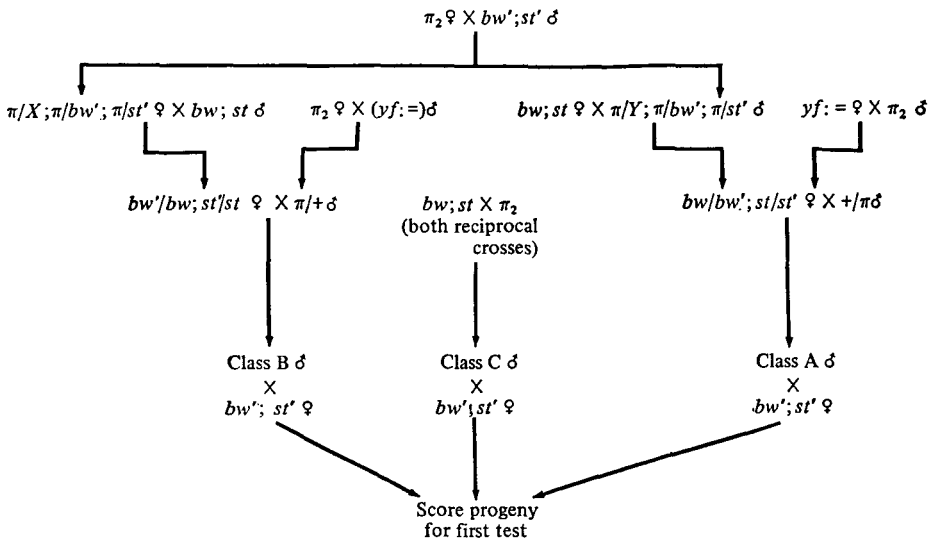

Fig. 2. Mating scheme for measuring the rate of six types of reciprocal translocations. The *bw'*; *st'* stock was used rather than *bw*; *st* in some matings to avoid deleterious homozygous effects. See text for further explanation.

There are six kinds of translocations detectable by this technique. Three of them, $T(X, 2)$, $T(X, 3)$, and $T(2, 3)$, linking the marked chromosomes were received from the mother; and the other three, $T(Y, 2)$, $T(Y, 3)$, and $T(2, 3)$, linking the unmarked chromosomes were received from the father. The rules for distinguishing these six translocations from each other and from non-translocation types are in Table 2.

As shown in Table 3, only two of a possible 15468 translocation events occurred, and one of them was in a cross B control. The overall per-gamete frequency of each type of translocation was $1{\cdot}3 \times 10^{-4}$. Therefore, these data provide no evidence for the induction of reciprocal translocations involving the second or third chromosome by hybrid dysgenesis.

(iv) X–Y *translocations and sex ratio distortion*

R. L. Berg (unpublished) using wild strains other than π_2 first observed that when hybrid males were crossed to *yf*: = females, occasionally exceptional non-*yellow*, *forked* daughters appeared. Genetic analysis and cytological analysis by R. Kreber showed that most of these were due to multiple-break translocations between the X and Y chromosomes in which the tip of the X chromosome

containing the wild type allele of *yellow* became linked to a *Y*-fragment. Repeated occurrence of certain breakpoints suggested that the primary event was not random chromosome breakage. Thus the exceptional y^+f daughters have an ordinary attached-*X*, plus the Y^{y+} fragment.

Table 2. *Rules for distinguishing six types of translocations*

Females				Males				
+ +	*bw* +	+ *st*	*bw st*	+ +	*bw* +	+ *st*	*bw st*	
				First test*				
1†	1	1	1	1	1	1	1	No translation
0	1	0	1	1	0	1	0	*T*(*X*, 2) or *T*(*Y*, 2)
0	0	1	1	1	1	0	0	*T*(*X*, 3) or *T*(*Y*, 3)
1	0	0	1	1	0	0	1	*T*(2, 3) from male or female
				Second test‡				
0	1	0	1	1	0	1	0	*T*(*Y*, 2)
1	1	1	1	1	1	1	1	*T*(*X*, 2)
0	0	1	1	1	1	0	0	*T*(*Y*, 3)
1	1	1	1	1	1	1	1	*T*(*X*,3
1	0	0	1	1	0	0	1	*T*(2, 3) from male
1	1	1	1	1	1	1	1	*T*(2, 3) from female

* Cross of the original *bw*/+ ; *st*/+ male to *bw′*; *st′* females.

† 1 = presence of indicated type; 0 = absence.

‡ Second test was performed only if first test indicated a translocation. Ten mated *bw*; *st* females from the first test were placed in fresh vials and their progeny scored.

Table 3. *Numbers of translocations obtained from dysgenic and control crosses*

	Number of males tested	*T*(*Y*, 2)	*T*(*X*, 2)	*T*(*Y*, 3)	*T*(*X*, 3)	*T*(2, 3)*	*T*(2, 3)†
Class A	1504	0	0	0	0	1	0
Class B	770	0	0	0	0	1	0
Class C	304	0	0	0	0	0	0

* From male. † From female.

The following experiments using the π_2 strain were designed to examine this phenomenon quantitatively, and to determine whether it follows the usual rules of hybrid dysgenesis. Hybrid males from crosses A and B with *yf*: = and π_2 as parents were individually mated to *yf*: = females, and all progeny emerging until the 16th day were counted. As a control, non-hybrid π_2 males were also tested in the same way.

As shown in Table 4, both types of hybrid male produced y^+f daughters at appreciable frequencies. Although most of the y^+f females were sterile, a fertile one from cross A was examined cytologically and found to be an *X*–*Y* translocation similar to those discovered by Berg. These results can be compared with those of the previous experiment which screened for a different class of

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translocation. Both experiments used the same dysgenic and control males, yet the $X-Y$ translocations detected here occurred at approximately 100 times the rate of the other classes of rearrangements. This observation confirms the previous finding that these events are not caused by random chromosome breakage.

Table 4. *$X-Y$ translocations and sex ratio distortion in π_2 hybrids*

	Number of males tested	Total progeny	Average frequency of y^+f daughters (%)	Sex ratio (% sons)	τ*
Cross A	91 (23†)	3384	1·63	46·0	−0·216
Cross B	50 (5)	2229	0·66	52·3	−0·185
π_2 non-hybrids	48 (0)	2875	0	56·2	—

* Kendall's τ measures the correlation between frequency of y^+f daughters and sex ratio; $P < 0{\cdot}05$.

† Number of males with at least one y^+f daughter.

The production of y^+f females adheres faithfully to the rules of hybrid dysgenesis. That is, it is strictly limited to hybrids, and is more frequent in hybrids from cross A than from cross B. Among the 91 males from cross A, 23 produced at least one y^+f daughter. Fisher's exact test shows that this is significantly more than the 5 of 50 cross B males or the 0 of 48 non-hybrid males, ($P < 0{\cdot}05$). These tests can be considered 'conservative' since for each comparison, the class with more translocation-producing males was also the class with fewer average offspring per male.

As with male recombination, the $X-Y$ translocations occurred in clusters. For example, one of the cross A males had 4 y^+f daughters and 14 ordinary yf: = daughters. After pooling the smaller broods of the cross A males to form a 2×25 contingency table, I performed a standard likelihood ratio test for homogeneity. The results confirmed that the production of y^+f females was clustered as opposed to binomial ($P < 0{\cdot}05$).

These data also provide evidence for yet another manifestation of hybrid dysgenesis – sex-ratio distortion. By analogy with segregation distortion of the second chromosome described earlier, one would expect the cross A males to distort against the π_2 chromosome (the X in this case) and produce too few sons. Furthermore, the rules of hybrid dysgenesis imply that the cross B males show less of this distortion, and that the non-hybrids show none at all. The data in Table 4 are in line with all of these predictions. Treating each male as a separate observation, the Mann–Whitney–Wilcoxon test was used to compare classes. As expected, the proportion of sons produced by the cross A males was significantly lower than that of either cross B or the non-hybrid males ($P < 0{\cdot}001$ for both tests). However, the difference between cross B and the non-hybrids was not significant ($P = 0{\cdot}15$).

As with the other phenomena discussed so far, there was significant non-

binomial variability among dysgenic males in the sex ratio of their offspring indicating the involvement of pre-meiotic events. For the cross A data, the test for homogeneity yielded $\chi^2_{90} = 238$; $P < 0{\cdot}001$.

Finally, by analogy with the male recombination and segregation distortion results, we might expect the frequency of y^+f females to be negatively correlated with the proportion of sons produced by the males within each class. The negative values of the non-parametric correlation are significantly different from zero, and confirm this expectation (see Table 4).

4. DISCUSSION

Most of the present results are in good agreement with other cases of hybrid dysgenesis, but two exceptions should be mentioned. First, my finding that both male recombination and segregation distortion have optima near 25° coincides with Kidwell *et al.* (1977) on male recombination, but not with those by Yannopoulos & Pelecanos (1977) who find that both traits increase monotonically with temperature. Second, Slatko & Hiraizumi (1978) report that at least some of the reciprocal cross effect on male recombination and segregation distortion is due to suppressors on the *X* chromosome. However, I find no such suppressors in the π_2 strain, and can explain the reciprocal cross effects only by extra-chromosomal factors. Other cases of non-reciprocal male recombination where *X*-linked suppressors can be ruled out were found by Kidwell (1978). Despite these points of disagreement, the similarities among the various cases of hybrid dysgenesis vastly outweigh the differences. The notion of a basic, underlying unity with only some strain-to-strain variation still seems most reasonable.

The list of abnormal characteristics in the dysgenic hybrid offspring of π_2 males now includes both male and female sterility, male recombination, distortion of segregation ratios and of sex ratios, and the production of certain kinds of translocations. In addition, M. J. Simmons studied mutagenesis in hybrids between π_2 and laboratory strains, and found elevated rates for *X*-chromosome lethal and visible mutations in the cross A hybrids (personal communication).

Some insight into the nature of hybrid dysgenesis can be gained by noting that all of these traits are germ-line abnormalities. In fact, detailed studies of the biology of female sterility (Engels & Preston, 1979; Schaefer, Kidwell & Fausto-Sterling, 1979; Kidwell & Novy, 1979) find a loss of germ cells, but no visible effects on somatic tissues. Similarly, Thompson, Woodruff & Schaefer (1978) report that male recombination and chromosome breakage are limited to the germ line. The effect is further localized by the finding that at least some of the events leading to these abnormalities occur pre-meiotically. The non-binomial or clustered occurrence of male recombination, translocations, segregation distortion and sex ratio distortion all point to pre-meiotic origins, as do the correlations observed between male recombination and segregation distortion, and between translocations and sex ratio distortion. The studies of female sterility cited above found pre-meiotic temperature effects, and Yannopoulos & Pelecanos

(1977) found that male recombination, segregation distortion, and meiotic chromosomal breakage all have pre-meiotic temperature-sensitive periods. The above observations all suggest that there is highly abnormal activity in the pre-meiotic germ cells of the dysgenic hybrids, but the specific nature of this abnormality and of hybrid dysgenesis in general remains a mystery.

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REFERENCES

ENGELS, W. R. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: Rules of inheritance of female sterility. *Genetical Research*. (In the Press.)

ENGELS, W. R. & PRESTON, C. R. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: The biology of female and male sterility. *Genetics*. (In the Press.)

HIRAIZUMI, Y. (1971). Spontaneous recombination in *Drosophila melanogaster* males. *Proceedings of the National Academy of Sciences, Washington* **68**, 268–270.

KENDALL, M. G. (1955). *Rank Correlation Methods*. London: Charles Griffen & Co.

KIDWELL, M. G. (1978). Hybrid dysgenesis in *Drosophila melanogaster*: no suppressor X effect on male recombination. *Journal of Heredity* **69**, 417–419.

KIDWELL, M. G. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: the relationship between the P-M and I-R interaction systems. *Genetical Research*. (In the Press.)

KIDWELL, M. G., KIDWELL, J. F. & SVED, J. A. (1977). Hybrid dysgenesis in *Drosophila melanogaster*: A syndrome of aberrant traits including mutation, sterility, and male recombination. *Genetics* **86**, 813–833.

KIDWELL, M. G. & NOVY, J. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: sterility resulting from gonadal dysgenesis in the P-M system. *Genetics*. (In the Press.)

LEHMANN, E. L. (1975). *Nonparametrics: Statistical Methods Based on Ranks*. San Francisco: Holden-Day Inc.

LINDSLEY, D. L. & GRELL, E. H. (1968). Genetic variations of *Drosophila melanogaster*. *Carnegie Institution of Washington*, Publication No. 627.

SCHAEFER, R. E., KIDWELL, M. G. & FAUSTO-STERLING, A. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: morphological and cytological studies of ovarian dysgenesis. *Genetics*. (In the Press.)

SLATKO, B. & HIRAIZUMI, Y. (1978). Genetic suppression of male recombination activity in *Drosophila melanogaster*. *Genetics* **88**, s92.

THOMPSON, JR, J. N. & WOODRUFF, R. C. (1978). Mutator genes: Pacemakers of evolution. *Nature* **274**, 317–321.

THOMPSON, JR, J. N., WOODRUFF, R. C. & SCHAEFFER, G. B. (1978). An assay of somatic recombination lines of *Drosophila melanogaster*. *Genetica* **49**. 77–80.

YANNOPOULOS, G. & PELECANOS, M. (1977). Studies on male recombination in a Southern Greek *Drosophila melanogaster* population. (*a*) Effect of temperature. (*b*) Suppression of male recombination in reciprocal crosses. *Genetical Research* **29**, 231–238.