Studies of $F_1$ Male Sterility from Outcrosses of Wild-type Seto - Japan Males of

Drosophila melanogaster

An Honors Thesis (ID 499)

by

Marybeth Helwig

Thesis Director
Dr. L. E. Engstrom

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INTRODUCTION

Hybrid sterility is a common property of hybrids between fully formed species and often a property of races evolving into distinct species (Dobzhansky and Pavlovsky, 1960, 1967; Dobzhansky and Spassky, 1959). While much of the research done on hybrid sterility with the genus Drosophila has utilized the species D. paulistorum, relatively little has been done using D. melanogaster. Kidwell (1979) and Sved (1976) have reported on a phenomenon termed "hybrid dysgenesis" in which crosses between laboratory strains result in various types of infertility in the offspring. Kidwell, Kidwell and Sved (1977) and, more recently, Sved (1979) have reviewed the hybrid dysgenesis syndrome and reported the following characteristics: nonreciprocality, male recombination, sterility of both male and female hybrids, mutation, transmission ratio distortion, changes in female recombination, chromosomal aberrations, and nondisjunction. The observations reported in these studies indicate that the phenomenon of sterility, mutation, and male recombinations are not simply properties of given strains but rather the results of the interactions of two strains, most often one laboratory marker and one wild type.

Ehrman (1959) has listed four types of hybrid sterility originally given by Dobzhansky (1951):

1. Chromosomal sterility, wherein differences in the gene arrangements on the chromosomes of the forms crossed lead to mechanical difficulties in the meiotic pairing and disjunctions of the chromosomes in the hybrids.

2. Genic sterility, caused by the discordant genic endowments contributed by the parents of the hybrid, resulting in a physiological disturbance of the process of gametogenesis.

3. Cytoplasmic sterility, which results in failure of gametogenesis in individuals developing from eggs deposited by mothers of cer-
tain genetic constitutions.

4. Intersexuality, where the hybrids are sterile because they are neither fully formed females or males.

Ehrman (1959) reports that F₁ sterility in D. paulistorium hybrids is due to the genic make-up of the sterile males themselves. In the same article she notes that the type of sterility produced in backcrosses to the parental strain can be attributed to maternal factors and appeared to be cytoplasmic in nature. This was later confirmed by Williamson and Ehrman (1970) who also showed the factor to be infectious and transferable by injection. Salas and Ehrman (1971) have reported at least one instance where hybrid sterility is due to an incompatibility of the Y chromosome of one strain with the cytoplasm of another.

L. E. Engstrom, in the process of related research, observed a sterility effect involving males of the F₁ generation of hybrids which had Seto strain male parents. Seto is a wild-type strain of Drosophila melanogaster first collected in Seto, Japan and maintained at the Midwest Stock Center at Bowling Green, Ohio. When males of the Seto strain were mated to another wild-type strain of D. melanogaster (Oregon-R), all of the male offspring tested were sterile. After numerous crosses of Seto males with females of various strains of D. melanogaster, it was concluded that the F₁ males were sterile regardless of what strain was used as the female parent in the parental generation. The effect also appeared to be independent of the particular genetic make-up (wild-type or mutant) carried by the non-Seto female parent.

Hybrid sterility effects of this type (involving males of the first filial generation) have been well documented in other species of Drosophila and may be the result of either cytoplasmic or chromosomal factors (Ehrman, 1959, 1964; Kernaghan and Ehrman, 1970; Salas and Ehrman, 1971; Williamson and Ehrman, 1970; Sved, 1976). The present study is an attempt to determine if the sterility in the Seto hybrids is chromosomal in nature (as opposed to cytoplasmic) and, if
so, to determine which chromosomes are involved.

**MATERIALS AND METHODS**

Two alternate hypotheses were developed as to the cause of the $F_1$ male sterility and test were conducted to determine which of the two was responsible for the observed sterility.

A) It was first hypothesized that the infertility may have been due to the interaction of non-Seto cytoplasm with the gene responsible for male sex-determination or for spermiogenesis in the $F_1$ flies. Procedure A outlines the process used to determine if this mechanism was responsible. This procedure utilized an attached-$X$ system and sequential backcrosses to Seto stock to increase the proportion of cytoplasm produced under Seto genetic control.

B) Alternately, it was suggested that the sterility was due to the interaction of non-Seto genes or chromosomes and Seto male sex-determining genes or the Seto genes controlling spermiogenesis. This was tested by constructing combinations of Seto autosomes and sex chromosomes with non-Seto autosomes and sex chromosomes and comparing the fertility of the flies carrying the various combinations. In these crosses the origin of the $X$ chromosome was not considered. This is outlined in part B of the procedure.

**Stocks:**

- $+ -$ Seto wild-type
- $+ -$ Oregon-$R$ wild-type
- $XX$ (C(1)RM) this stock has been Oregonized by repeated backcrosses to Oregon-$R$
- $X/y;Pm;D/Sb$ this stock carries Oregon-$R$ sex chromosomes

All stocks were originally obtained from Bowling Green State University (Mid-American Stock Center) and mutant genes are described in Lindsley and Grell (1968).

**Procedure A: Test for Cytoplasmic Effects**

The crosses performed to determine whether cytoplasmic influences exist or not are outlined in Figure 1. The crosses produce males possessing Seto $X$ and $Y$ sex chromosomes ($X^S/Y^S$) or Oregon $X$ and Seto $Y$ sex chromosomes ($X^O/Y^S$). These males develop from eggs containing cytoplasms representing the products of heterozygous combinations of autosomes ($F_1$ females). The $F_2$ males will, themselves,
possess various combinations of autosomes. Thus, if cytoplasmic effects exist, it might be expected that differences in fertility would be demonstrated by males possessing identical sex chromosome compositions.

**Figure 1**

Crosses performed to produce males possessing various autosome and cytoplasmic makeups. II = second autosome, III = third autosome, superscript "o" = Oregon-R chromosome, superscript "s" = Seto chromosome.

Parental \(^{\wedge}\)XX/Y\(^{\wedge}\); II\(^{O}\)/II\(^{O}\); III\(^{O}\)/III\(^{O}\) X \(+\) Seto \(\sigma^s\)  

\[\text{cross 1}\]

\(F_1\) \(+\) Oregon-R \(\sigma^s\) X \(^{\wedge}\)XX/Y\(^{\wedge}\); II\(^{O}\)/II\(^{S}\); III\(^{O}\)/III\(^{S}\) \(X\) \(+\) Seto \(\sigma^s\)  

\[\text{cross 2A}\]

\(F_2\) \(X^o/Y^s\); combinations of II and III \(X^S/Y^S\); combinations of II and III  

All \(F_2\) males were individually mated to virgin Oregon-R or Seto females.

**Procedure B:** Test for Chromosomal (Genic) Effects:

The crosses performed to determine if chromosomal effects were responsible for the hybrid sterility are outlined in Figures 2-4. Because of the limited space available in the body of the tables, only those offspring used in subsequent crosses are shown in the tables. Other offspring may be inferred from the crosses. Each \(F_2\) genotype indicated is crossed with the genotypes which carry the Cy/Pm;D/Sb chromosomes which it does not; thus, each female is crossed with two types of males and each male with two types of females. See Figure 3. The flies which result from these crosses carry all possible combinations of Seto and non-Seto (Oregon-R) autosomes and sex chromosomes. See Figure 4. By crossing these flies to Seto and Oregon-R and checking the fertility, a determination is made of which chromosomes are involved in the sterility effect.
Figure 2

Superscript "s" indicates Seto origin
Superscript "o" indicates Oregon-R origin
Cy/Pm = second chromosomes from Cy/Pm; D/Sb (autosomal)
D/Sb = third chromosomes from Cy/Pm; D/Sb (autosomal)
II = second chromosome (autosome)
III = third chromosome (autosome)
CIE = an X chromosome

(P1)  
\[ CIE/X^0; Cy/Pm; D/Sb (X) X^S/Y^S; II^S/III^S/III^S \]

(F1)  
\[ CIE/X^S; Pm/II^S; D/III^S \]
\[ (+) \]
\[ X^S/Y^S; II^S/III^S/III^S \times \]
\[ (+) \]
\[ CIE/X^S; Cy/II^S; D/III^S \]
\[ (+) \]
\[ CIE/X^S; Cy/II^S; Sb/III^S \]
\[ (+) \]
\[ X^S/Y^S; Cy/II^S; Sb/III^S \]
\[ (+) \]
\[ X^S/Y^S; Cy/II^S; D/III^S \]
\[ (+) \]
\[ X^S/Y^S; Pm/II^S; Sb/III^S \]
\[ (+) \]
\[ X^S/Y^S; Pm/II^S; D/III^S \]

(F2)  
\[ CIE/X^S; Cy/II^S; Sb/III^S \]
\[ (+) \]
\[ CIE/X^S; Cy/II^S; D/III^S \]
\[ (+) \]
\[ CIE/X^S; Pm/II^S; Sb/III^S \]
\[ (+) \]
\[ CIE/X^S; Pm/II^S; D/III^S \]
\[ (+) \]
\[ X^S/Y^S; Cy/II^S; Sb/III^S \]
\[ (+) \]
\[ X^S/Y^S; Cy/II^S; D/III^S \]
\[ (+) \]
\[ X^S/Y^S; Pm/II^S; Sb/III^S \]
\[ (+) \]
\[ X^S/Y^S; Pm/II^S; D/III^S \]

Figure 3

Examples of F2 Crosses

\[ CIE/X^S; Cy/II^S; Sb/III^S \times X^S/Y^S; Pm/II^S; D/III^S \]
\[ X^S/Y^S; Pm/II^S; D/III^S \]
\[ X^S/Y^S; Cy/II^S; Sb/III^S \times CIE/X^S; Pm/II^S; D/III^S \]

\[ X^S/Y^S; Cy/II^S; Sb/III^S \times CIE/X^S; Pm/II^S; D/III^S \]
Figure 4

(F3)  
C1B/Xs;Cy/Pm;III^s/III^s  
C1B/Xs;II^s;D/Sb  
C1B/Xs;II^s;III^s/III^s  
C1B/Xs;Cy/Pm;D/Sb  
X^o/Xs;Cy/Pm;III^s/III^s  
X^o/Xs;II^s;II^s;D/Sb  
X^o/Xs;II^s;III^s/III^s  
X^o/Xs;Cy/Pm;D/Sb 
X^s/Xs;Cy/Pm;III^s/III^s 
X^s/Xs;II^s;II^s;D/Sb 
X^s/Xs;II^s;III^s/III^s 
X^s/Xs;Cy/Pm;D/Sb 
X^o/Yo;Cy/Pm;III^s/III^s  
X^o/Yo;II^s;II^s;D/Sb  
X^o/Yo;II^s;III^s/III^s  
X^o/Yo;Cy/Pm;D/Sb  
X^o/Y;Cy/Pm;III^s/III^s  
X^o/Y;II^s;II^s;D/Sb  
X^o/Y;II^s;III^s/III^s  
X^o/Y;Cy/Pm;D/Sb  
X^s/Yo;Cy/Pm;III^s/III^s  
X^s/Yo;II^s;III^s/III^s  
X^s/Yo;Cy/Pm;D/Sb  
X^s/Y;Cy/Pm;III^s/III^s  
X^s/Y;II^s;II^s;D/Sb  
X^s/Y;II^s;III^s/III^s  
X^s/Y;Cy/Pm;D/Sb  

These flies were crossed to both Soto and Oregon-R flies and the fertility checked.
RESULTS

A. Determination of Cytoplasmic Effects

\[ X^S / Y^S; \text{ combinations of II and III} \]
\[ \text{Result} \quad \text{Seto} \quad \text{OR} \quad \text{sterile} \quad \text{fertile} \]
\[ \text{Result} \quad \text{Seto} \quad \text{OR} \quad \text{sterile} \quad \text{fertile} \]
\[ \begin{array}{cccc}
X^S / Y^S; \text{ combinations of II and III} & \text{Seto} & \text{OR} & \text{sterile} & \text{fertile} \\
\text{II} & \text{II} & \text{II} & \text{II} & \text{II} \\
\text{X} & \text{OR} & \text{sterile} & \text{fertile} \\
\end{array} \]

E. Determination of Chromosomal Effects

\[ F_3 \quad \text{Result} \quad \text{# of individuals tested} \]
\[ \text{crosses to OR} \quad \text{crosses to Seto} \quad \text{# of individuals tested} \]
\[ \begin{array}{cccc}
\text{CLE} / X^S; Cy / Pm; III^S / III^S & \text{fertile} & 4 & \text{fertile} \\
\text{CLE} / X^S; \text{II}^S / II^S ; D / Sb & \text{fertile} & 4 & \text{fertile} \\
\text{CLE} / X^S; \text{II}^S / II^S ; III^S / III^S & \text{fertile} & 4 & \text{fertile} \\
\text{CLE} / X^S; Cy / Pm; D / Sb & \text{fertile} & 5 & \text{fertile} \\
\text{X}^O / X^S; Cy / Pm; III^S / III^S & \text{fertile} & 4 & \text{fertile} \\
\text{X}^O / X^S; II^S / II^S ; D / Sb & \text{fertile} & 4 & \text{fertile} \\
\text{X}^O / X^S; II^S / II^S ; III^S / III^S & \text{fertile} & 6 & \text{fertile} \\
\text{X}^O / X^S; Cy / Pm; D / Sb & \text{fertile} & 4 & \text{fertile} \\
\text{X}^O / X^S; Cy / Pm; III^S / III^S & \text{fertile} & 4 & \text{fertile} \\
\text{X}^O / X^S; II^S / II^S ; D / Sb & \text{fertile} & 7 & \text{fertile} \\
\text{X}^O / X^S; II^S / III^S / III^S & \text{fertile} & 8 & \text{fertile} \\
\text{X}^O / X^S; Cy / Pm; D / Sb & \text{fertile} & 4 & \text{fertile} \\
\text{X}^O / Y^O; Cy / Pm; III^S / III^S & \text{fertile} & 3 & \text{fertile} \\
\text{X}^O / Y^O; II^S / II^S ; D / Sb & \text{fertile} & 3 & \text{fertile} \\
\text{X}^O / Y^O; II^S / II^S ; III^S / III^S & \text{fertile} & 4 & \text{fertile} \\
\text{X}^O / Y^O; Cy / Pm; D / Sb & \text{fertile} & 4 & \text{fertile} \\
\end{array} \]
B. Determination of Chromosomal Effects (continued)

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<th>Result of # of</th>
<th>Result of # of</th>
</tr>
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<tr>
<td>X^0_Y^S;Cy/Pm;III^S/III^S</td>
<td>no offspring*</td>
<td>4</td>
</tr>
<tr>
<td>X^0_Y^S;II^S/III^S;D/Sb</td>
<td>no offspring*</td>
<td>4</td>
</tr>
<tr>
<td>X^0/Y^S;II^S/III^S/III^S</td>
<td>no offspring*</td>
<td>4</td>
</tr>
<tr>
<td>X^0/Y^S;Cy/Pm;D/Sb</td>
<td>no offspring*</td>
<td>3</td>
</tr>
<tr>
<td>X^S/Y^O;Cy/Pm;II^S/III^S</td>
<td>fertile</td>
<td>3</td>
</tr>
<tr>
<td>X^S/Y^O;II^S/III^S;D/Sb</td>
<td>fertile</td>
<td>3</td>
</tr>
<tr>
<td>X^S/Y^O;II^S/III^S/III^S</td>
<td>fertile</td>
<td>6</td>
</tr>
<tr>
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<td>3</td>
</tr>
<tr>
<td>X^S/Y^S;Cy/Pm;III^S/III^S</td>
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<td>3</td>
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<tr>
<td>X^S/Y^S;II^S/III^S;D/Sb</td>
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<td>3</td>
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<tr>
<td>X^S/Y^S;II^S/III^S/III^S</td>
<td>fertile</td>
<td>5</td>
</tr>
<tr>
<td>X^S/Y^S;Cy/Pm;D/Sb</td>
<td>fertile</td>
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DISCUSSION

The results indicate that the sterility of the F_1 males is chromosomal and involves the interaction of Seto Y chromosomes with non-Seto X chromosomes.

Part A of the results provides evidence that the controlling factor in the sterility is not cytoplasmic. Despite the fact that all the F_2 males developed from heterozygous cytoplasm and possessed a variety of combinations of autosomes, all males which had the Seto Y in combination with an Oregon-R X were sterile; whereas, the F_2 males with both sex chromosomes being Seto were fertile. If cytoplasmic effects were involved a certain percentage of the F_2 males with both X and Y of Seto origin would have been expected to be sterile due to interactions of these sex chromosomes with cytoplasms which were of Oregon-R
Part E of the results clearly indicates that it is the Seto Y chromosome in combination with the Oregon-R X which is responsible for the effect and that autosomes II and III are not involved.

No offspring were produced by any of the crosses involving males possessing the OR X and Seto Y together with any combination of autosomes. The males with reversed sex chromosome combination (Y from OR; X from Seto) were fertile. All other combinations of sex chromosomes and autosomes were fertile.

In the case of the sterile crosses the infertility is attributed to the males since (1) eggs were present in all cases, (2) the stocks of females used had previously shown no sterility and (3) subsequent matings of females from the infertile crosses to normal males were fertile.

The specific cause of the sterility is not indicated but most probably lies in the Seto Y chromosomes being deficient for some sperm function since earlier crosses indicated that the F₁ male sterility was constant if parental females of several strains other than Seto were used.

The Seto X chromosome may correct the condition if the gene(s) defective on the Seto Y are present as normal allele(s) on the X. Grøll (1969) has found that different combinations of X heterochromatin with Y-chromosomes derived from different strains resulted not only in varied segregation patterns of XYY chromosomes, but also with certain combinations in sterility, semisterility, and lethality. Such results suggest a possible explanation for the sterility observed in this study. Namely, the heterochromatic regions of Seto X chromosomes support normal spermiogenesis and sperm function; however, the heterochromatic regions of X-chromosomes of the other strains tested do not carry this supportive function.

Sved (1976) has put forward the hypothesis that hybrid dysgenesis is the
result of a disruption of spatial organization of chromosomes, specifically to a failure of the paternally-derived chromosomes to associate normally with the nuclear membrane contributed by the female parent.

W.R. Engles and C.R. Preston describe a type of sterility which affects both sexes and occurs in the hybrid offspring of males of a wild strain (2) and laboratory females. They report that hybrids from the reciprocal cross and non-hybrids from the parental strain are fertile. The infertility of the sterile hybrid females was reported due to a lack of egg-laying and is at least partially temperature dependant. The male sterility reported is nonreciprocal and temperature dependant, but affected a much lower fraction of the individuals.

Initially it appears that the Seto hybrid sterility described in this paper is a type of hybrid dysgenesis which is not identical to any previously described but similar to the general syndrome involving the F₁ progeny of crosses between males from various wild-derived strains and females from a number of laboratory stocks. The sterility involved is generally nonreciprocal. (See Kidwell, Kidwell, and Sved, 1977). However, the sterility observed in this study departs from the general hybrid dysgenesis syndrome as described by Sved (1979) in that only sex chromosomes appear to be responsible for the sterility of hybrids.

Because the sample size in this investigation was rather small, there is no indication of the generality or completeness of the sterility produced. Mass crosses of males carrying the infertile combination of sex chromosomes are warranted.

Physiological studies of the sterile males themselves would yield indications of the specific cause of the infertility. There are several possible causes of the sterility which include: difficulties in meiosis, disturbance of spermiogenesis in the sterile male, abnormalities of the reproductive system of the sterile males preventing sperm transfer, or, if motile sperm are produced, interferences in sperm-egg interactions.
References cited:


