CHROMOSOMAL SENSITIVITY TO MEIOTIC DRIVE IN DROSOPHILA MALES

By

Bruce David McKee

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology and Genetics Program

1981

ABSTRACT

CHROMOSOMAL SENSITIVITY TO MEIOTIC DRIVE IN DROSOPHILA MALES

By

Bruce David McKee

In *Drosophila melanogaster*, males carrying $sc^{4}sc^{8}$, an *X* chromosome deficient for almost all of the basal heterochromatin, experience high *X-Y* nondisjunction and skewed sex chromosome segregation ratios. *Y* and *XY* classes are recovered poorly because of sperm dysfunction. In this study, the nature of chromosomal sensitivity to this meiotic drive system was addressed. The recovery of various normal and rearranged chromosomes was assessed by crossing flies of appropriate genotypes.

Several conclusions emerge from these studies. 1) The recovery of all chromosomes -- marked and unmarked *Y*'s, *X* chromosomes including euchromatic and heterochromatic deficiencies, and major autosomes -- is disrupted by $sc^{4}sc^{8}$. 2) The recovery probability of a chromosome from a $sc^{4}sc^{8}$ male is an inverse function of its length. 3) Autosomal and sex chromosomal segregation ratios are independent of each other. 4) Drive levels are independent of the amount of sex chromatin in the genome. 5) Heterochromatically duplicated *X* chromosomes do not induce meiotic drive, implying that unpaired heterochromatin is not responsible for the meiotic disruptions in $sc^{4}sc^{8}$ males. 6) Levels of drive and nondisjunction in $sc^{4}sc^{8}$ males can be independently modified by *Y* chromosome or autosomal background.

These conclusions have several implications for understanding the mechanism of sex chromosomal meiotic drive. The length dependence effect could be explained by assuming that $sc^{4}sc^{8}$ disrupts production of a chromosome processing material, causing a shortage and leading to competition among chromosomes. However, the lack of interaction between sex chromosomes and autosomes and the failure of additional sex chromatin to enhance drive argue against the notion that chromosomes in sc^4sc^8 males must compete for a scarce resource. An alternative explanation is that mispairing of unequal sized homologs at meiosis I causes a failure to inactivate the unpaired stretch of the larger chromosome. This stretch is then an "armed bomb" which can destroy any sperm which carry it. This hypothesis fails to account for autosomal sensitivity to sc^4sc^8-induced drive. It also predicts drive induction by heterochromatically duplicated *X*'s, contrary to observation. Furthermore, since large free pieces of *X* heterochromatin are unable to restore *Y* recovery to normal, this hypothesis implies euchromatic participation in normal *X*-*Y* pairing, again contrary to observation. It is argued that meiotic drive is caused by separation of *X* genes from a basal *X* controlling site, perhaps the same site implicated in some cases of dominant male sterility.

I dedicate this dissertation to my parents, James and Alice McKee, and to my wife Anne.

ACKNOWLEDGEMENTS

The author wishes to gratefully acknowledge the advice and guidance of Dr. Leonard G. Robbins and the technical assistance of Ms. Nancy Veenstra. Thanks are also due to my wife Anne for assisting with editing and typing and to Ellen Swanson, Roger Denome, Dr. Thomas Friedman, Dr. Peter Carlson, Dr. Loren Snyder, and Dr. Donald Beaver for reading and commenting upon the manuscript. Special thanks are due to Dr. Thomas Friedman for advice and encouragement.

TABLE OF CONTENTS

RESULTS

RESULTS

RESULTS

RESULTS

RESULTS

CHAPTER 6 SUMMARY AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In *Drosophila melanogaster* males, *X* chromosomes deficient for the basal heterochromatin, such as $In(1)sc^{4L}sc^{8R}$ ($sc^{4}sc^{8}$), cause meiotic nondisjunction of the *X* and *Y* chromosomes and abnormal sex chromosome segregation ratios (Gershenson 1933; Sandler and Braver 1954; Peacock and Miklos 1973). Recovery of *Y* sperm is depressed relative to *X* sperm and *XY* sperm are recovered very poorly relative to nullo-*X*, nullo-*Y* sperm (Sandler and Braver 1954). The absence in $sc^{4}sc^{8}$ of most of the *X* heterochromatic "collochores" (discrete *X*-*Y* pairing sites found at several locations in the heterochromatin of normal *X* and *Y* chromosomes) may be responsible for its failure to pair with the *Y* in some spermatocytes (Cooper 1964). When pairing fails to occur, the *X* and *Y* move to the same pole, thus generating *XY* and nullo-*X*, nullo-*Y* meiotic products. There is no evidence for meiotic loss; reciprocal secondary spermatocytes occur in equal frequencies (Peacock 1965). Although it is difficult to rule out post-meiotic chromosome loss, the recovery of the *X* in well over 50% of the offspring in some crosses (Peacock, Miklos, and Goodchild 1975) argues that chromosome loss can not be the whole story. The ratio of *X* to nullo-*X*, nullo-*Y* classes is substantially the same in secondary spermatocytes and in the progeny (Peacock 1965; Peacock, Miklos, and Goodchild 1975), implying that *X*-bearing sperm are recovered as well as nullo-*X*, nullo-*Y* sperm and that chromosomes are not lost post-meiotically (since that would inflate the nullo-*X*, nullo-*Y* class relative to the *X* class). Since the egg hatch in matings involving sc^4sc^8 males is reasonably normal, zygotic death can be ruled out (Peacock 1965). Electron microscopic examination of testes from sc^4sc^8 males reveals aberrant spermatid development. The most commonly observed abnormality is failure of individualization, with syncytial spermatid breakdown and elimination in the waste sac (Peacock, Miklos, and Goodchild 1975). The number of abnormal sperm per bundle varies with the severity of drive and is reasonably close to the number predicted from progeny counts. Thus, gamete dysfunction is evidently the mechanism of sc^4sc^8-induced meiotic drive.

Similar developmental abnormalities are observed in spermatids from males heterozygous for *Sd*, a naturally occurring second chromosome mutant which causes sperm ratios heavily biased toward the *Sd* chromosome. In some cases, over 99% of the progeny of an *Sd* heterozygous male receive the *Sd* chromosome from their father (Sandler, Hiraizumi, and Sandler 1959; Hartl and Hiraizumi 1976). As with sc^4sc^8 drive, sperm carrying the homolog of the driven element seem to self-destruct. In the testes of high meiotic drive *Sd* males, half the developing sperm in each bundle of 64 appear defective (Peacock, Tokuyasu, and Hardy 1972; Tokuyasu, Peacock, and Hardy 1977).

In *Sd* males, this self-destruct response can be traced to a specific second chromosome locus called *Rsp* (Responder). Normal chromosomes carry a sensitive allele of *Rsp*. All naturally occurring *Sd* chromosomes carry an insensitive variant (Hartl and Hiraizumi 1976). Current evidence favors the idea that in *Sd* heterozygotes the *Sd* allele damages its homolog, rendering it lethal to the developing sperm. An alternative view is that *Sd* fails to do something essential for sperm development normally done to its homolog by Sd^+ (Hartl and Hiraizumi 1976). However, deficiency for *Sd* mimics Sd^+, and deficiency for *Rsp* renders a chromosome insensitive to *Sd* (Sandler and Carpenter 1972; Ganetzky 1977). Wild type chromosomes may well carry no allele at the *SD* locus. Although they do carry an *Rsp* locus, the function of that locus can not be required for sperm development after anaphase since its deletion has no detectable effect except to render a chromosome resistant to *Sd*.

No specific loci have been identified in the sc^4sc^8 case. All bb^- (bobbed minus, a deficiency for the *rDNA*) *X* deficiencies tested thus far have induced both nondisjunction and drive (Lindsley and Grell 1968; Peacock and Miklos 1973; Yamamoto and Miklos 1977). Thus, a bobbed locus deficiency is a candidate for the role of drive inducer. However, an extra bobbed locus can be added to the sc^4sc^8 genotype in the form of a heterochromatic free *X* duplication (an *X* chromosome missing almost all the euchromatin) without any apparent improvement in the recovery of the *Y* (Haemer 1978). Furthermore, the severity of both drive and nondisjunction seems to vary with length of deficiency even though all the drive inducers tested thus far are completely bobbed lethal. The implication is that drive is due to deficiency for all or part of a large locus or a group of loci in the centric heterochromatin near the bobbed gene. Since only a few *X* heterochromatic deficiencies have been tested, nothing more definite can be concluded.

Chromosomal Sensitivity to Meiotic Drive

Very little is known at present about the nature of chromosomal sensitivity to meiotic drive in sc^4sc^8 males. In fact, the literature is ambiguous as to whether the sensitivity of *Y* chromosomes is a property of the *Y* or of the translocated *X* genes used to mark *Y* chromosomes. Gershenson's original study (1933) using an unmarked *Y* chromosome reported poor recovery of *XY* sperm but not of *Y* sperm. All subsequent studies have demonstrated poor recovery of *Y* sperm as well, but all have used marked *Y*'s exclusively. Chapter 1 reports a series of experiments which resolves this ambiguity by demonstrating poor *Y* recovery from sc^4sc^8 males carrying unmarked *Y* chromosomes.

Uncertainty also exists as to the unit of sensitivity of the *Y* chromosome. The sensitivity of the *Y* might be determined by a single response gene, as with *Rsp*, or by a group of response genes. Or, conceivably, sensitivity to drive could be a function of all *Y* chromatin. Chapter 3 reports a series of experiments aimed at characterizing the "unit of sensitivity" of the *Y* chromosome. Centric fragments carrying various segments of the *Y* were tested for relative sensitivity in the presence of sc^4sc^8. The results are completely consistent with the view that all *Y* chromatin is drive-sensitive since the degree of sensitivity of a fragment is a function of its length. These experiments eliminate the possibility of a single locus responder but do not rule out polygenic sensitivity, since a relatively limited number of fragments are available for testing.

Uncertainties also exist concerning *X* chromosome sensitivity. Peacock (1965) and Peacock and Miklos (1973) found similar ratios of *X* to nullo-*X*, nullo-*Y* classes in secondary spermatocytes and in progeny of sc^4sc^8 males, implying that sc^4sc^8 does not distort its own recovery. However, in high drive males, one can find sperm bundles in which more than half of the sperm are defective (Peacock, Miklos, and Goodchild 1975) meaning that some *X* and/or nullo-*X*, nullo-*Y* sperm are malfunctioning. When a third sex chromosome, either a second *Y* (Sandler and Braver 1954) or a free *X* duplication (Haemer 1978) is added to the genotype of a sc^4sc^8 male, the result is suicide drive of the sc^4sc^8 *X*. It is recovered in well under half, often less than a quarter, of the progeny. In sc^4sc^8/*Y*/*Y* males, the two *Y*'s pair and disjoin from one another leaving the univalent *X* to move randomly to one pole or the other. Despite its unpaired condition, the *X* is not lost during the meiotic divisions and presumably is included in the nuclei of half the developing spermatids (Cooper 1964). An alternative possibility that has not been rigorously ruled out is post-meiotic loss of the *X* such as has been found in some attached-*XY* males where the attached-*XY* segregates into a micronucleus which later degenerates (Hardy 1975). Micronuclei have not been reported in sc^4sc^8 males but since their occurrence was unknown at the time of the light microscope studies of sc^4sc^8, they might have been overlooked. No cytological examination of sc^4sc^8/*Y*/*Dp* males has been performed, but the genetic data are similar to those from sc^4sc^8/*Y*/*Y* males: regular disjunction of the heterochromatic elements and random assortment and poor recovery of sc^4sc^8.

Genetic evidence for meiotic drive, rather than chromosome loss, as the explanation for poor *X* recovery in sc^4sc^8/*Y*/*Dp* males is presented in Chapters 1 and 2. With different *Y* chromosomes, different levels of recovery of both *X* and *Y* chromosomes are observed. The correlation between *X* recovery and *Y* recovery is very strong in these males. This correlation implies that the same forces and, presumably, the same mechanism are operative in both cases. Since poor *Y* recovery must be caused by meiotic drive and not by chromosome loss (as loss of the *Y* would produce nullo-*X*, nullo-*Dp* sperm which are not observed), poor *X* recovery is probably also a case of meiotic drive. The definitive cytology still needs to be done on these males, but until then this evidence argues strongly against chromosome loss.

Further evidence for drive acting against the *X* as well as the *Y* has come from experiments with another sex chromosomal meiotic drive system, $T(1;4)B^S$. This is a reciprocal translocation between the *X* and the tiny fourth chromosome with an *X* break point in the proximal euchromatin (Lindsley and Grell 1968). The bulk of the *X* euchromatin is attached to the centromere of a fourth chromosome, and the remaining *X* base is capped with a fourth chromosome tip. Disjunction is regular in $T(1;4)B^S$ males, with the *X* base disjoining from the *Y* and the fourth chromosome centromeres disjoining from each other. Reciprocal meiotic products are not recovered equally. The longer element of each bivalent (the *Y* and 4^PX^D) is recovered poorly. Recovery probabilities from the two bivalents are independent of each other (Novitski and Sandler 1957). Evidently, viability interactions, so common in zygotic lethals, do not occur in this gametic lethal system.

In the drive systems just described, reduced recovery of the *X* as well as of the *Y* is observed. In all these cases, drive is acting against the euchromatic portion of the *X*, the portion in which almost all of the genes are located. sc^4sc^8 is deficient for at least 90% of the heterochromatin and is, therefore, an almost completely euchromatic chromosome. The 4^PX^D element of $T(1;4)B^S$ consists of most of the tiny fourth plus about four-fifths of the *X* euchromatin. No evidence exists as to whether or not drive can act against the heterochromatic portion of the *X*. When a free *X* duplication containing most of the heterochromatin is added to a sc^4sc^8 genotype, it disjoins from the *Y*. Under these circumstances, one can ask only whether *Dp* sperm are recovered better or worse than *Y* sperm. In fact, they are recovered far better, with two to three *Dp* sperm recovered for every *Y* sperm (Haemer 1978). This does not mean that drive acts against the *Y* only. It may be the case that drive affects both *Y* and *Dp* but disrupts recovery of the larger *Y* more than the smaller *Dp*.

Experiments described in Chapter 1 attempt to settle this point. In one experiment, recoveries of two small free *X* duplications are measured in sc^4sc^8/*Y*/*Dp* males. The duplications are small enough so that they do not disjoin (except occasionally) from the *X* or *Y*. This makes it possible to measure the relative recovery of otherwise identical sperm classes with and without the duplication. The results are consistent with but do not prove mild disruption of recovery of these rather small chromosomes. The second approach is to compare the recoveries of two reasonably large but different sized free duplications, one of *X* origin and the other of *Y* origin, against a common standard in sc^4sc^8/B^SY/*Dp* males. The result is that the B^SY:*Dp* recovery ratio is proportional to the length of the duplication,implying that the absolute recovery of *Dp* sperm declines with increasing size of duplication. However, since one can measure relative but not absolute recoveries, this interpretation is not certain. An alternative is that drive acts only against the larger of two paired homologs and only to the degree that the homologs differ in size.

No information is available in the literature concerning autosomal sensitivity to sc^4sc^8-induced drive. A test for autosomal sensitivity is described in Chapter 4. sc^4sc^8 males heterozygous for a reciprocal but asymmetric translocation between the second and third chromosomes are crossed to females heterozygous for the same translocation. The breakpoints of the translocation are such that sperm carrying three or five major autosomal arms are generated in addition to sperm carrying the usual four arms. Since the females produce complementary egg classes, the experiment permits recovery of these unusual sperm types and determination of the numbers of each type in the presence and in the absence of sc^4sc^8.

If sc^4sc^8 interferes with some aspect of chromosome processing which involves the whole genome, then one expects the sperm with five autosomal arms to have reduced recovery relative to sperm with three or four autosome arms. The results described in Chapter 4 demonstrate that autosomes as well as sex chromosomes are sensitive to sc^4sc^8-induced drive. The recovery of autosomally duplicated sperm relative to euploid sperm is considerably worse in sc^4sc^8/*Y* than in control *X*/*Y* males. Furthermore, recovery of the autosomal deficiency class is improved relative to either of the euploid classes by the presence of sc^4sc^8. These relationships hold true for all sex-chromosomal sperm classes. The fact that the recovery ratio of deficiency sperm to euploid sperm is greater in the presence of sc^4sc^8 implies that both *X* and *Y* sperm with euploid autosomal constitution suffer sperm dysfunction. Thus it now seems likely that *X* sperm produced by sc^4sc^8 males malfunction even if drive levels are not extraordinarily high.

These data also provide evidence against sperm viability interactions between sex chromosomes and autosomes in sc^4sc^8 males. The relative frequencies of autosomally duplicated, euploid, and deficient sperm are approximately the same in all sex chromosome classes. This result argues against the notion, discussed in more detail later, that sex chromosomes compete for a scarce material in sc^4sc^8 males. If competition were occurring, we would expect to find lower ratios of duplication to euploid and euploid to deficiency classes among *XY* sperm than among *Y* or *X* sperm. This is not the case; strict independence of sex chromosomes and autosomes seems to be the rule.

The conclusion of all these studies is that the probability of recovery of a sperm from a sc^4sc^8 male is an inverse function of its chromatin content. This may mean that sc^4sc^8 disrupts chromatin processing so that chromosomes become partial gametic lethals. Or, it may mean that sperm physiology is altered in some other way that renders sperm sensitive to the amount of perfectly normal chromatin. Since it is not known at present whether or not chromatin is altered in any way by sc^4sc^8 no decision between these alternatives can be made.

Modifiers of Meiotic Drive

It is often possible to gain important insights into the mechanism of a phenomenon by studying its modifiers. Sex chromosome meiotic drive systems are subject to modification by temperature at meiosis (Zimmering 1963; Zimmering and Perlman 1962; Zimmering and Green 1965), source of Y chromosome (Zimmering 1959; Zimmering 1960; Peacock and Miklos 1973), number of *Y* chromosomes (Sandler and Braver 1954), source of autosomes (Zimmering 1959; Zimmering 1960; Peacock and Miklos 1973), and amount of heterochromatin (Haemer 1978).

Lowered termperature tends to reduce meiotic abnormalities. When sc^4sc^8 males are raised at 18 or 19 degrees instead of the usual 25 degrees, both nondisjunction and drive are greatly reduced (Zimmering 1963). The reduction of nondisjunction is due partly to an increase in the probability of *X-Y* pairing and partly to random assortment of unpaired chromosomes at 18 degrees (Peacock 1965; Peacock, Miklos, and Goodchild 1975). Lowered temperature also moderates drive in $T(1;4)B^S$ males (Zimmering

1963; Zimmering and Perlman 1962) and reduces drive against the X in sc^4sc^8/Y/Y males (Zimmering and Green 1965).

Genetic background has also proved to be important in determining the level of meiotic drive. The amount of autosomal heterochromatin is a strong determinant of drive levels in sc^4sc^8 males. Deficiency for second chromosome heterochromatin suppresses nondisjunction and drive (Haemer 1978), but autosomal inversions have no substantial impact on drive levels (Ramel 1968). Sd has been found to interact strongly with sc^4sc^8. In Sd^+ progeny of sc^4sc^8/Y; Sd/Sd^+ males, recovery of Y and XY sperm is unusually high, almost equalling that of X and nullo-X, nullo-Y sperm, while in the Sd progeny the usual distorted ratios are seen (Miklos, Yanders, and Peacock 1972). It is as if a small fraction of gametes are immune to both drive systems. The degree of drive in T(1;4)B^S males depends on the source of the Y chromosome and autosomes. Segregation ratios are nearly normal when the autosomes and the Y are derived from a "low drive" stock but quite abnormal when they come from a "high drive" stock (Zimmering 1959; Zimmering 1960). Levels of drive and nondisjunction show a similar dependence on both Y and autosomal source in sc^4sc^8 males. When individual males in one cross are ranked by frequency of nondisjunction, it is found that high nondisjunction males are also characterized by high meiotic drive and low fertility and that males low in nondisjunction are low in drive and high in fertility. These correlations imply that inter-male variation reflects segregation of modifiers, presumably autosomal (Miklos, Yanders, and Peacock 1972; Peacock and Miklos 1973). The experiments described in Chapter 2 test the effects of low and high drive Y's and low and high drive autosomes on nondisjunction and drive in sc^4sc^8/Y, sc^4sc^8/Y/Dp, and sc^4sc^8/Y/Y males. The result is that both *Y* chromosomes and autosomes independently modify nondisjunction frequency and meiotic drive level in $sc^{4}sc^{8}$ males. It is also found that modifiers have parallel effects on *Y* chromosome recovery in $sc^{4}sc^{8}$/*Y* and $sc^{4}sc^{8}$/*Y*/*Dp* siblings, confirming the supposition that these two types of males suffer from the same defect. It is also shown that there is no necessary connection between sex chromosome nondisjunction and meiotic drive. Despite completely regular disjunction of the *Y* and *Dp* in $sc^{4}sc^{8}$/*Y*/*Dp* males, they are subject to the same modification of drive levels as are $sc^{4}sc^{8}$/*Y* males in whom nondisjunction is frequent. Another result is that *X* and *Y* chromosome recoveries covary in $sc^{4}sc^{8}$/*Y*/*Dp* males. A very strong correlation between recoveries of these two chromosomes was found across a wide range of recovery coefficients. This implies that *X* and *Y* chromosomes are recovered poorly for the same reason -- gamete dysfunction.

Mechanisms of Meiotic Drive

The parallel effects of both temperature and autosomal background on disjunction and drive in $sc^{4}sc^{8}$ males lend credence to the idea that nondisjunction and drive are caused by the same fundamental problem -- mispairing of *X* and *Y*. This hypothesis holds that unpaired and weakly paired chromosomes tend to be improperly processed for spermiogenesis. The consequence is developmental failure of the spermatids that carry them. The absence of most of the collochores from $sc^{4}sc^{8}$ is supposed to be responsible for its tendency to pair only weakly with the *Y*. This weak pairing leads to both nondisjunction and meiotic drive. Lowered temperature strengthens pairing forces, thus decreasing both nondisjunction and drive. Support for this theory comes from observations on the meiotic behavior of male-specific meiotic mutants. All twenty EMS-induced mutants isolated in a mutant screen for lines with high *X*-*Y* nondisjunction also caused reduced *Y* chromosome recovery (Baker and Carpenter 1972). Two of these mutants were mapped roughly to the proximal euchromatin. All the mutants proved unstable and were lost within a few months. Additional evidence for this interpretation comes from meiotic analysis of sc^4sc^8/*Y*/*Dp* and sc^4sc^8/*Y*/*Y* males. In these males the *X* essentially never pairs. Although it is included in 50% of the spermatid nuclei, its recovery is poor (Sandler and Braver 1954; Cooper 1964; Haemer 1978).

There are a number of objections to this pairing model. Meiotic pairing and disjunction is evidently inadequate to insure normal chromosome recovery. All *Y* sperm in sc^4sc^8/*Y* males derive from spermatocytes in which the *X* and *Y* paired and disjoined from each other. Yet many of these sperm fail to function. It may be that this pairing is weaker than normal and, while adequate to insure disjunction, does not provide a tight enough bond to insure normal chromosome processing. If so, why doesn't the *X* experience recovery difficulties? A similar difficulty arises in the analysis of meiosis in sc^4sc^8/*Y*/*Dp* males. Here the heterochromatic duplication, with its full complement of pairing sites, pairs and disjoins regularly from the *Y*. However, the recovery of the *Y* is poor (Haemer 1978). Similarly, disjunction is regular in $T(1;4)B^S$ males, but segregation ratios are aberrant (Novitski and Sandler 1957).

Another version of the pairing model is based on Baker and Carpenter's (1972) suggestion that chromosomes enter meiosis carrying "armed bombs" destined to explode if not defused prior to the spermatid stage. Normally, the *X* and *Y* defuse each other's bombs during meiotic pairing. This mutual facilitation depends on size matching of the two chromosomes so that each armed bomb on one chromosome can line up opposite a defusing site on the other chromosome. The heterochromatic deficiency in $\underline{sc^4sc^8}$ renders it less likely to defuse all of the $\underline{Y}$ chromosome's bombs. The Y, however, is long enough to defuse all of the $\underline{X}$'s bombs. This explains why the $\underline{Y}$, but not the $\underline{X}$, experiences recovery difficulties in $\underline{sc^4sc^8}/\underline{Y}$ males. In $\underline{sc^4sc^8}/\underline{Y}/\underline{Y}$ males, the two Y's are the same size and so can defuse each other's bombs. The absolute recovery of $\underline{Y}$ sperm should be very good in this genotype, if it could be measured. The armed bombs of unpaired chromosomes, such as $\underline{sc^4sc^8}$ in $\underline{sc^4sc^8}/\underline{Y}/\underline{Y}$ and $\underline{sc^4sc^8}/\underline{Y}/\underline{Dp}$ males, can not be properly defused. Thus the recovery of unpaired chromosomes is poor despite the absence of meiotic loss. Peacock and Miklos' pairing-dysfunction model (1973) is similar in most respects to this armed bomb model.

Although the armed bomb model is phrased in non-biological terms, it is not difficult to imagine biological candidates for the armed bomb role. For example, an armed bomb might be an actively transcribed gene. Given the evidence (reviewed below) for transcriptional shut-off during spermiogenesis, it is possible that persistence of gene activity past the normal shut-off point is detrimental to sperm development. Normally, $\underline{X}$ and $\underline{Y}$ chromosomes would help each other shut off transcription during meiotic pairing but $\underline{sc^4sc^8}$ disrupts this interaction. An alternative armed bomb candidate would be a stretch of insufficiently condensed chromatin. Normal sperm heads contain highly condensed chromatin. This conformation is facilitated by the transition from lysine-rich to arginine-rich histones during spermiogenesis (Das, Kaufmann, and Gay 1964). Perhaps $\underline{sc^4sc^8}$ interferes with the histone transition (as suggested by Kettaneh and Hartl (1976) for $\underline{Sd}$) by disrupting a necessary precondition related to pairing. There is no evidence for either of these suggestions.

They are made merely to point out concrete biological models consistent with the more abstract theory under discussion.

Although the armed bomb model explains much of the genetic data, it suffers from one major drawback -- no one has ever observed pairing between the X and Y at other than the restricted heterochromatic sites known as collochores (Cooper 1964). The failure of a free X duplication carrying a full complement of collochores to restore normal Y recovery implies that at least some of the pairing sites must be euchromatin. The side by side, point for point pairing required for the X and Y under the armed bomb model has been observed with other chromosomes but never for the X and Y.

The armed bomb model and the pairing-dysfunction model share an emphasis on pairing as fundamental to proper chromosome processing. Other models can be devised which explain most of the data, yet place less emphasis on pairing. For example, it is possible that the heterochromatic deficiency disrupts production or distribution of some essential chromosome processing material (e.g., a sperm histone). The ensuing shortage of the material would cause chromosomes to compete for adequate supplies of it. Any chromosome unable to garner enough of it would become a gametic lethal. Suppose that every binding site for the material has the same probability of binding it and that each binding site on a chromosome must be occupied for that chromosome to be non-lethal. Under shortage conditions, the longer a chromosome, the less likely it is to have all its binding sites occupied. The sensitivity of a chromosome to sc^4sc^8-induced drive would be proportional to its length. This model would agree with the armed bomb model in predicting length dependence of chromosome sensitivities, but for different reasons. Under the latter model the longer of two homologs suffers recovery disruption (to the degree that they differ in length) because the bombs on the overhanging piece are not defused. Under the competition model, both homologs are affected but the larger one is more sensitive than the shorter one. Pairing is fundamental to the armed bomb model, because without it bombs can not be defused. The role of pairing in the competitive model is not entirely clear but certainly not fundamental. Perhaps chromosomes with less than the normal amount of the scarce material also experience pairing difficulties. Or perhaps the apparent connection between nondisjunction and drive is a coincidence owing to the proximity of pairing sites and the drive inducing site.

Another plausible explanation is that the heterochromatic deficiency causes a shortage of time rather than material. Perhaps the length of time available for a key meiotic process is dependent somehow on the length of the *X* chromosome. For example, suppose that the signal to finish one step in chromosome processing and move on to the next step comes when the *X* has finished the step. Suppose further that an unusually short *X* finishes the step unusually early, before other, longer chromosomes have been able to finish. The severity of the consequences of this failure would depend upon the length of material unprocessed and thus upon the overall length of the affected chromosome. This time shortage does not lead to competition between chromosomes since time can not be sequestered. Thus the time shortage model differs from the material shortage model in its consequences for chromosome interactions.

Several aspects of these models are amenable to experimental testing.

Tests of Models

One test of the armed bomb model is based on the relationship between size discrepancy of *X* and *Y* chromosomes and drive against the longer element. Size mismatch results in an unpaired region on the longer chromosome which, according to the armed bomb model, should kill developing sperm. In all the drive examples examined thus far, the *Y* has been the longer element of the *X*-*Y* bivalent. What happens if the *X* is longer than the *Y*? Tests for meiotic drive in males carrying heterochromatically duplicated *X* chromosomes are described in Chapter 5. These unusually long *X* chromosomes have one dose of heterochromatin near the centromere and one dose near the tip. Despite the considerable *X*-*Y* size discrepancy, no drive was observed. It seems, then, that the meiotic disruptions associated with sc^4sc^8 are due not to size mismatching per se but either to the absence of some function normally located in the *X* heterochromatin or to its separation from the rest of the chromosome (as in $sc^4sc^8/Y/Dp$ males).

A second test of the armed bomb model and of the competitive model involves drive against the unpaired sc^4sc^8 *X* in $sc^4sc^8/Y/Y$ and $sc^4sc^8/Y/Dp$ males. Accoring to the armed bomb model, poor recovery of this chromosome is due to its unpaired, and therefore undefused, condition. There is no reason to think that the level of interaction between the two heterochromatic chromosomes would affect the recovery of the *X*. There is no place in the model for chromosomal interactions other than direct pairing ones. The recovery of sc^4sc^8 should be the same in $sc^4sc^8/Y/Y$ and $sc^4sc^8/Y/Dp$ males when background genotype and environmental conditions are the same.

Under the competitive model, recovery of the *X* chromosome should be inversely related to the amount of sex chromatin in the genome; the more sex chromatin, the fiercer the competition and the smaller the average ration of the scarce material. Since a *Y* chromosome is considerably larger than a free *X* duplication, drive levels should be higher (and *X* chromosome recovery lower) in $sc^4sc^8/Y/Y$ males than in $sc^4sc^8/Y/Dp$ males.

Some of the results in Chapter 3 are consistent with the prediction of the competitive model. However, none of the results in which autosomal background is fully controlled are consistent with it. The best evidence is that *X* chromosome recovery is independent of amount of sex chromatin in the genome, implying absence of competition.

Another test for competition involves adding a fourth sex chromosome to a sc^4sc^8 genome and monitoring its effect on *X* recovery. Most sc^4sc^8 males with four sex chromosomes are sterile, but $sc^4sc^8/Y^S/B^SY/Dp$ males do produce some offspring. *X* recovery in these males is no worse than in $sc^4sc^8/B^SY/Dp$ or $sc^4sc^8/B^SY/Y^S$ siblings. Thus, increasing the amount of sex chromatin in the genome does not exacerbate drive levels.

A third test for competition was described previously. To briefly recapitulate, sc^4sc^8 males carrying a reciprocal asymmetric translocation between the second and third chromosomes exhibit drive sensitivity of autosomes as well as sex chromosomes. If chromosomes compete for a scarce resource, one would expect the amount of autosomal chromatin to influence the frequencies of the various sex chromosome classes and vice versa. However, this is not the case. Autosomal and sex chromosome recoveries are independent.

The absence of competition implies that the meiotic disruptions induced by sc^4sc^8 are not due to shortage of a chromosome processing material. This result is consistent with but does not prove the armed bomb model. It is also consistent with other shortage models which do not imply competition (e.g., time shortage). However, no critical tests of such models have been devised.

Meiotic Drive and the Genetics of Spermiogenesis

The chief difficulty in explaining male meiotic drive systems in *Drosphila melanogaster* is the evidence that post-meiotic germ cell development is not dependent upon expression of any genetic functions carried by those cells. If any such function existed, it would be possible to find mutants that can not be transmitted by heterozygous males (gametic lethals). None have been found in Drosophila (Lindsley and Lifschytz 1972). There is also no post-meiotic chromosomal requirement. Clearly the *X* and *Y* are dispensible since each is absent from half the sperm. That autosomes are also dispensible is proved by the recovery of sperm deficient for one autosomal arm and duplicated for another as segregants from whole arm reciprocal translocations (Muller and Settles 1927). Males carrying compound second and third chromosomes routinely produce nullo-*2* and nullo-*3* sperm which function perfectly well in fertilization. In fact, sperm carrying only the tiny fourth chromosome have been recovered in progeny of such males (Lindsley and Grell 1969). Biochemical studies (Olivieri and Olivieri 1965; Gould-Somero and Holland 1974) indicate that transcription does not occur in spermatids or sperm of Drosophila, although some transcription clearly does occur in mammalian spermatids (Monesi 1965; Moore 1971).

The fact that no genetic functions are expressed post-meiotically does not mean that post-meiotic development is free of genetic control. Most male-sterile mutations in Drosophila interrupt development after meiosis (Linsley and Lifschytz 1972). Translational activity continues unabated after meiosis despite the transcriptional shut-off (Brink 1968; Gould-Somero and Holland 1974). Apparently, messages synthesized in the spermatocyte are stored for later use in spermatids.

Given the evidence for absence of post-meiotic gene expression, explanations of meiotic drive can not invoke damage to essential spermatid functions. Instead one is tempted by the opposite conclusion, that genetic repression is essential for sperm development and that meiotic drive is caused by inappropriate post-meiotic gene expression. No direct evidence for this idea exists. A related explanation is that meiotic drive is caused by a failure of spermatid chromosomes to achieve adequate condensation. Kettaneh and Hartl (1976) have shown that spermatids in Sd homozygotes do not undergo the histone transition from lysine-rich to arginine-rich histones necessary for proper chromatin condensation. Electron micrographs reveal inadequate chromatin condensation in many sperm of Sd males (Peacock, Tokuyasu and Hardy 1972; Tokuyasu, Peacock and Hardy 1977; Kettaneh and Hartl 1980). Sex chromosome meiotic drive also involves sperm dysfunction although it is not clear whether or not chromatin condensation is normal (Peacock, Miklos and Goodchild 1975).

Meiotic Drive, Sterility, and X Inactivation

In both Sd (Hartl, Hiraizumi and Crow 1967) and sc^4sc^8 (Peacock and Miklos 1973) an inverse correlation between severity of drive and degree of male fertility has been observed. Perhaps severe meiotic drive could cause complete sterility. There are indications that certain types of chromosomally-based male sterility are related to meiotic drive. In Drosophila and other organisms, many *X*-autosome translocations are male sterile. Addition of a duplication covering the region of the *X* breakpoint does not restore fertility to sterile *T(X;A)*'s, indicating that the sterility is dominant. Many *X*-*Y* translocations are also sterile, but an extra *Y* usually rescues them, suggesting that the *Y* breakpoint has interrupted a fertility factor. Almost all autosome-autosome translocations are fertile in both sexes. The distribution of breakpoints between the fertile and sterile *X*-autosome translocations is informative. Fertile *X*-autosome translocations either have both breakpoints near the tips or have an *X* breakpoint in the proximal heterochromatin and an autosomal break anywhere. In both cases the bulk of the *X* euchromatin remains intact (Lifschytz and Lindsley 1972).

Lifschytz and Lindsley (1972) have explained these results by proposing that *X* genes are normally inactivated earlier than autosomal genes in the primary spermatocyte and that *X*-autosome translocations interrupt this timing by separating *X* genes from a proximal controlling site. Considerable evidence exists for early *X* inactivation in a wide variety of male heterogametic organisms.

It is interesting that at least two *X;4* translocations with central *X* breakpoints cause meiotic drive while *X;4* translocations with terminal *X* breaks do not (Novitski and Sandler 1957; Zimmering 1960; Chapter 5, below). Perhaps the fundamental lesion here is the same as that involved in translocation male sterility; the difference between sterility and meiotic drive being a matter of degree. Quantitatively, the difference is not a large one; $T(1:4)B^S$ males are not very fertile (Novitski 1970). If the largely heterochromatic fourth chromosome is closer to the *X* in terms of inactivation cycle than are the two major autosomes, these observations would imply that prevention of early inactivation of portions of the *X* chromosome can lead either to meiotic drive or to sterility, depending on the severity of the disruption.

Another parallel between sex chromosome meiotic drive and chromosomal sterility can be seen in the phenotypes of basal *X* deficiencies. All heterochromatic deficiencies encompassing the bobbed locus which have been examined thus far cause nondisjunction and meiotic drive. Deficiencies encompassing both bobbed and suppressor of forked (*su(f)*), the most proximal known gene in the *X* euchromatin, are male sterile, at least in the presence of certain *Y* chromosomes (Lifschytz and Lindsley 1972). Since these deficiencies are missing essential genes, they are male lethal except in the presence of *Y* chromosomes duplicated for the proximal *X* euchromatin, such as $mal^{+}Y$ and $B^{S}Y$. There is a negative correlation between the size of the duplication and male fertility, an effect reminiscent of the impact of additional sex chromatin on fertility in $sc^{4}sc^{8}$ males. The parallel would be stronger if we knew that the fertile *Df;Dp* combinations experienced drive, but the appropriate tests have not yet been done.

Another interesting connection between sex chromosome meiotic drive and male sterility has recently been observed (Lindsley, unpublished data). Males carrying both a *Y*-autosome translocation and $sc^{4}sc^{8}$ are sterile even if the translocation is fertile in the presence of a normal *X*. Attempts to map the *X* heterochromatic locus responsible for this effect are inconclusive so far. It is not clear whether this phenomenon is best seen as $sc^{4}sc^{8}$ modifying the phenotype of *Y*-autosome translocations or vice versa. However, it does strengthen the view that meiotic drive and chromosomal male sterility are closely related phenomena.

Materials and Methods

The methods used in this study are those of classical genetics -- analysis of progeny counts from crosses of individuals with appropriate genotypes. The materials are chromosome rearrangements; they are described in the text as the experiments involving them are introduced. All crosses were done on standard cornmeal-molasses-yeast-carragheenin medium. Except where noted, crosses were done in single pairs.

mei

abou

stu

dri

KY

(Ge

re

Whe

Bra

Ge

rou

los

X

Ho

is

ti

al

s

b

e

CHAPTER 1

SENSITIVITY OF SEX CHROMOSOMES TO MEIOTIC DRIVE

Despite considerable research into the nature of $sc^{4}sc^{8}$-induced meiotic drive, (reviewed in the Introduction), some ambiguity remains about the chromosomal targets against which meiotic drive acts. This study examines the sensitivity of *X* and *Y* chromosomes to $sc^{4}sc^{8}$-induced drive. All investigators agree that in $sc^{4}sc^{8}/Y$ males the recovery of *XY* sperm is severly reduced relative to its reciprocal (nullo-*X*, nullo-*Y*) (Gershenson 1933; Sandler and Braver 1954; Peacock 1965). Some doubt remains, however, about the recovery of *Y* sperm relative to *X* sperm. When marked *Y* chromosomes are used, *Y* recovery is depressed (Sandler and Braver 1954; Peacock and Miklos 1973). However, using an unmarked *Y*, Gershenson (1933) found equality of *X* and *Y* classes. Ambiguity also surrounds the question of *X* chromosome sensitivity. The absence of meiotic loss points to gamete dysfunction as the mechanism responsible for poor *X* recovery in $sc^{4}sc^{8}/Y/Y$ males (Sandler and Braver 1954; Cooper 1964). However, post-meiotic loss has not been ruled out. If it can be estabished that $sc^{4}sc^{8}$ is sensitive to drive in $sc^{4}sc^{8}/Y/Y$ males, the sensitivity of *X* heterochromatin will remain in doubt because $sc^{4}sc^{8}$ is an almost entirely euchromatic chromosome. It is necessary to measure the sensitivity of *X* heterochromatic duplications to $sc^{4}sc^{8}$-induced drive.

Marked *Y* chromosomes certainly are convenient for genetic studies, but interpretation of results obtained with them is clouded by the presence of the translocated *X* genes. It is possible, especially given

Gershenson's results, that poor recovery of marked *Y*'s from sc^4sc^8 males is due to the translocation rather than to the *Y* itself. It is known that some translocations involving the *X* chromosome can induce meiotic drive. Males carrying a translocation which moves about four-fifths of the *X* euchromatin to the fourth chromosome ($T(1;4)B^S$) produce distorted gametic ratios with poor recovery of the 4^PX^D element (the translocation half containing the fourth chromosome centromere and the distal section of the *X*) and of the *Y* (a marked *Y*) (Novitski and Sandler 1957). The translocated pieces in marked *Y*'s are certainly much smaller than in $T(1;4)B^S$ and do not normally induce meiotic drive. But, in the presence of drive inducers like sc^4sc^8 or $T(1;4)B^S$, they might exhibit unusual sensitivity to drive.

In order to determine whether the sensitivity of a marked *Y* is due to the translocated *X* markers or to the *Y* itself, a reexamination of sc^4sc^8-induced drive was undertaken using unmarked *Y* chromosomes. The experiments were aimed at measuring recovery of the *Y* in sc^4sc^8/Y and $sc^4sc^8/Y/Dp$ males. The latter genotype was tested because a previous report (Haemer 1978) had indicated poor recovery of the *Y* despite regular disjunction from the free *X* duplication.

Uncertainties also remain about sensitivity of the *X* chromosome to sc^4sc^8-induced drive. Peacock (1965) reported that in sc^4sc^8/Y males the ratio of *X* to nullo-*X*, nullo-*Y* nuclei after the second meiotic division is the same as the ratio of *X* to nullo-*X*, nullo-*Y* classes among the progeny. This suggests that no loss of *X*-bearing sperm (relative to nullo-*X*, nullo-*Y* sperm) occurs. However, electron microscopy reveals that in high drive males more than half of the sperm in some bundles are defective, suggesting that some non-*Y* sperm are malfunctioning (Peacock, Miklos, and Goodchild 1975). Recovery of the *X* is poor in $sc^{4}sc^{8}/Y/Dp$ (Haemer 1978) and $sc^{4}sc^{8}/Y/Y$ (Sandler and Braver 1954) males. Cytological studies (Cooper 1964) reveal that the *X* behaves as a univalent in $sc^{4}sc^{8}/Y/Y$ males, but is not lost during the meiotic divisions. Thus, the evidence points to malfunction of *X*-bearing sperm in these males. As a further test of this, *X* recovery was compared in high drive and in low drive $sc^{4}sc^{8}/Y/Dp$ males. If the recovery of the *X* parallels that of the *Y* in these crosses, then the two chromosomes must be responding to the same forces. The absence of nullo-*X*, nullo-*Y* sperm implies that poor *Y* recovery is due to meiotic drive and not chromosome loss. Parallel behavior of the *X* and *Y* would imply that the *X* is also a victim of meiotic drive.

The evidence demonstrating poor recovery of $sc^{4}sc^{8}$ from certain types of males indicates that the *X* euchromatin is sensitive to meiotic drive. However, since $sc^{4}sc^{8}$ contains almost no heterochromatin, it is not known whether or not the *X* heterochromatin is similarly sensitive. When a free *X* duplication containing all the heterochromatin but very little of the euchromatin is added to a $sc^{4}sc^{8}$ genotype, the males experience considerable meiotic drive (Haemer 1978). As the recovery of the free duplication is the best of the three chromosomes, it is impossible to say whether any duplication-bearing sperm malfunction. Two approaches to this problem were taken. The first approach was to measure the sensitivity of free *X* duplications so small that they do not disjoin from the *Y*. The recovery of the duplication can be determined under these conditions because one can measure the recovery of otherwise identical sperm with and without the free duplication. This can not be done with the larger free duplications which disjoin from the *Y*; any sperm which lac

dup

mal

a Y

lar

tha

sis

lack the free duplication carry the *Y* and are not otherwise identical to duplication sperm, all of which lack the *Y*.

In the second approach, chromosome recoveries were compared between males carrying sc^4sc^8, a *Y*, and a large free *X* duplication and sc^4sc^8, a *Y*, and a smaller free *Y* duplication (but large enough to disjoin regularly from the *Y*). Substantial differences in *Y* recovery would indicate that the size of the free duplication makes a difference, a result consistent with the view that drive affects all sex chromosomes.

RESULTS

Y Chromosome Sensitivity -- Crosses to Free-X Females

The first set of experiments test for drive sensitivity of unmarked *Y* chromosomes in sc^4sc^8/Y males. The presence of a bobbed locus (*rDNA*) on the *Y* chromosome makes it possible to distinguish *Y*-bearing from non-*Y*-bearing progeny. Although normal *X*'s also carry a bobbed locus, sc^4sc^8 is completely deficient for it. In the first experiment, sc^4sc^8, $y\ w^a\ bb^-$/*Y* males were crossed to females of the genotype *y w bb/y w bb/Dp(1;f)3*, $y^+\ bb^+$. These females are homozygous for a moderate bobbed allele, a partial *rDNA* deficiency. The bobbed locus on the free duplication covers this deficiency. Half the disjunctional eggs are *X* and half are *XDp*. In the *X* eggs, progeny from all four sperm classes can be distinguished but only two of them (the *XY* and *Y* classes) have normal viability. The other two classes are bobbed and cannot be used to estimate sperm frequencies. In the *XDp* eggs, all four sperm classes are viable but the *Y* can not be detected. A few simple calculations provide reasonable estimates of all four sperm classes. First, to estimate the recovery of *XY* and *Y* sperm, the numbers of $y\ w^a$ females and *y w* males respectively are used. Second, to estimate the recovery of *X* sperm, the $y\ w^a$ females are subtracted from the w^a females (because the w^a females include both *X* and *XY* classes while the $y\ w^a$ females come from *XY* sperm only). Third, *y w* males are subtracted from *w* males to obtain an estimate of the recovery of nullo-*X*, nullo-*Y* sperm.

Table 1 presents the results for four unmarked *Y* chromosomes and two marked *Y*'s. Several points emerge from an examination of Table 1. The first, and most important, is that poor recovery of *Y* sperm is observed whenever sc^4sc^8/Y males are tested, no matter what kind of *Y*

Table 1 Y Chromosome Recovery from $sc^4 sc^8/Y$ Males

		Recoveries: Y Chromosomes			
Sperm Genotype	Progeny Phenotype	Y_1	Y_2	Y_3	Y_4
X	y w^a bb Females	446	85	4	96
XY	y w^a Females	58	30	7	81
X or XY	w^a Females	1504	961	121	766
0	y w bb Males	64	25	20	158
Y	y w Males	982	715	65	471
0 or Y	w Males	1370	907	129	797

Estimates of Sperm Frequencies	Y_1	Y_2	Y_3	Y_4	w^+Y	B^SY
X	1446	931	114	685	149	1113
XY	58	30	7	81	10	152
0	388	192	64	326	71	1287
Y	982	715	65	471	70	688
Nondisjunction -- 0/(X+0)	.21	.17	.36	.32	.32	.54
Recovery Ratios -- Y:X	.68	.77	.57	.69	.47	.62
-- XY:0	.15	.16	.11	.25	.14	.12

$sc^4 sc^8$, y w^a bb^-/Y males were crossed to y w bb/y w bb/Dp(1;f)3, y^+ bb^+ females. The estimates of sperm class frequencies are calculated as outlined in the text. For the marked Y chromosomes, the sperm class frequencies are actual numbers, not estimates.

chromosome (marked or unmarked) they carry. All four unmarked *Y*'s and both marked *Y*'s gave *Y*:*X* ratios well below 1, ranging from 0.47 to 0.77. The second point is that nondisjunction is frequent in all cases, but is highest for B^SY and lowest for Y_1 and Y_2. The third point is that drive is invariably more severe in the nondisjunctional class (*XY*:*0*) than in the disjunctional class (*Y*:*X*). The final point is that recovery ratios show a rough inverse correlation with nondisjunction frequencies (although there are some exceptions). Thus, these data reaffirm the oft-remarked correlation between nondisjunction and drive (Peacock and Miklos 1973).

Y *Chromosome* *Sensitivity* -- *Crosses* *to* *Attached-X* *Females*

To be sure that the poor recovery of the *Y* in these crosses was not an artifact of the experimental design, a second set of crosses involving the same males but different females was undertaken. Table 2 presents the results for crosses of sc^4sc^8/Y males to three different kinds of attached-*X* females : (1) *C(1)DX*, bb^-/B^SY, (2) *C(1)RM*/B^SY, and (3) *C(1)RM*/*0*. In the second cross, all four sperm types produce viable progeny, but the *Y* can not be detected. In cross (1) nullo-*X*, nullo-*Y* sperm can not be recovered because *C(1)DX* is bb^-. All surviving females come from *Y*-bearing sperm. Since crosses (1) and (2) share a common class ($sc^4sc^8/B^SY/Y$ or *0*), that class can be used as a standard to derive a weighting ratio. The estimated number of *Y* sperm in cross (2) is the number of *Y* sperm in cross (1) multiplied by the ratio of males in the two crosses. The recovery of nullo-*X*, nullo-*Y* sperm is total females in cross (2) minus the estimated number of females derived from *Y* sperm in that cross. The only surviving males in cross (3) come from *XY* sperm since sc^4sc^8 is bb^-. Making use of the fact that crosses (2) and (3) share a common

Table 2 Recovery of an Unmarked Y from sc^4sc^8 Males Using Attached-X Females

			Sperm Class			
Cross	Maternal Genotype	Egg Genotype	sc^4sc^8, bb^-	sc^4sc^8, bb^-/Y	0	Y
(1)	C(1)DX, bb^-/B^SY	C(1)DX	Inviable Metafemales 0		Inviable bb^- 0	C(1)DX/Y Females 0
		B^SY	sc^4sc^8/B^SY or $sc^4sc^8/B^SY/Y$ Males 573		Inviable Nullo-X 0	
(2)	C(1)RM/B^SY	C(1)RM	Inviable Metafemales 0		C(1)RM/0 or C(1)RM/Y Females 971	
		B^SY	sc^4sc^8/B^SY or $sc^4sc^8/B^SY/Y$ Males 904		Inviable Nullo-X 0	
(3)	C(1)RM/0	C(1)RM	Inviable Metafemales 0		C(1)RM/0 or C(1)RM/Y Females 1558	
		0	Inviable bb^- 0	sc^4sc^8/Y Males 53	Inviable Nullo-X 0	
Estimated Sperm Class Frequencies			871	33	365	606

class (C(1)RM/Y or 0), the estimated number of XY sperm in cross (2) is the number of XY sperm recovered in cross (3) weighted by the ratio of females in the two crosses. The recovery of X sperm is total males in cross (2) minus the estimated number of males derived from XY sperm. The resulting estimates are listed in the last line of Table 2. These estimates are not very different from those obtained using the same males but normal X females (Table 1). In both cases, Y recovery is substantially worse than X recovery and XY recovery is very poor. There can be no doubt that the sensitivity of Y chromosomes to sc^4sc^8-induced meiotic drive is a property of the Y itself and not of the translocated X genes used to mark many Y chromosomes.

Y Chromosome Sensitivity -- sc^4sc^8/Y/Dp Males

It has been reported that the addition of a largely heterochromatic free-X duplication to a sc^4sc^8 genotype results in poor Y recovery despite regular disjunction of the Y and Dp (Haemer 1978). To find out whether poor Y recovery in this genotype is a property of the Y itself or of translocated X markers, several unmarked Y's and two marked Y's were tested in crosses of sc^4sc^8, y w^a bb^-/Y/Dp(1;f)3, y^+ bb^+ males to y w bb/y w bb females. The females were chosen to permit easy detection of Y-Dp nondisjunction by the occurrence of y w^a bb (sc^4sc^8/y w bb) female progeny. Very few such females were observed in any of the crosses (Table 3). The reciprocal nondisjunctional class (YDp) is detectable by phenotype only in the marked Y crosses, where it proved to be very rare. To make sure that these nondisjunctional males are also rare in the unmarked Y crosses, the y^+ w offspring males in line 1 were tested for fertility. All were sterile, indicating that the Y and the Dp disjoined from each other regularly in these crosses as well.

Table 3 Recovery of X and Y Chromosomes from sc^4sc^8/Y/Dp(1;f)3 Males

	Frequencies of Sperm Classes						Recovery Ratios	
Y Chromosome	X	XDp	XY	Y	Dp	YDp	Y:Dp	X:0
Y_1	0	235	543	628	1035	0	.55	.47
Y_2	3	1224	2099	2256	2998	-	.68	.63
Y_3	0	339	860	908	1876	-	.46	.43
Y_4	4	465	1204	1307	2801	-	.44	.41
w^+Y	0	265	528	585	865	1	.61	.55
B^SY	1	149	672	516	1694	1	.28	.37

Each line presents the results of a cross of the type sc^4sc^8, y w^a bb^-/Y/Dp(1;f)3, y^+ bb^+ X y w bb/y w bb. The six Y's tested are the same ones as in Table 1. The Y:Dp recovery ratio is (XY + Y)/(XDp + Dp). The X:0 recovery ratio is females:males. The 0 in line 1, YDp column is based on a fertility test of all the y^+ male offspring.

In all crosses, the recovery of the *Y* is worse than that of the duplication, with *Y*:*Dp* recovery ratios ranging from 0.28 to 0.68. The failure to recover nullo-*X*, nullo-*Y* sperm rules out *Y* chromosome loss; thus, poor *Y* recovery must be due to meiotic drive. Since crosses involving unmarked as well as marked *Y* chromosomes exhibit this effect, it can not be attributed to the influence of translocated *X* genes. Poor *Y* recovery from $sc^4sc^8/Y/Dp$ males (just as with sc^4sc^8/Y males) is a property of the *Y* chromosome itself. This is not to say, however, that the translocated *X* genes make no contribution to chromosome sensitivity. Both in Table 1 and Table 3, B^SY shows the highest drive, suggesting that the *X* duplication enhances its sensitivity.

X Chromosome Sensitivity -- Euchromatin

The data in Table 3 indicate that recovery of the *X* in $sc^4sc^8/Y/Dp$ males is much lower than expected and is of the same order as that found with $sc^4sc^8/Y/Y$ males (Sandler and Braver 1954). The failure to observe meiotic loss of the unpaired *X* in $sc^4sc^8/Y/Y$ males (Cooper 1964) suggests that its poor recovery in these males and in $sc^4sc^8/Y/Dp$ males is due to sperm dysfunction. Further evidence in favor of the idea that *X*-bearing sperm malfunction in these males can be found in Table 3. Here we find that with different *Y*'s, the *Y*:*Dp* and *X*:*0* recovery ratios both vary over quite a considerable range. In any one cross, however, the *X*:*0* ratio is very close to (usually slightly below) the *Y*:*Dp* ratio. This correlation argues that the forces causing poor *Y* recovery are the same as the forces causing poor *X* recovery. Knowing that the mechanism is sperm dysfunction in the case of the *Y*, we are justified in presuming that *X* sperm also malfunction in these males. This argument is not watertight, but until the definitive electron microscopy is done, it is a reasonable conclusion.

X Chromosome Sensitivity -- Heterochromatin

The evidence for *X* chromosome drive sensitivity in *sc*^{4}*sc*^{8}/*Y*/*Y* and *sc*^{4}*sc*^{8}/*Y*/*Dp* males reveals nothing about the sensitivity of *X* heterochromatin, because *sc*^{4}*sc*^{8} is an almost entirely euchromatic chromosome. In crosses involving *Dp(1;f)3*, a largely heterochromatic *X* fragment, nothing can be said about the recovery of the duplication except that it exceeds that of the *Y*. The difficulty is that the duplication disjoins from the *Y*, making it impossible to measure the effect of adding the free duplication to a sperm without changing any other aspect of the genome. All *Dp* sperm lack the *Y* and all non-*Dp* sperm carry a *Y*. To circumvent this problem, *Dp(1;f)164* and *Dp(1;f)1144*, two small free duplications approximately the size of the fourth chromosome, were tested. Males of the genotypes *sc*^{4}*sc*^{8}/*B*^{S}*Y*/*Dp(1;f)164* and *sc*^{4}*sc*^{8}/*B*^{S}*Y*/*Dp(1;f)1144* were generated from crosses of *sc*^{4}*sc*^{8}/*sc*^{4}*sc*^{8}/*B*^{S}*Y* females to attached-*XY*/*Dp(1;f)164* and attached-*XY*/*Dp(1;f)1144* males and were crossed to *y*/*y* females. The results, presented in Table 4, indicate that the free duplication segregates randomly in the vast majority of meioses. The results are equivocal as to sensitivity of the free duplications. The recovery of *Dp1144* is 0.94 which is significantly different from 1. The recovery of *Dp164* is 0.98, not significantly different from 1. This may mean that the duplications have different intrinsic sensitivities. An alternative explanation is that the duplication disjoins from one of the other chromosomes more frequently in the *164* case than in the *1144* case. Occasional disjunction would result in an excess of *Dp* offspring and a shortage of *XDp*, *YDp*, and *XYDp* offspring relative to the non-*Dp* classes. This distortion is evident in both crosses, but is more frequent in the *164* case than in the *1144* case. Whenever the duplication disjoins from a

Table 4 Recovery of Small Free X Duplications

	Frequencies of Sperm Classes								
Paternal Genotype	X	XDp	XY	XYDp	0	Dp	Y	YDp	Recovery of Dp
$sc^4sc^8/B^SY/Dp1144$	958	849	241	157	1120	1198	653	602	.94
$sc^4sc^8/B^SY/Dp164$	858	695	97	44	1215	1450	344	279	.98

sc^4sc^8, $y\ w^a\ bb^-/B^SY/Dp(1;f)1144$, y^+ males and sc^4sc^8, $y\ w^a\ bb^-/B^SY/Dp(1;f)164$, y^+ males were generated from crosses of $sc^4sc^8/sc^4sc^8/B^SY$ females to attached-$XY/Dp(1;f)1144$ and attached-$XY/Dp(1;f)164$ males and were crossed to y/y females.

chromosome, its recovery will be much greater than 1 since its disjunctional homolog, either the *X* or the *Y*, is much larger than and therefore more sensitive to drive than the *Dp*. The small fraction of meioses in which the duplication disjoins will tend to inflate the overall recovery of the duplication. The slight difference in apparent sensitivities of the two duplications may be due to the slightly greater tendency of *Dp164* to disjoin from its homologs.

A second approach to the problem of *X* heterochromatic sensitivity was to compare the relative sensitivities of *Dp(1;f)3* (a large heterochromatic *X* duplication) and Y^S (a somewhat smaller *Y* chromosome fragment) against a common standard, B^SY. Sibling males of the genotypes $sc^4sc^8/B^SY/Y^S$ and $sc^4sc^8/B^SY/Dp(1;f)3$ were generated from a cross of $sc^4sc^8/sc^4sc^8/Dp(1;f)3$ females to $y\ w/B^SY/Y^S$ males. These males were then crossed to *y w bb/y w bb* females. The data are presented in Table 5. It is evident from the absence of $y\ w^a\ bb$ females among the progeny of these crosses that both fragments disjoin regularly from B^SY. However, recovery ratios in the two crosses are not the same. The recovery of B^SY relative to Y^S is considerably worse than its recovery relative to *Dp(1;f)3*.

Table 5 Comparison of Drive Sensitivities of Y^S and Dp(1;f)3 Relative to B^SY

	Frequencies of Sperm Classes							Recovery Ratios		
Paternal Genotype	X	XY^S	XB^SY	XDp	Y^S	Dp	B^SY	$B^SY:Y^S$	$B^SY:Dp$	X:0
$sc^4sc^8/Y^S/B^SY$	0	565	91	---	1945	---	390	.19	---	.26
sc^4sc^8/B^SY/Dp(1;f)3	0	---	77	361	----	983	288		.27	.34

sc^4sc^8, y w^a $bb^-/Y^S/B^SY$ and sc^4sc^8, y w^a bb^-/B^SY/Dp(1;f)3, y^+ bb^+ males were generated from a cross of sc^4sc^8/sc^4sc^8/Dp(1;f)3 females to y w bb/B^SY/Y^S males and were crossed to y w bb/y w bb females.

de

pr

ge

ar

th

in

a

h

DISCUSSION

The experiments described in the first section of this chapter demonstrate that poor recovery of *Y* chromosomes from sc^4sc^8 males is a property of the *Y* chromosomes themselves and not of the translocated *X* genes used as markers. When sc^4sc^8/*Y* males with unmarked *Y* chromosomes are crossed either to attached-*X* or to regular females, the recovery of the *Y* is lower than that of the *X*. Similarly, sc^4sc^8/*Y*/*Dp* males carrying an unmarked *Y* show poor *Y* recovery.

The question of *X* chromosome sensitivity is somewhat more problematic, although a partial answer can be given. The evidence presented here indicates that poor recovery of the *X* from sc^4sc^8/*Y*/*Y* and sc^4sc^8/*Y*/*Dp* males is most likely a consequence of dysfunction of *X*-bearing sperm. The alternative, post-meiotic chromosome loss, is unlikely because the recovery of the *X* chromosome responds to the same modifying forces that determine the level of *Y* chromosome recovery, a phenomenon certainly not due to chromosome loss. There remains some uncertainty about the recovery of the *X* chromosome in sc^4sc^8/*Y* males. Light microscopic studies (Peacock 1965; Peacock and Miklos 1973) argue for full *X* recovery, but electron microscopy (Peacock, Miklos and Goodchild 1975) demonstrates sperm dysfunction of non-*Y* sperm in some instances. Uncertainty also remains concerning drive sensitivity of *X* heterochromatin. The results of the small free duplication experiment are consistent with mild sensitivity of both *Dp1144* and *Dp856* -- a sensitivity partially masked by the tendency of both duplications to disjoin occasionally from the other chromosomes. The results are also consistent with other hypotheses. For example, *Dp1144* but not *Dp164* may be sensitive. Alternatively, *Dp1144* might be lost meiotically on occasion.

The results obtained with large free duplications are consistent with the view that all sex chromatin is sensitive to drive and that the length of a chromosome determines its degree of sensitivity. However, it is impossible to demonstrate by genetic means alone that the least sensitive element of a genotype is susceptible to meiotic drive.

Three other points concerning this study deserve mention. One is that *Y*:*Dp* recovery ratios from $\underline{sc^4sc^8}/\underline{Y}/\underline{Dp}$ males (Table 3) are invariably lower than *Y*:*X* recovery ratios from $\underline{sc^4sc^8}/\underline{Y}$ males (Table 1) carrying the same *Y*. In light of suggestions that faulty *X*-*Y* pairing is responsible for drive in $\underline{sc^4sc^8}/\underline{Y}$ males (Baker and Carpenter 1972; Peacock and Miklos 1973), it is interesting that supplying the *Y* with a regular pairing partner in the form of a free *X* duplication which carries a full complement of pairing sites does nothing to enhance *Y* recovery. In fact, it seems to make matters worse.

The second point is that recoveries of the *Y* and *X* from $\underline{sc^4sc^8}/\underline{Y}/\underline{Dp}$ males are not independent of each other. In every cross the recovery of *XY* sperm is worse than expected on the basis of independence. (X^2 is significant at the .05 level.) Evidently, *X* and *Y* chromosomes interact when present in the same sperm. This contradicts Haemer's (1978) finding of independence in crosses of this sort. The reason for this difference is unclear. Perhaps the overall higher drive levels in the present experiments account for the occurrence of interaction in them but not in Haemer's experiments.

Finally, the largely random disjunction of the unpaired small free *X* duplications *1144* and *164* (Table 4) in spermatocytes nondisjunctional for *X* and *Y* is intriguing in light of Peacock's (1965) finding of nonrandom disjunction of *X* and *Y* when they fail to pair. The forces causing the unpaired X and Y to migrate to the same pole do not affct the free duplications. Perhaps they are too small to be included in this system.

CHAPTER 2

GENETIC BACKGROUND EFFECTS ON LEVELS OF MEIOTIC DRIVE

Sex chromosome meiotic drive systems in *Drosophila melanogaster* are subject to modification by genetic background effects, including source of *Y* chromosome (Zimmering 1960; Peacock, Miklos, and Goodchild 1973), source of autosomes (Zimmering 1960; Peacock, Miklos, and Yanders 1972), amount of autosomal heterochromatin (Haemer 1978), and amount of *X* heterochromatin (Haemer 1978). Males carrying $T(1;4)B^S$ (an *X*;*4* translocation with the *X* breakpoint in the proximal euchromatin) produce highly distorted sperm ratios in some genetic backgrounds but not others (Novitski and Sandler 1957; Zimmering 1960). With "A-type" autosomes and *Y* chromosome, the recoveries of the *Y* chromosome and the 4^PX^D element are impaired. With "E-type" autosomes and *Y* chromosome, sperm ratios are normal. Mixtures of E and A type chromosomes produce intermediate levels of distortion. In males carrying the *X* heterochromatic deficiency $In(1)sc^{4L}sc^{8R}$ (sc^4sc^8), the frequencies of *X*-*Y* nondisjunction and of recovery disruption of *XY* and *Y* sperm vary depending on the *Y* chromosome used and upon the segregation of uncontrolled, presumably autosomal, modifiers (Peacock 1965; Peacock, Miklos, and Yanders 1972; Peacock and Miklos 1973). The addition of a *Y* chromosome or a heterochromatic free *X* duplication to a sc^4sc^8 genotype enhances recovery disruption. In sc^4sc^8/*Y*/*Y* males, the two *Y* chromosomes pair and disjoin regularly (Cooper 1964). The univalent *X* is recovered poorly (Sandler and Braver 1954) despite absence of meiotic loss (Cooper 1964). In sc^4sc^8/*Y*/*Dp* males (where *Dp* is one of several largely heterochromatic *X* fragments called free duplications) the *Y* and *Dp* disjoin from each other, and recoveries of both the *X* and *Y* are depressed (Haemer 1978).

No systematic effort to sort out the effects of *Y* chromosomes and of autosomes on the sc^4sc^8 system has previously been made. Experiments exhibiting *Y* effects on nondisjunction and meiotic drive in sc^4sc^8 males (Peacock and Miklos 1973) have not included controls on autosomal background. In the present study autosomes and *Y* chromosomes were varied independently to obtain answers to the following questions concerning drive in sc^4sc^8 males. 1) Can different *Y* chromosomes affect levels of drive and nondisjunction in sc^4sc^8 males independent of autosomal background? 2) Can different sets of autosomes affect levels of drive and nondisjunction independent of sex chromosome content? 3) Does modification by *Y* or autosomal background cause parallel changes in levels of nondisjunction and drive in sc^4sc^8/Y males? 4) Does modification by *Y* chromosome or autosomal background cause parallel changes in *Y* chromosome recovery from sc^4sc^8/Y and $sc^4sc^8/Y/Dp$ flies? 5) Does modification by *Y* or autosomal background cause parallel changes in recovery of both *Y* and *X* chromosomes from $sc^4sc^8/Y/Dp$ flies? 6) Does modification by *Y* or autosomal background cause parallel changes in *X* recovery in $sc^4sc^8/Y/Dp$ and $sc^4sc^8/Y/Y$ males?

These questions are interesting because they bear upon several theoretical issues. One issue is the nature of the connection between pairing and meiotic drive. Drive levels correlate with nondisjunction in sc^4sc^8/Y males where *X*-*Y* pairing interactions are weak (Peacock 1965; Peacock and Miklos 1973). This has been taken to imply a causal relationship between weak pairing and meiotic drive (Baker and Carpenter

1972; Peacock and Miklos 1973). If chromosome recoveries respond in parallel fashion to modification in sc^4sc^8/Y, $sc^4sc^8/Y/Dp$, and $sc^4sc^8/Y/Y$ males, it is reasonable to conclude that the primary lesion in these males is the same despite superficial phenotypic differences. It would also be reasonable to conclude that meiotic drive levels can vary independently of nondisjunction since there is no nondisjunction in $sc^4sc^8/Y/Dp$ or $sc^4sc^8/Y/Y$ males.

A second issue is the reason for poor *X* recovery in $sc^4sc^8/Y/Y$ and $sc^4sc^8/Y/Dp$ males. Parallel modification of *X* and *Y* chromosome recovery in $sc^4sc^8/Y/Dp$ males would argue that *X* and *Y* chromosomes are recovered poorly for the same reason (i.e., sperm dysfunction, not chromosome loss). If modification by source of *Y* chromosome can affect *X* recovery as well as *Y* recovery, that would argue that drive levels are influenced by indirect (non-pairing) interactions between chromosomes since the *X* and *Y* do not pair in these males.

The relative levels of *X* and *Y* recovery from $sc^4sc^8/Y/Dp$ and $sc^4sc^8/Y/Y$ males are of interest because they have implications for the occurrence of competition between sex chromosomes. If the additional sex chromatin in $sc^4sc^8/Y/Y$ males reduces recovery of the *X*, that implies that chromosomes compete for a scarce resource in sc^4sc^8 males. Better relative *Y* recovery from $sc^4sc^8/Y/Y$ males than from $sc^4sc^8/Y/Dp$ males implies either that closer size matching of sex chromosomes leads to better recovery (presumably through pairing interactions) or that absolute chromosome recovery is a function of chromosome length. Greater similarity in size of two paired homologs may lead to greater *relative* recovery of the larger element but not necessarily greater *absolute* recovery. These issues are explored in more depth in the Discussion.

RESULTS

Experimental Design

Three experiments were undertaken. Experiments 1 and 2 (diagrammed in Figure 1) were very similar, differing only in choice of marked Y (w^+Y or B^SY) and in time of execution. In both experiments sc^4sc^8/Y, sc^4sc^8/marked Y, sc^4sc^8/Y/marked Y, $sc^4sc^8/Y/Dp(1;f)3$, and sc^4sc^8/marked Y/Dp(1;f)3 males were generated as siblings from a single cross. In each experiment three such crosses were performed using Y chromosomes and autosomes from different laboratory stocks. Thus in each experiment, fifteen different types of males were generated and tested simultaneously. The data from experiments 1 and 2 are presented in Tables 6-8. The third experiment was a comparison of drive and nondisjunction levels in males with identical sex chromosomes but different autosomes.

Y Chromosome Modification in sc^4sc^8/Y Males

Table 6 reveals that different Y-autosome sets do indeed cause different levels of nondisjunction and drive. In both experiments 1 and 2, the chromosomes from stock 1 are associated with much lower nondisjunction and higher recovery values than the chromosomes from either stock 2 or stock 3. The latter two stocks have similar disjunction and recovery coefficients.

Are the differences between stock 1 and the other two stocks attributable to the autosomes or to the Y chromosomes? Two comparisons bear on this question. The first is a comparison of crosses with the same Y chromosome (either w^+Y or B^SY) but autosomes drawn from stocks 1, 2, or 3. Although simultaneous crosses involving the same X and Y do not give identical results, the similarities are certainly more striking than the

Experiment 1

$\underline{y}\ \underline{w}/\underline{y}\ \underline{w}/\underline{w^{+}Y}$ X $(\underline{X}/\underline{Y})_{i}$

$\underline{y}\ \underline{w}/\underline{w^{+}Y}/\underline{Y_{i}}$ X $\underline{sc^{4}sc^{8}}/\underline{sc^{4}sc^{8}}/\underline{Dp(1;f)3}$

$$\begin{array}{l} \underline{sc^{4}sc^{8}}/\underline{Y_{i}} \\ \underline{sc^{4}sc^{8}}/\underline{Y_{i}}/\underline{w^{+}Y} \\ \underline{sc^{4}sc^{8}}/\underline{w^{+}Y} \end{array} \quad \text{X} \quad \underline{y}\ \underline{w}\ \underline{bb}/\underline{y}\ \underline{w}\ \underline{bb}/\underline{Dp(1;f)3}$$

$$\begin{array}{l} \underline{sc^{4}sc^{8}}/\underline{w^{+}Y}/\underline{Dp(1;f)3} \\ \underline{sc^{4}sc^{8}}/\underline{Y_{i}}/\underline{Dp(1;f)3} \end{array} \quad \text{X} \quad \underline{y}\ \underline{w}\ \underline{bb}/\underline{y}\ \underline{w}\ \underline{bb}$$

Experiment 2

$\underline{y}\ \underline{w}/\underline{y}\ \underline{w}/\underline{B^{S}Y}$ X $(\underline{X}/\underline{Y})_{i}$

$\underline{y}\ \underline{w}/\underline{B^{S}Y}/\underline{Y_{i}}$ X $\underline{sc^{4}sc^{8}}/\underline{sc^{4}sc^{8}}/\underline{Dp(1;f)3}$

$$\begin{array}{l} \underline{sc^{4}sc^{8}}/\underline{Y_{i}} \\ \underline{sc^{4}sc^{8}}/\underline{Y_{i}}/\underline{B^{S}Y} \\ \underline{sc^{4}sc^{8}}/\underline{B^{S}Y} \end{array} \quad \text{X} \quad \underline{y}\ \underline{w}\ \underline{bb}/\underline{y}\ \underline{w}\ \underline{bb}/\underline{Dp(1;f)3}$$

$$\begin{array}{l} \underline{sc^{4}sc^{8}}/\underline{B^{S}Y}/\underline{Dp(1;f)3} \\ \underline{sc^{4}sc^{8}}/\underline{Y_{i}}/\underline{Dp(1;f)3} \end{array} \quad \text{X} \quad \underline{y}\ \underline{w}\ \underline{bb}/\underline{y}\ \underline{w}\ \underline{bb}$$

Figure 1   Mating Scheme for Experiments 1 and 2

6. Nondisjunction and Drive in sc^4sc^8/Y Males

Table 6 Nondisjunction and Drive in sc^4sc^8/Y Males

			Sperm Classes				Nondis-junction	Recovery Ratios	
Experiment	Source of Autosomes	Y Chromosome	X	XY	Y	0	0/(X+0)	Y:X	XY:0
1	Stock 1	Y_1	931	30	715	192	.17	.77	.16
		w^+Y	121	14	73	78	.39	.60	.18
2	Stock 1	Y_1	1305	44	1080	290	.19	.82	.15
		B^SY	934	170	557	1176	.56	.60	.14
1	Stock 2	Y_2	114	7	65	64	.36	.57	.11
		w^+Y	273	19	180	149	.35	.66	.13
2	Stock 2	Y_2	2722	101	1506	1422	.34	.55	.07
		B^SY	995	142	506	1416	.59	.51	.10
1	Stock 3	Y_3	685	81	471	326	.32	.69	.25
		w^+Y	287	22	210	195	.40	.73	.11
2	Stock 3	Y_3	1707	100	1024	826	.33	.60	.12
		B^SY	1113	152	688	1287	.54	.62	.12

sc^4sc^8/Y males were crossed to y w bb/y w bb/Dp(1;f)3 females differences. In experiment 1, there are no significant differences among the three sc^4sc^8/w^+Y crosses in terms of nondisjunction or meiotic drive. In experiment 2, the differences in drive and nondisjunction are significant, mostly because of the abnormally high nullo-*X*, nullo-*Y* class in the stock 2 cross. However, this difference does not account for the major effect in Table 6 -- namely, the consistently low drive and nondisjunction of the Y_1 crosses. Those differences disappear when the *Y* chromosome is held constant. Evidently, they were caused by the *Y* chromosome and not by the autosomes. This conclusion is confirmed by the second comparison in which autosomes are held constant and *Y* chromosome is allowed to vary. In Table 1, six pairwise comparisons involving different *Y* chromosomes but constant autosomes (e.g., line 1 with line 2, line 3 with line 4, etc.) can be made. Nondisjunction and recovery coefficients for members of a pair are not in general the same, and in several instances are widely divergent. Clearly, the *Y* chromosome can have a drastic effect on levels of drive and nondisjunction when autosomes are held constant.

These results confirm those of previous investigators (see Peacock and Miklos 1973) in showing a strong correlation between nondisjunction and drive. In Table 6, stock 1 shows consistently lower nondisjunction and higher *Y*:*X* recovery than the other two stocks. Similarly, B^SY shows both higher nondisjunction and lower *Y* chromosome recovery than w^+Y.

Y Chromosome Recovery in $sc^4sc^8/Y/Dp$ Males

Another genotype in which it is possible to monitor *Y* chromosome recovery is $sc^4sc^8/Y/Dp$. Males of this genotype were generated as siblings to the sc^4sc^8/Y males discussed above. Do these $sc^4sc^8/Y/Dp$ males show the same inter-stock patterns of *Y* recovery as their sc^4sc^8/Y

brothers. The data in Table 7 reveal that they do. Just as in Table 6, recovery coefficients are consistently higher for crosses in which *Y* and autosomes have been derived from stock 1 than for crosses with chromosomes from stocks 2 or 3. Once again, stocks 2 and 3 are very close to each other.

Are these differences attributable to the autosomes or to the *Y* chromosomes? The data in Table 7 permit comparison of simultaneous crosses involving the same *Y* chromosome (either $w^{+}Y$ or $B^{S}Y$) but different sets of autosomes. Again, the differences among these crosses are minor compared to the differences observed when both the *Y* chromosomes and autosomes vary. There are no significant differences in experiment 1. In experiment 2, the *Y*:*Dp* recovery ratios are significantly different, but this difference is not repeated in the *X*:*0* recovery ratios, which are the same. The autosomes alone do not contribute significantly to the main effect -- the low drive in Y_1 crosses. When autosomes are held constant and pairwise comparisons between crosses involving the same autosomes (lines 1 and 2, lines 3 and 4, etc.) are made, the differences in *Y* recovery between members of a pair are generally substantial, sometimes large. The *Y* chromosome has a considerable impact on levels of meiotic drive in these males as well as in sc^4sc^8/Y males.

The parallel results for sc^4sc^8/Y and $sc^4sc^8/Y/Dp$ males provide support for the idea that the same defect is responsible for the meiotic anomalies in both genotypes. Evidently, the defect is not simply absence of an *X* heterochromatic function, since the duplication contains all the heterochromatin missing from sc^4sc^8. Rather, it must be the separation of the heterochromatin from the bulk of *X* euchromatin that causes these problems.

Table 7 Sperm Recovery from sc^4sc^8/Y/Dp(1;f)3 Males

Experiment	Source of Autosomes	Y Chromosome	Sperm Classes				Recovery Ratios	
			XY	XDp	Y	Dp	Y:Dp	X:0
1	Stock 1	Y_1	1224	2099	2256	2998	.68	.63
		w^+Y	80	126	167	241	.67	.50
2	Stock 1	Y_1	141	322	367	795	.45	.40
		B^SY	66	329	308	936	.30	.32
1	Stock 2	Y_2	339	860	908	1876	.46	.43
		w^+Y	31	79	78	142	.49	.50
2	Stock 2	Y_2	122	411	483	1299	.35	.30
		B^SY	87	431	330	1144	.26	.35
1	Stock 3	Y_3	465	1204	1307	2801	.44	.41
		w^+Y	154	273	340	482	.65	.52
2	Stock 3	Y_3	86	338	381	961	.36	.32
		B^SY	40	326	234	871	.23	.33

sc^4sc^8/Y/Dp(1;f)3 males were crossed to y w bb/y w bb females.

These results also demonstrate that it is possible to modify meiotic drive levels in sc^4sc^8 males without at the same time modifying the sex chromosome disjunctional pattern. In every sc^4sc^8/Y/Dp cross performed, the Y and the free duplication disjoined reliably from each other, and sc^4sc^8 assorted randomly. Drive levels nevertheless varied over a considerable range.

X and Y Chromosome Recovery in sc^4sc^8/Y/Dp Males

The data in Table 7 show that X chromosome recovery is always depressed in sc^4sc^8/Y/Dp males. They also reveal a strong correlation between recovery of the Y and the X. As pointed out in Chapter 1, the same forces causing recovery disruption of the Y are also acting on the X. These data supply more cases supporting this correlation. The data in Table 7 also show that the recovery of XDp sperm is not always lower than that of Y sperm as it is in all the unmarked Y cases. B^SY is more sensitive than the X and Dp together, perhaps because of the X genes on B^SY.

These data also permit another look at the question of independence of X and Y chromosome recovery. The sc^4sc^8/Y/Dp experiments discussed in Chapter 1 all revealed an interaction between X and Y chromosomes such that XY sperm fared worse than expected. The new data in Table 7 (experiment 2) confirm this in all cases but one. Non-independence is especially striking in the B^SY crosses which also show the highest drive levels of any cross. This helps to confirm the earlier suggestion that degree of interaction may be related to overall drive level. The one independent case in Table 7 also helps to confirm this notion as it contains the Y chromosome and autosomes from stock 1, the "low drive" stock.

Chromosome Recoveries from $sc^4sc^8/Y/Y$ Males

These experiments were designed to permit evaluation of $sc^4sc^8/Y/Y$ males along with the other genotypes. However, $sc^4sc^8/w^+Y/Y$ males were completely sterile. $sc^4sc^8/Y/B^SY$ males were fertile but did not produce very many offspring. The data are displayed in Table 8. No distinctions among stocks can be made on the basis of these data as the numbers are too small. Still, two conclusions can be reached. One is that the recovery of B^SY relative to that of the unmarked Y chromosomes is considerably better than its recovery relative to the free X duplication (Table 7). This suggests either that the sensitivity of sex chromosomes to meiotic drive is a function of length or that the sensitivity of the larger of two sex chromosome homologs is a function of their difference in length. The experiments described in Chapter 3 confirm and extend the notion of length dependence although they do not permit a decision between these two alternatives.

The second conclusion is that the degree of X chromosome recovery in $sc^4sc^8/Y/Y$ males is in the same general range found for $sc^4sc^8/Y/Dp$ males. However, the numbers in the $sc^4sc^8/Y/Y$ cross are not large enough to permit a decision as to whether there is some difference between the two genotypes. This is a question of considerable theoretical import and is taken up again in Chapter 3.

Modification by Autosomes

The differences between stock 1 and stocks 2 and 3 in terms of effects on disjunction and meiotic drive are mostly attributable to the Y chromosomes. One reason for the minor influence of autosomes may be that the test males were generated by crossing males from different laboratory stocks to identical females and then crossing their sons to

Table 8 Chromosome Recovery from $sc^4sc^8/Y/B^SY$ Males

Source of Autosomes and Y Chromosomes	Sperm Classes				Recovery Ratios	
	XY	XB^SY	Y	B^SY	B^SY:Y	X:0
Stock 1	68	56	180	105	.65	.43
Stock 2	125	88	340	266	.76	.35
Stock 3	32	20	143	85	.60	.23

$sc^4sc^8/Y/B^SY$ males were crossed to y w bb/y w bb/Dp(1;f)3 females.

identical females. Any between stock differences would be considerably attenuated after the equivalent of two generations of backcrossing.

That autosomal modifiers are segregating in these crosses is suggested by the sometimes considerable differences among males in a single cross. These differences can not reflect sampling error alone since they affect *X* and *Y* chromosome recovery in a parallel fashion in $sc^4sc^8/Y/Dp$ crosses. In two such crosses, individual males were ranked in terms of recovery of *XDp* sperm relative to *Dp* sperm and were divided into categories based on this ranking (Table 9). Recovery of *Y* sperm relative to *Dp* sperm was then determined for males in each category. It was found that the *Y*:*Dp* ratio paralleled the *XDp*:*Dp* ratio, suggesting that these two ratios measure the same underlying meiotic disruption. These between-male differences might reflect segregation of autosomal modifiers. Alternatively, uncontrolled non-genetic factors might be responsible for the differences.

Convincing evidence for autosomal modification of sc^4sc^8-induced meiotic drive comes from a set of crosses designed for another purpose. The purpose of the experiments was to evaluate the drive sensitivity of small free *X* duplications (Chapter 1). $sc^4sc^8/sc^4sc^8/B^SY$ females were crossed to attached-*XY*/*Dp(1;f)164* and attached-*XY*/*Dp(1;f)1144* males. Both of the free duplications are quite small and frequently fail to disjoin from the attached-*XY*. Males carrying sc^4sc^8 and B^SY with or without the free duplication were recovered from both crosses and were mated to *y*/*y* females. The results are displayed in Table 10. The differences between the two sc^4sc^8/B^SY crosses are considerable: the *Y*:*X* ratio is .67 in one case and .49 in the other while the *XY*:*0* ratios are .22 and .082, respectively. The nondisjunction fractions are .54 and .58, respectively. These differences must be due to different autosomes

Table 9 Within Cross Correlations between X and Y Recovery

Cross 1

XDp:Dp Ratio	Y:Dp Ratio
below 0.6	.59
.60 - .69	.72
.70 - .79	.72
.80 - .99	.92
above 1.0	1.17

Cross 2

XDp:Dp Ratio	Y:Dp Ratio
below 0.3	.38
.30 - .39	.38
.40 - .49	.46
above .50	.59

Males from two sc^4sc^8/Y/Dp(1;f)3 crosses were ranked into the categories in the XDp:Dp column. Then the Y:Dp ratio was calculated for each category. Crosses 1 and 2 are from lines 1 and 5 respectively of Table 7.

Table 10 Autosomal Modification of Nondisjunction and Meiotic Drive in sc^4sc^8 Males

	Sperm Classes								Nondis-junction	Recovery Ratios	
Paternal Genotype	X	XDp	XY	XYDp	0	Dp	Y	Dp	0:(X+0)	Y:X	XY:0
sc^4sc^8/B^SY	1734	----	454	----	2029	----	1162	----	.54	.67	.22
$sc^4sc^8/B^SY/Dp1144$	958	849	241	157	1120	1298	653	602	.56	.69	.17
sc^4sc^8/B^SY	1695	----	193	----	2349	----	841	----	.58	.49	.08
$sc^4sc^8/B^SY/Dp164$	858	695	97	44	1215	1450	344	279	.63	.40	.05

The males were generated from crosses of $sc^4sc^8/sc^4sc^8/B^SY$ females to attached-XY/Dp(1;f)1144 (rows 1 and 2) and to attached-XY/Dp(1;f)164 (rows 3 and 4) males. The females were y/y.

coming from the attached-*XY*/*Dp* stocks since the sex chromosome constitution of the flies is identical. The presence of a free duplication in these crosses has no effect on the drive and disjunction ratios; sibling sc^4sc^8/B^SY and $sc^4sc^8/B^SY/Dp$ males give the same results. An interesting feature of these data is that the drive ratios appear to be more sensitive to modification than does the amount of nondisjunction. This contrasts with previous reports of modification (Zimmering 1963; Peacock and Miklos 1973; also see Table 6 above) in which nondisjunction is the more sensitive parameter.

DISCUSSION

The major results of this study are 1) both autosomes and *Y* chromosomes can influence levels of nondisjunction and meiotic drive in sc^4sc^8 males, 2) recovery of *Y* chromosomes is modified in the same direction in sc^4sc^8/Y and $sc^4sc^8/Y/Dp$ males, 3) recoveries of *X* and *Y* chromosomes are modified in the same direction in $sc^4sc^8/Y/Dp$ males, and 4) replacing the free duplication with a second *Y* improves the relative recovery of B^SY.

These results have several implications for understanding the mechanisms responsible for meiotic drive in sc^4sc^8 males. One is that the lesion responsible for meiotic drive and nondisjunction in sc^4sc^8/Y males must be the same as the one responsible for drive in $sc^4sc^8/Y/Dp$ males. This is not surprising since both genotypes include a heterochromatically deleted *X* chromosome. It is interesting, however, that restoring the normal amount of heterochromatin by adding a free *X* duplication to the genotype does nothing to improve *Y* chromosome recovery.

A second implication is that meiotic drive levels can vary independently of the disjunctional pattern. In $sc^4sc^8/Y/Dp$ males, where the *Y* and *Dp* disjoin regularly, drive levels are at least as sensitive to *Y* modification as in sc^4sc^8/Y males where disjunction is irregular and also subject to modification.

A third implication is that the *Y* chromosome participates in the determination of overall drive levels in the spermatocyte. *Y* chromosome modification of drive levels in $sc^4sc^8/Y/Dp$ males does not affect only the recovery of the *Y* chromosome, but the recovery of the *X* as well. Since the *X* probably does not participate in meiotic pairing (judging from its random segregation) in these males, it follows that recovery levels can be determined by other than direct pairing interactions.

A fourth implication is that sex chromosome meiotic drive sensitivity appears to display length dependence. The relative recovery of $B^{S}Y$ is better when it is paired with a longer chromosome (a second *Y*) than when it is paired with the smaller free duplication. In the latter cross, since one chromosome is *X*-derived and the other is a *Y*, the difference may be due to the differing genetic contents of the two chromosomes rather than to their lengths. No firm conclusion concerning length dependence can be drawn until more cases have been examined. In the following chapter, the drive sensitivity of several *Y* chromosome fragments is examined.

in

re

dr

In

co

dr

re

ta

th

s

O

q

o

Y

c

t

i

s

t

CHAPTER 3

SENSITIVITY OF Y CHROMOSOME FRAGMENTS TO MEIOTIC DRIVE

All of the sex chromosome meiotic drive systems examined thus far in male *Drosophila melanogaster* share at least one common feature: poor recovery of the *Y* chromosome. Sensitivity of *Y* chromosomes to meiotic drive has been demonstrated in $T(1;4)B^S$ (Novitski and Sandler 1957), $In(1)sc^{4L}sc^{8R}$ (sc^4sc^8) (Gershenson 1933; Sandler and Braver 1954; Peacock 1965), and Recovery Disrupter (Erickson 1965). The question addressed in the following study is: what property of the *Y* chromosome renders it sensitive to meiotic drive? Perhaps the *Y* chromosome contains a discrete response function analogous to the Responder locus in the *SD* system (Sandler and Hiraizumi 1960; Ganetzky 1977). Or several such functions could contribute to the overall sensitivity of the *Y*. Or *Y* chromatin in general may be sensitive to drive. To answer this question, the sensitivities of several centric *Y* fragments were assessed.

If a single response function exists it must be on one arm or the other (Y^S or Y^L) of the submetacentric *Y* chromosome. Therefore, either Y^S or Y^L but not both should prove sensitive when tested as separate chromosomes. If both fragments prove sensitive, then there are at least two response loci, one on each arm. By measuring the relative sensitivities of a series of *Y* fragments, it was possible to test the idea that sensitivity to meiotic drive is a diffuse function of *Y* chromatin rather than a property of a discrete response locus.

The experiments described below involve males carrying sc^4sc^8, an

X d

X-Y

X a

Bra

(Co

(Pe

chr

di

ju

pr

th

in

te

fy

ex

b

s

t

f

S

t

Y

X deficient for the basal heterochromatin. This chromosome causes high *X*-*Y* nondisjunction and depressed recovery of *Y* and *XY* sperm relative to *X* and nullo-*X*, nullo-*Y* sperm respectively (Gershenson 1933; Sandler and Braver 1954). Cytology reveals that chromosomes are not lost meiotically (Cooper 1964; Peacock 1965) and that many sperm fail to develop properly (Peacock, Miklos, and Goodchild 1975). When sc^4sc^8 males carry two *Y* chromosomes (Sandler and Braver 1954; Cooper 1964), the two *Y*'s pair and disjoin from each other while sc^4sc^8 acts as a univalent. Regular disjunction of the *Y* chromosomes in these males is thought to be due to the presence of collochores (*X*-*Y* pairing sites) on both arms of the *Y*. Given that collochores are observed on both Y^S and Y^L, it was expected that in the $sc^4sc^8/Y^S/Y^L$ males and in the other three-sex-chromosome genotypes tested in the present study, the heterochromatic elements would disjoin from each other. This expectation proved correct. Deviations from the expected one to one recovery ratios were used to calculate differences between the fragments in sensitivity to meiotic drive. Pairwise comparisons of fragments permitted a ranking of the fragments in terms of sensitivity. Fragment features such as length and genetic content were tested for correlation with drive sensitivity.

The experiments also permitted measurement of *X* recovery. In $sc^4sc^8/Y/Y$ and in the sc^4sc^8/Y fragment/*Y* fragment genotypes tested in this study, recovery of the unpaired *X* is quite low. Since the various *Y* chromosomes differ considerably in length, it was possible to ask whether *X* recovery varies in a systematic fashion with the total amount of sex chromatin in the genome. If sex chromosomes in spermatocytes of sc^4sc^8 males must compete for adequate supplies of a scarce but essential resource, the consequence would be an inverse relationship between total sex

I c

de

sex chromatin content and X chromosome recovery. Thus, measurement of X chromosome recovery permits a test of the idea that X heterochromatic deficiencies cause scarcity of a chromosome processing material.

defi

miss

Y ar

ato

met

der

di

bw

to

ch

t

e

h

MATERIALS AND METHODS

The chromosomes used in this study include: $In(1)sc^{4L}sc^{8R}$, an *X* deficient for the basal heterochromatin; B^SY; Y^Ly^{3M}, a bb^+ *Y* chromosome missing *KS*, derived by recombination between the short arm of an intact *Y* and the distal heterochromatin of $In(1)sc^{S1}$ and described by its creators (Crew and Lamy 1940) as an acrocentric rod the size of Y^L in mitotic metaphase (but surely somewhat longer); $Y^L{\cdot}y^+B2$, a bb^- *Y* missing *KS*, derived by recombination between the short arm of an intact *Y* and the distal heterochromatin of $In(1)sc^{8L}EN^R$; Y^S, a spontaneous derivative of bw^+Yy^+ lacking *KL* and described as a small two-armed chromosome in mitotic metaphase (Baker and Spofford 1959); $Y^S{\cdot}Y^S$, a two-armed *V*-shaped chromosome with two doses of *KS* and measuring almost twice the length of the short arm; and *Dp(1;f)3*, an *X*-ray induced deletion of most of the euchromatin of the X that carries bobbed and almost all the rest of the heterochromatin. See Lindsley and Grell (1968) for further details.

Di

pr
sc
ma
ar
or
su
ci
19
sc
nc
di
pr
w
ar
su
ca

th
di
bo
i
th
s
w

RESULTS

Disjunction and Drive in $sc^4sc^8/Y^S/Y^L$ Males

To find out whether *Y* fragments disjoin from each other in the presence of sc^4sc^8, and to determine *Y* fragment drive sensitivities, $sc^4sc^8/Y^S/Y^L$ males were mated to *y w bb*/*y w bb* females. Sibling control males carrying a normal *X*, Y^S, and Y^Ly^{3M} were also tested. The results are presented in Table 11. In the control all six types of sperm with one or two sex chromosomes were recovered with reasonable frequencies suggesting that all three chromosomes participate in a trivalent association as is usually found in males with three sex chromosomes (Cooper 1964). In the experimental cross ($sc^4sc^8/Y^S/Y^L$), the failure to recover $sc^4sc^8/y\ w\ bb$ females or $y\ w\ bb/Y^Ly^{3M}/Y^S$ males implies that sc^4sc^8 does not disjoin from either of the *Y* fragments and that Y^L and Y^S always disjoin from each other. Thus the pairing sites on Y^S and Y^L, although presumably evolved for *X*-*Y* pairing, are perfectly capable of pairing with each other when carried on separate chromosomes. The results here are completely analogous to those obtained with $sc^4sc^8/Y/Y$ males and suggest that no functional differences exist among the pairing sites carried on the *X*, Y^S, and Y^L.

If the *Y* chromosome contains a single discrete response function, then either Y^S or Y^L but not both should be sensitive to sc^4sc^8-induced drive. If, on the other hand, response is a function of length, then both Y^S and Y^L should be sensitive, but Y^L should be more sensitive since it is longer. The results in Table 1 indicate that Y^L is more sensitive than Y^S; the recovery of Y^L relative to Y^S is 0.43. This result is consistent either with the presence of a discrete response locus on Y^L or with the idea that chromosome sensitivity is a function of length.

Table 11 Disjunction and Sperm Recovery from $X/Y^S/Y^L$ Males

Paternal Genotype	Sperm Classes X	XY^S	XY^L	Y^S	Y^L	Y^SY^L	Recovery Ratios $Y^L:Y^S$	$X:0$
$y\ w\ bb/Y^S/Y^Ly^{3M}$	691	738	927	996	1518			.94
$sc^4sc^8/Y^S/Y^Ly^{3M}$	0	930	335	2017	936	0	.43	.42

In the control (first line) $y\ w\ bb/Y^S/Y^Ly^{3M}$ males were crossed to sc^4sc^8/y females. Because of the poor viability of $y\ w^a\ bb$ females ($sc^4sc^8/y\ w\ bb$), the number of X sperm was estimated by subtracting $y\ w^a$ ($y\ w\ bb/sc^4sc^8/Y^S$) females from y ($y\ w\ bb/y/Y^S$ or $y\ w\ bb/y$) females. No $Y^L:Y^S$ recovery ratio was calculated because of the inability to distinguish Y^L from Y^LY^S sperm. The experimental cross (second line) was $sc^4sc^8/Y^S/Y^Ly^{3M}$ males X $y\ w\ bb/y\ w\ bb$ females. The absence of Y^SY^L sperm was deduced from the sterility of all $y^{3M}\ w$ progeny.

These data alone do not permit a decision between the diffuse and discrete models.. They do imply that if there is a single response locus, it must be on Y^L.

Relative Sensitivity of Y^L and Y

Since there is no direct way to determine the sensitivity of the Y^S chromosome, a less direct way of measuring short arm sensitivity is needed. One solution is to compare the sensitivities of Y^L and a complete Y. If Y^S is insensitive, then all the sensitivity of the Y is concentrated in Y^L. Y^L and a complete Y should be equally sensitive. Table 12, line 1 presents data from a cross involving $sc^4sc^8/Y^Ly^{3M}/B^SY$ males. The low fertility of these males is responsible for the relatively small numbers. However, enough flies were recovered to demonstrate that Y^Ly^{3M} is less sensitive than B^SY; (X^2 is significant at the .05 level).

A second approach, which bypasses the fertility problem, is to compare the recoveries of Y^Ly^{3M} and B^SY in two different crosses involving a common standard, Y^S. $sc^4sc^8/Y^S/Y^Ly^{3M}$ and $sc^4sc^8/Y^S/B^SY$ males were generated as brothers and were crossed to *y w bb*/*y w bb* females. The results, in lines 2 and 3 of Table 12, are that the recovery of Y^L relative to Y^S and the recovery of B^SY relative to Y^S is 0.22. This difference is significant at the .01 level. There can be no doubt that B^SY is more sensitive than Y^Ly^{3M}.

Is it possible that the differences in Y chromosome recovery observed in these experiments are due not to length differences, but to different overall drive levels? Since the males in Table 12 are all siblings and since the experiments were done at the same temperature, any differences in drive levels would have to be caused by the Y chromosomes. However, it is certainly the case that different Y chromosomes can cause different

Table 12 Comparison of Drive Sensitivities of Y^Ly^{3M} and B^SY in sc^4sc^8 Males

	Sperm Classes						Recovery Ratios	
Paternal Genotype	XY^S	XY^L	XB^SY	Y^S	Y^L	B^SY	$Y_1:Y_2$	X:0
$sc^4sc^8/B^SY/Y^Ly^{3M}$	----	73	51	----	145	121	.79	.46
$sc^4sc^8/Y^S/Y^Ly^{3M}$	611	236	---	1423	592	---	.41	.42
$sc^4sc^8/Y^S/B^SY$	553	---	103	1396	---	319	.22	.38

The paternal males were generated as siblings from a cross of $sc^4sc^8/sc^4sc^8/B^SY$ females to $y\ w\ bb/Y^S/Y^Ly^{3M}$ males and were in turn crossed to $y\ w\ bb/y\ w\ bb$ females. The $Y_1:Y_2$ recovery ratio is the ratio of recovery of the more sensitive to the less sensitive *Y* chromosome in each cross (e.g., $B^SY:Y^Ly^{3M}$ in the first line).

le
so
re
to
of
(C
me
a
W
c
g
r

levels of drive (see Chapter 2). Perhaps $B^{S}Y$ causes more drive than Y^{L} so that when these chromosomes are each paired with Y^{S} the relative recovery of $B^{S}Y$ is worse than that of Y^{L}. A test of this explanation is to compare *X* recovery in these crosses. The *X* segregates independently of the *Y* chromosomes yet responds to the same meiotic drive conditions (Chapter 2). Thus the recovery of the *X* supplies a second, independent measure of drive. The results in Table 12 provide strong evidence against the idea that meiotic drive levels in each cross are different. While the *Y* chromosome recovery ratios range from .22 to .79, the *X* chromosome recovery ratios range only from .38 to .46. Pairwise contingency tests show that none of the *X* chromosome recovery values is significantly different from any of the others. Differences in *Y* chromosome recovery ratios can not be attributed to changes in overall drive levels. They must be due to different sensitivities of Y^{L} and $B^{S}Y$.

Relative Sensitivity of Y^{S} and $Y^{S}\cdot Y^{S}$

A reasonable explanation of this sensitivity difference is that deletion of the short arm reduces the drive sensitivity of a *Y* chromosome. However, the sensitivity difference could be due to different amounts of translocated *X* material on $B^{S}Y$ and $Y^{L}y^{3M}$. Another way to assess the sensitivity of the short arm is to compare the effects of Y^{S} and a chromosome duplicated for the short arm ($Y^{S}\cdot Y^{S}$) on the relative drive sensitivity of a common standard, $Y^{L}y^{3M}$. If Y^{S} contains none of the sensitivity of the *Y*, the sensitivity of $Y^{L}y^{3M}$ relative to $Y^{S}\cdot Y^{S}$ will be the same as its sensitivity relative to Y^{S}. Table 13 presents data from crosses involving half-brother $sc^{4}sc^{8}/Y^{S}/Y^{L}y^{3M}$ and $sc^{4}sc^{8}/Y^{S}\cdot Y^{S}/Y^{L}y^{3M}$ males. The recovery of $Y^{L}y^{3M}$ relative to $Y^{S}\cdot Y^{S}$ is 0.61 while its recovery relative to Y^{S} is 0.47, a highly significant difference. These experiments show that both

Table 13 Comparison of Drive Sensitivities of Y^S and $Y^S \cdot Y^S$ Relative to $Y^L y^{3M}$

	Sperm Classes						Recovery Ratios		
Paternal Genotype	XY^S	$XY^S \cdot Y^S$	XY^L	Y^S	$Y^S \cdot Y^S$	Y^L	$Y^L : Y^S$	$Y^L : Y^S \cdot Y^S$	X:0
$sc^4 sc^8 / Y^S / Y^L y^{3M}$	1092	----	423	2137	-----	1106	.47	---	.47
$sc^4 sc^8 / Y^S \cdot Y^S / Y^L y^{3M}$	----	1008	592	----	2561	1582	---	.61	.39

The paternal males were generated as half-brothers from crosses of $sc^4 sc^8 / sc^4 sc^8 / Y^L y^{3M}$ females to $X \cdot Y^L / Y^S$ and $X \cdot Y^L / Y^S \cdot Y^S$ males and were crossed to y w bb/y w bb females.

Y^L and Y^S are sensitive to sc^4sc^8-induced drive and that Y^L is more sensitive than Y^S, consistent with its greater length.

Drive Sensitivity of the Bobbed Locus

Since both Y^S and Y^Ly^{3M} carry *rDNA* genes, one wonders what contribution the *rDNA* makes to their relative sensitivities. One way to estimate this contribution is to compare the sensitivities of Y^Lbb^+ and Y^Lbb^- relative to Y^S. Since Y^Lbb^+ is longer than Y^Lbb^- it should be more sensitive. The results of crosses involving half-sibling $sc^4sc^8/Y^S/Y^Lbb^+$ and $sc^4sc^8/Y^S/Y^Lbb^-$ males, displayed in Table 14, bear out this expectation. The sensitivity difference between Y^Lbb^+ and Y^Lbb^- is substantial; the recovery of Y^Lbb^+ relative to Y^S is only .45 while that of Y^Lbb^- relative to Y^S is .70. Deletion of the bobbed locus reduces the sensitivity of a *Y* chromosome. Does this result imply that the bobbed locus is the *Y* chromosome response function? If so, then Y^S (which is bb^+) should be more sensitive than Y^Lbb^-. This is clearly not the case. A more reasonable interpretation is that the sensitivity difference between Y^Lbb^+ and Y^Lbb^- reflects their length difference.

Sensitivity of a Free X Duplication

To determine how an *X* chromosome fragment fits into this graded set of *Y* fragments, the sensitivity of *Dp(1;f)3*, an *X* chromosome deficient for almost all the euchromatin was assessed versus both Y^S and $Y^S{\cdot}Y^S$ using B^SY as standard. Four types of males were tested: $sc^4sc^8/B^SY/Y^S$ and $sc^4sc^8/B^SY/Dp(1;f)3$ siblings and $sc^4sc^8/B^SY/Y^S{\cdot}Y^S$ and $sc^4sc^8/B^SY/Dp(1;f)3$ siblings. The results in Table 15 show that Y^S is less sensitive than the *Dp*. The recovery of B^SY relative to Y^S is only .19 while its recovery relative to *Dp(1;f)3* is .27, a highly significant difference.

Table 14 Comparison of Drive Sensitivities of $Y^L bb^+$ and $Y^L bb^-$ Relative to Y^S

	Sperm Classes				Recovery Ratios	
Paternal Genotype	XY^S	XY^L	Y^S	Y^L	$Y^L:Y^S$	X:0
$sc^4 sc^8/Y^S/Y^L y^{3M}$, bb^+	1703	699	3560	1698	.45	.45
$sc^4 sc^8/Y^S/Y^L \cdot y^+ B2$, bb^-	1851	1320	2818	1961	.70	.66

The paternal males were generated as half-brothers from crosses of $sc^4 sc^8/sc^4 sc^8/Y^S$ females to $X \cdot Y^S/Y^L y^{3M}$ and $X \cdot Y^S/Y^L \cdot y^+ B2$ males and were crossed to y/y females.

Table 15 Comparison of Drive Sensitivities of Y^S, $Y^S \cdot Y^S$, and Dp(1;f)3 Relative to B^SY

	Sperm Classes								Recovery Ratios		
Paternal Genotype	XY^S	$XY^S \cdot Y^S$	XB^SY	XDp	Y^S	$Y^S \cdot Y^S$	B^SY	Dp	$B^SY:Y^S$	B^SY:Dp	X:0
$sc^4sc^8/Y^S/B^SY$	565	----	91	---	1945	----	390	----	.19	----	.26
sc^4sc^8/B^SY/Dp(1;f)3	---	----	77	361	----	----	288	983	---	.27	.34
$sc^4sc^8/Y^S \cdot Y^S/B^SY$	---	388	95	---	----	1422	419	---	.28	---	.26
sc^4sc^8/B^SY/Dp(1;f)3	---	----	61	398	----	----	335	1054	---	.27	.33

The males in lines 1 and 2 are siblings, generated from a cross of sc^4sc^8/sc^4sc^8/Dp(1;f)3 females to y w/B^SY/Y^S males. The males in lines 3 and 4 are also siblings, derived from a cross of sc^4sc^8/sc^4sc^8/Dp(1;f)3 females to y w/$B^SY/Y^S \cdot Y^S$ males. The males in lines 1 and 2 are half-brothers to those in lines 3 and 4. All crosses were to y w bb/y w bb females.

The
The
to
cl
<u>sc</u>
in

se
le
t
a
r

X

c

The data also indicate that $\underline{Y^S \cdot Y^S}$ and Dp(1;f)3 are equally sensitive. The recovery of $\underline{B^S Y}$ relative to $\underline{Y^S \cdot Y^S}$ is .28 and its recovery relative to Dp(1;f)3 is .27. These data also provide further support for the claim that $\underline{Y^S \cdot Y^S}$ is more sensitive than $\underline{Y^S}$ since the two crosses of $\underline{sc^4 sc^8}/\underline{B^S Y}/\underline{Dp(1;f)3}$ males give the same results, and the two crosses involving Y fragments do not.

The results presented above are consistent with the idea that the sensitivity of a sex chromosome in $\underline{sc^4 sc^8}$ males is a function of its length. In all comparisons of a shorter chromosome with a longer one, the longer one proved to be more sensitive. This could reflect either an even distribution of discrete response loci on the Y or a diffuse response function.

X Chromosome Recovery

In $\underline{sc^4 sc^8}/\underline{Y}/\underline{Y}$ (Sandler and Braver 1954) males and in the three-sex-chromosome genotypes tested in this study, the sex ratio is highly skewed toward males. If the poor recovery of $\underline{sc^4 sc^8}$ (and of the Y fragments) is caused by shortage of an essential chromosome processing material, then X recovery should be inversely proportional to total sex chromatin in the genome. The experiments displayed in Tables 12 and 15 provide tests of this prediction. In Table 12, sex chromatin content ranges from one X and almost two Y's ($\underline{sc^4 sc^8}/\underline{B^S Y}/\underline{Y^L y^{3M}}$) to one X and one Y ($\underline{sc^4 sc^8}/\underline{Y^S}/\underline{Y^L y^{3M}}$). However, there are no significant differences in X recovery between any two of the males. In Table 15, $\underline{sc^4 sc^8}/\underline{Y^S}/\underline{B^S Y}$ and $\underline{sc^4 sc^8}/\underline{Y^S \cdot Y^S}/\underline{B^S Y}$ males show identical X recovery values despite their differences in sex chromatin amount. Tables 13 and 14 are not useful for this purpose because autosomal background is not fully controlled.

To examine this question further, two additional experiments were performed to compare *X* chromosome recovery in males with large differences in sex chromatin content. In the first experiment, $sc^4sc^8/B^SY/Y$ and $sc^4sc^8/Y/Dp(1;f)3$ males were generated as brothers from a cross of $sc^4sc^8/B^SY/Dp(1;f)3$ males to *C(1)DX/Y* females. In the second experiment, $sc^4sc^8/B^SY/Y$, $sc^4sc^8/Y/Dp(1;f)3$, and $sc^4sc^8/B^SY/Dp(1;f)3$ sibling males were generated from a cross of $sc^4sc^8/sc^4sc^8/Dp(1;f)3$ females to $y\ w/Y/B^SY$ males. The results of both experiments are presented in Table 16. In the first experiment, there is no significant difference between $sc^4sc^8/B^SY/Y$ and $sc^4sc^8/Y/Dp(1;f)3$ males in terms of *X* recovery (.52 and .49 respectively) although the differences in *Y* recovery are large. In the second experiment, *X* recovery is .43 from $sc^4sc^8/Y/B^SY$, .41 from $sc^4sc^8/Y/Dp$ and .31 from $sc^4sc^8/B^SY/Dp$ males. The $sc^4sc^8/B^SY/Dp$ result is significantly different from the other two but it is in the wrong direction for demonstrating an inverse relationship between sex chromosome content and *X* recovery.

$sc^4sc^8/B^SY/Y^S/Dp(1;f)3$ Males

Another test of the competition hypothesis is to compare *X* recovery in sibling males carrying either three or four sex chromosomes. When $sc^4sc^8/sc^4sc^8/Dp(1;f)3$ females were crossed to $y\ w/B^SY/Y^S$ males, the offspring included $sc^4sc^8/B^SY/Y^S/Dp(1;f)3$ males. They were crossed to *y w bb/y w bb* females. The results, presented in Table 17, indicate that no sperm carrying only sc^4sc^8 were recovered. The addition of a fourth sex chromosome does not change the univalent behavior of sc^4sc^8. Since the *X* segregates randomly, its recovery can be used as an absolute measure of meiotic drive. *X* recovery from $sc^4sc^8/B^SY/Y^S/Dp(1;f)3$ males is .31, not significantly different from either the .28 recovery observed for

Table 16 Comparison of Sibling sc^4sc^8/Y/Y and sc^4sc^8/Y/Dp Males

Experiment	Paternal Genotype	Sperm Classes						Recovery Ratios		
		XB^SY	XY	XDp	B^SY	Y	Dp	B^SY:Y	Y:Dp	X:0
1	sc^4sc^8/Y/B^SY	117	115	---	260	188	----	1.24	----	.52
	sc^4sc^8/Y/Dp(1;f)3	---	499	1377	---	1360	2474	----	.48	.49
2	sc^4sc^8/Y/B^SY	210	262	----	447	661	----	.71	---	.43
	sc^4sc^8/Y/Dp(1;f)3	---	230	555	---	655	1244	---	.49	.41
	sc^4sc^8/B^SY/Dp(1;f)3	46	---	218	179	---	663	---	.26	.31

The paternal males in experiment 1 are siblings as are those in experiment 2. In all crosses, the females were *y w bb*/*y w bb*. The unmarked *Y* is different in the two crosses which may explain why it is recovered worse than B^SY in experiment 1 but better in experiment 2.

Table 17 Sperm Recovery from $sc^4sc^8/B^SY/Dp(1;f)3/Y^S$ Males

Progeny Phenotypes								
Females				Males				
$y\ w^a\ B$	B	$y\ w^a$	w^a	y w B	B	y w	w	X Chromosome Recovery
5	7	42	30	23	8	128	110	.31

sc^4sc^8, $y\ w^a\ bb^-/B^SY/Y^S/Dp(1;f)3$, $y^+\ bb^+$ males were crossed to y w bb/y w bb females.

$\underline{^{4}\mathrm{sc}\ \mathrm{s}}$

sibl

$\underline{^{4}\mathrm{sc}\ \mathrm{s}}$

$\underline{\mathrm{B}^{\mathrm{S}}\mathrm{Y}}$

rec

si

gu

Tr

hy

$sc^4sc^8/B^SY/Y^S$ siblings or the .34 figure observed for $sc^4sc^8/B^SY/$*Dp(1;f)3* siblings (Table 15). The addition of sex chromatin to the genome of a sc^4sc^8 male does not increase drive levels.

Although it is impossible to detect Y^S in the presence of either B^SY or *Dp(1;f)3*, since both are bb^+, some conclusions can be made about recovery of chromosomes other than the *X*. First, Y^S has the best recovery since the Y^S class exceeds the *Dp* and Y^SDp classes (which are indistinguishable) combined. Second, the recovery of B^SY (43/310) is the worst. These are exactly the relationships expected under the length dependence hypothesis.

DISCUSSION

The major conclusion from this study is that the response of the *Y* chromosome to meiotic drive is not a property of a single, discrete response function. The results are consistent with the idea that drive response is a diffuse function of *Y* chromatin so that the sensitivity of a *Y* chromosome is proportional to its length. As a limited number of *Y* fragments are available for testing, it is not possible to rule out the idea that meiotic drive sensitivity is controlled by several discrete loci.

These results are consistent with at least two hypotheses concerning the effects of relative and absolute size of chromosomes on severity of meiotic drive in sc^4sc^8 males. Under one hypothesis, only the longer of two pairing partners is subject to recovery disruption. The shorter one is protected by pairing along its length. The degree of recovery disruption of the longer chromosome is proportional to the length difference between it and the shorter element. To borrow Baker and Carpenter's (1972) colorful metaphor, each chromosome carries an array of "armed bombs" (pairing sites) which must be defused by meiotic pairing. The shorter of two homologs is able to defuse all its bombs because it is fully paired. The longer homolog must be unpaired along some length and can not be fully defused. This hypothesis implies that drive is much milder in a genotype such as $sc^4sc^8/B^SY/Y^Ly^{3M}$ where the two *Y* elements are close to the same size than in $sc^4sc^8/B^SY/Y^S$ where the two *Y* elements are vastly different in size. In either genotype, the unpaired *X* would go through meiosis with unreacted pairing sites and would be recovered poorly, although no worse in one genotype than the other.

An alternative hypothesis is that $sc^{4}sc^{8}$ disrupts recovery of all sex chromosomes, the degree of disruption being proportional to the length of the chromosome. $sc^{4}sc^{8}/B^{S}Y/Y^{L}$ males exhibit a relatively high *Y* chromosome recovery ratio because $B^{S}Y$ and Y^{L} are of similar sizes and subject to similar levels of drive. The absolute recovery of $B^{S}Y$ sperm would be no better in these males than in $sc^{4}sc^{8}/B^{S}Y/Y^{S}$ males.

Shortage of an essential chromosome processing material is one mechanism that could give rise to length-dependent chromosome recovery. If it is assumed that each binding site for the material must be occupied for a chromosome to be non-lethal, and if the number of binding sites is a function of length, then the probability of at least one site being unoccupied is proportional to length. Shortage of the material would create competition between chromosomes. One consequence of competition would be the more sex chromatin present in a spermatocyte, the less processing material is available to each chromosome. Drive levels would be proportional to the amount of sex chromatin in the genome.

Most of the data, and more important, the best data, are inconsistent with this prediction. *X* recovery is the same in $sc^{4}sc^{8}/B^{S}Y/Y^{L}$, $sc^{4}sc^{8}/Y^{S}/Y^{L}$, and $sc^{4}sc^{8}/Y^{S}/B^{S}Y$ siblings (Table 12) even though total sex chromatin content (and *Y* chromosome recovery ratios) differ considerably among the three genotypes. $sc^{4}sc^{8}/Y/B^{S}Y$ males have the same *X* recovery as $sc^{4}sc^{8}/Y^{S}\cdot Y^{S}/B^{S}Y$ males (Table 15). These males are only half-brothers but results from their $sc^{4}sc^{8}/B^{S}Y/Dp(1;f)3$ full brothers showed that no autosomal modifiers of any importance were segregating in these crosses. In two experiments comparing sibling $sc^{4}sc^{8}/Y/B^{S}Y$ and $sc^{4}sc^{8}/Y/Dp(1;f)3$ males, the only significant difference in *X* recovery was in the wrong direction (Table 16). Finally, addition of a fourth sex chromosome to the genotype to make $sc^4sc^8/B^SY/Y^S/Dp(1;f)3$ males does not affect *X* chromosome recovery (Table 17). These results imply that sex chromosomes do not compete for a scarce resource in sc^4sc^8 males. If they did, one would expect to find an inverse relationship between sex chromatin content and meiotic drive levels. No such relationship exists.

These results are consistent with the armed bomb model. If the poor recovery of the *X* chromosome in these males is due to its failure to pair, one would not expect the amount of *Y* chromatin to matter. *X* recovery should be a constant, or should vary unsystematically with, changes in *Y* chromosome lengths. This is what is found. The major difficulty with this view is that it calls for pairing interactions between parts of chromosomes that do not appear, under the microscope, to be involved in meiotic pairing. The poor recovery of the *Y* in $sc^4sc^8/Y/Dp$ males implies that the free duplication does not have enough pairing sites to defuse all (or even most) of the *Y* chromosome's bombs. Since a normal *X* does, this implies that the remaining pairing sites must be euchromatic. Yet no one has ever observed any involvement of the *X* euchromatin in *X*-*Y* meiotic pairing.

CHAPTER 4

SENSITIVITY OF AUTOSOMES TO MEIOTIC DRIVE

In *Drosophila melanogaster* males, the *X* heterochromatic deficiency $In(1)sc^{4L}sc^{8R}$ (sc^4sc^8) disrupts recovery of sex chromosomes by causing sperm dysfunction (Gershenson 1933; Sandler and Braver 1954). Both the *Y* chromosome (in sc^4sc^8/*Y* and sc^4sc^8/*Y*/*Dp*) and the *X* (in sc^4sc^8/*Y*/*Dp* and sc^4sc^8/*Y*/*Y*) are subject to this recovery disruption (Gershenson 1933; Sandler and Braver 1954; Peacock, Miklos, and Goodchild 1975; Haemer 1978). No evidence exists as to whether or not recovery of autosomes is also disrupted. The question addressed in the following study is: does sc^4sc^8 disrupt autosomal recovery?

Since all sperm normally have the same autosomal content, they would be equally sensitive to length dependent recovery disruption in ordinary sc^4sc^8 males. To detect autosomal sensitivity, a sc^4sc^8 genotype which generates sperm with unequal amounts of autosomal chromatin was constructed. Males heterozygous for a reciprocal translocation between the major autosomes with one break near the tip of chromosome *2* and the other in the centric heterochromatin of chromosome *3* generate sperm carrying different amounts of autosomal chromatin. If autosomes are sensitive to sc^4sc^8-induced meiotic drive, recovery of sperm classes from sc^4sc^8 males carrying such a translocation should be inversely proportional to their autosomal chromatin content. The results described below indicate that this is the case. The implication is that *X* heterochromatic deficiencies disrupt a developmental process affecting all chromosomes.

MATERIALS AND METHODS

$\underline{T(2;3)bw^{V4}}$ (Lindsley and Grell 1968) has one break near the tip of chromosome $\underline{2}$ in the vicinity of the brown locus and a second break in the centric heterochromatin of chromosome $\underline{3}$. One half-translocation, $\underline{2^L \cdot 2^R 3^L}$, is unusually long, consisting of the equivalent of three autosome arms. The other half-translocation, $\underline{3^R}$, is unusually short. (For simplicity the translocated tip of $\underline{2^R}$ is neglected in this discussion -- it is too small to have any significant effect). Expected patterns of chromosome segregation in $\underline{T(2;3)bw^{V4}}$ heterozygotes are illustrated in Figure 1. Alternate segregations produce two types of euploid gametes, either normal sequence or translocated. Adjacent I segregations produce $\underline{2^L \cdot 2^R 3^L};\underline{3}$ (duplication) gametes and $\underline{2};\underline{3^R}$ (deficiency) gametes. Adjacent II segregations, which should be relatively infrequent or perhaps nonexistent (Glass 1933; Glass 1935; Roberts 1976) generate $\underline{2^L \cdot 2^R 3^L};\underline{2}$ and $\underline{3^R};\underline{3}$ gametes. The aneuploid gametes generated by adjacent segregations lead to viable progeny only when they combine with reciprocal aneuploid gametes from the other sex.

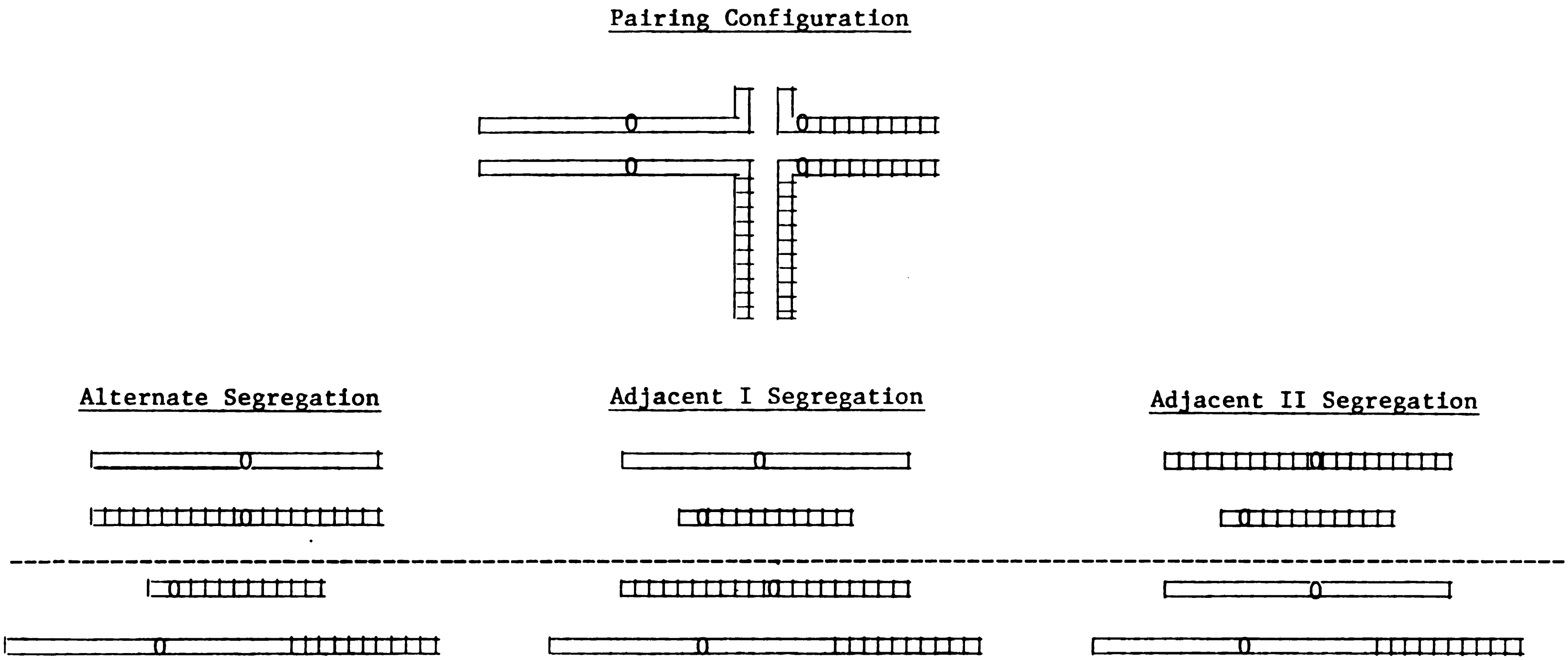


Figure 2 Pairing and Disjunction in $T(2;3)bw^{V4}$ Heterozygotes

RESULTS

Absence of Adjacent II Segregations

A preliminary question of some importance is whether or not adjacent II segregants are recovered from males heterozygous for T(2;3)bw^{V4}. Glass found very few or none (1933; 1935). To corroborate his finding, T(2;3)bw^{V4} was marked with Star (S, an eye texture mutant), and males heterozygous for T(2;3)bw^{V4}, S and E(S) (Enhancer of Star, a dominant second chromosome mutant that strongly enhances the Star phenotype) were crossed to females heterozygous for the unmarked T(2;3)bw^{V4}. The recovery of S/E(S) progeny (distinguished by very small, rough eyes) would signal the occurrence of adjacent II segregation since they must come from $2^L \cdot 2^R 3^L;2$ sperm (see Figure 1). No S/E(S) progeny were found among 345 offspring, implying that adjacent II segregation does not occur in at least one sex. All aneuploid gametes derive from adjacent I segregations.

Sperm Recovery from T(2;3)bw^{V4} Males

When males and females heterozygous for T(2;3)bw^{V4} are crossed, the progeny will include individuals derived from sperm with three, four, or five major autosomal arms. If sc^{4}sc^8 disrupts autosomal recovery it should bias recovery ratios in favor of sperm classes with the least amount of autosomal material. Males carrying sc^{4}sc^8 or a normal sequence X and T(2;3)bw^{V4} or non-translocated autosomes were generated as siblings by the mating scheme diagrammed in Figure 3. This produced sc^{4}sc^8/y$^+$Y T(2;3)bw^{V4}/St males and controls (y/y$^+$Y T(2;3)bw^{V4}/St, sc^{4}sc^8/y$^+$Y TM2/St, and y/y+Y TM2/St) as siblings. These males were then crossed in single pair matings to y/y T(2;3)bw^{V4}/SM1, Cy females. Figure 4A is a diagram of the cross for y/y$^+$Y T(2;3)bw^{V4} males. A diagram for sc^{4}sc^8/y$^+$Y

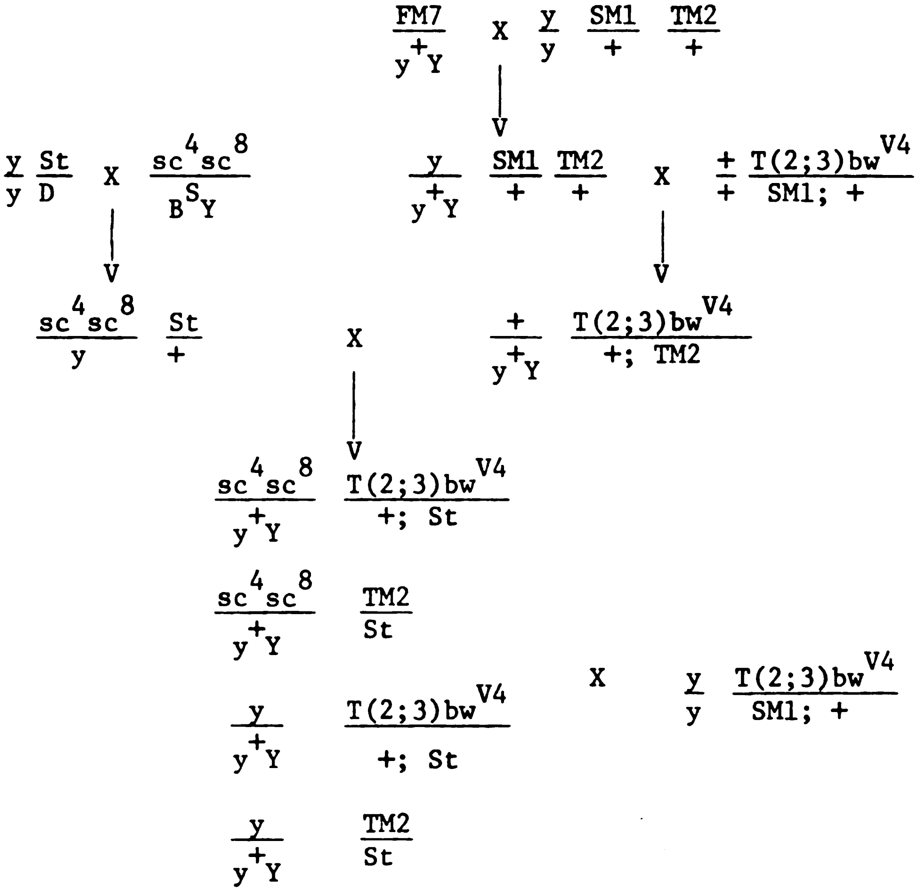


Figure 3 Crosses to Generate $sc^{4}sc^{8}$;$T(2;3)bw^{V4}$ Males and Controls

	Egg Classes			
Sperm Classes	$y;SM1;+$	$y;2^L\cdot 2^R 3^L;+$	$y;SM1;3^R$	$y;2^L\cdot 2^R 3^L;3^R$
$y;+;St$	yCySt Females			ybwSt Females
$y;2^L\cdot 2^R 3^L;St$			yCybwSt Females	
$y;+;3^R$		ybw Females		
$y;2^L\cdot 2^R 3^L;3^R$	yCybw Females			ybw Females
$y^+Y;+;St$	CySt Males			bwSt Males
$y^+Y;2^L\cdot 2^R 3^L;St$			CybwSt Males	
$y^+Y;+;3^R$		bw Males		
$y^+Y;2^L\cdot 2^R 3^L;3^R$	Cybw Males			bw Males

$y/y^+Y;T(2;3)bw^{V4}/+;St$ males were crossed to $y/y;T(2;3)bw^{V4}/SM1;+$ Females

Figure 4A Diagram of Cross between Two Translocation Heterozygotes

	Egg Classes			
Sperm Classes	$y;SM1;+$	$y;2^L\cdot 2^R 3^L;+$	$y;SM1;3^R$	$y;2^L\cdot 2^R 3^L;3^R$
$y;+;TM2$	yCyUbx Females			ybwUbx Females
$y;+;St$	yCySt Females			ybwSt Females
$y^+Y;+;TM2$	CyUbx Males			bwUbx Males
$y^+Y;+;St$	CySt Males			bwSt Males

$y/y^+Y:TM2/St$ males were crossed to $y/y;T(2;3)bw^{V4}/SM1$ females.

Figure 4B Diagram of Control Cross

$T(2;3)bw^{V4}$ males would be similar, but twice as large since *XY* and nullo-*X*, nullo-*Y* sperm are generated as well as X and Y sperm. The autosomal combinations are identical in the two crosses. A diagram of the control cross in which the males carry *TM2* (instead of the translocation) and a normal *X* appears in Figure 4B. Once again, the sc^4sc^8 cross is the same except that *XY* and nullo-*X*, nullo-*Y* classes are present as well as *X* and *Y*.

The data from these crosses appear in Tables 18 and 20. Table 18 presents the data for the translocation males, both sc^4sc^8 and regular *X*, and Table 20 presents the non-translocation male data.

Several conclusions are evident from the data in Table 18. The first is that alternate segregants outnumber adjacent segregants in both crosses; by 1925 to 458 in the normal *X* cross and by 1275 to 443 in the sc^4sc^8 cross. The second is that in the normal *X* cross reciprocal products are recovered in the expected ratios for both types of segregation. The expected ratio among the alternate (euploid) segregants is two normal sequence sperm to one translocation sperm. 1925 normal sequence sperm and 952 translocation sperm were recovered. Among the adjacent segregants, the expected ratio is one $2^L \cdot 2^R 3^L;3$ to one $2;3^R$ sperm. The observations were 213 and 245 respectively.

If translocation homozygotes survive, they would be indistinguishable from the deficiency class in this cross. Since Glass (1933) found homozygotes to be weakly viable, this possibility was examined in a separate cross with appropriately marked autosomes. No translocation homozygotes were recovered out of 1215 flies. Thus the viability of $T(2;3)bw^{V4}$ homozygotes is zero in these crosses.

In the sc^4sc^8 cross, reciprocal products of alternate segregation were recovered in the expected two to one ratio: 1478 normal sequence

Table 18 Results from Crosses between T(2;3)bw^{V4} Heterozygotes

Progeny Phenotype		Paternal Genotype	
Females	Sperm Genotype	$\frac{sc^4 sc^8}{y^+Y}$ $\frac{T(2;3)bw^{V4}}{+;\ St}$	$\frac{y}{y^+Y}$ $\frac{T(2;3)bw^{V4}}{+;\ St}$
y bw St	X;+;St	316	457
y Cy St	X;+;St	279	456
y bw Cy	X;$2^{L}\cdot 2^{R}3^{L}$;3^{R}	315	488
y bw	X;+;3^{R}	158	122
y bw Cy St	X;$2^{L}\cdot 2^{R}$;St	42	110
bw St	XY;+;St	19	---
Cy St	XY;+;St	31	---
bw Cy	XY;$2^{L}\cdot 2^{R}3^{L}$;3^{R}	27	---
bw	XY;+;3^{R}	14	---
bw Cy St	XY;$2^{L}\cdot 2^{R}3^{L}$;St	5	---
Males			
y bw St	+;St	243	---
y Cy St	+;St	256	---
y bw Cy	$2^{L}\cdot 2^{R}3^{L}$;3^{R}	38	---
y bw	+;3^{R}	124	---
y bw Cy St	$2^{L}\cdot 2^{R}3^{L}$;St	2	---
bw St	Y;+;St	156	516
Cy St	Y;+;St	178	496
bw Cy	Y;$2^{L}\cdot 2^{R}3^{L}$;3^{R}	161	464
bw	Y;+;3^{R}	85	123
bw Cy St	Y;$2^{L}\cdot 2^{R}3^{L}$;St	13	103

The females in both crosses were y/y;T(2;3)bw^{V4}/SM1, Cy; +

sperm to 753 translocation sperm. But reciprocal products of adjacent I segregation were not recovered equally. Summed over all sex chromosome classes, 381 $\underline{2;3^R}$ and 62 $\underline{2^L \cdot 2^R 3^L;3}$ sperm were recovered. This six to one ratio contrasts sharply with the one to one ratio obtained in the normal *X* cross.

The ratios of deficiency to euploid classes and of duplication to euploid classes are also affected by $\underline{sc^4 sc^8}$. In the control these ratios are both approximately one to four in the offspring (using the euploid translocation class as denominator), which implies a one to two ratio in the gametes of both sexes (assuming equal nondisjunction in both sexes). In the presence of $\underline{sc^4 sc^8}$, the deficiency to euploid ratio in the offspring is one to two which implies a one to one ratio in the sperm (assuming that the egg ratio stays at one to two). The duplication to euploid ratio in $\underline{sc^4 sc^8}$ is one to nine in the progeny (using only the *X* and *Y* classes, for reasons described below) which corresponds to a one to four or five sperm ratio. Relative to the euploid class, $\underline{sc^4 sc^8}$ increases the viability of deficiency sperm and decreases the viability of duplication sperm. This implies that in $\underline{sc^4 sc^8}$ males the probability of recovery of a sperm is inversely proportional to the amount of autosomal chromatin it contains.

A particularly striking feature of these data is the absence of interaction between autosomal and sex chromosomal meiotic drive. Despite the gross violations of Mendelian ratios, autosomal and sex chromosomal recovery ratios are mutually independent. This is illustrated in Table 19 which presents the data in an orthogonal array. Note that, with two exceptions in the nullo-*X*, nullo-*Y* class (discussed below), the expected number in each class (assuming independence) agrees remarkably well with

Table 19 Orthogonal Presentation of Results from sc^4sc^8;T(2;3)bw^{V4} Males

Sperm Sex Chromosome Genotype	+;St	$2^L\cdot2^R3^L;3^R$	$+;3^R$	$2^L\cdot2^R3^L;St$	Sums
sc^4sc^8	595 (604)	315 (310)	158 (158)	42 (37)	1110
sc^4sc^8/y^+Y	50 (52)	27 (27)	14 (14)	5 (3)	96
0	499	38	124	2	663
y^+Y	334 (323)	161 (166)	85 (85)	13 (20)	593
Sums	979	503	257	60	1799

Sums and expected values (in parentheses) are calculated without nullo-X, nullo-Y flies because of the viability problems discussed in the text.

the observed number.

The two exceptions mentioned above are the nullo-*X*, nullo-*Y*; $2^{L}\cdot 2^{R}3^{L}$;3^{R} and nullo-*X*, nullo-*Y*;$2^{L}\cdot 2^{R}3^{L}$;*3* sperm which both show very poor recovery. The few flies derived from these sperm which did survive were late-hatching, thin-bristled, and tended to get stuck in the food. A plausible explanation is partial dominant lethality due to variegation of the paternally transmitted $2^{L}\cdot 2^{R}3^{L}$ element in *XO* males. The phenotype suggests Minute and there is a strong Minute locus at *58D*, just a few bands proximal to the breakpoint. Variegation is implied by the fact that the recovery of $2^{L}\cdot 2^{R}3^{L}$ sperm is abnormal only in the *XO* males. It is interesting that the same element shows normal recovery when transmitted from the mother. This is an apparent example of the parental source effect (discussed by Spofford 1976).

To test this explanation, nullo-*X*, nullo-*Y*;$2^{L}\cdot 2^{R}3^{L}$ males were generated by another route. $sc^{4}sc^{8}/y;T(2;3)bw^{V4}/SM1$ females were crossed to $y/y^{+}Y;T(2;3)bw^{V4}/St$ males. *X* chromosome four-strand double exchanges generate nullo-*X* eggs which, when fertilized by *X* sperm, give rise to *XO* males. Some of these males carry a paternal $2^{L}\cdot 2^{R}3^{L}$ element and their recovery is depressed. No nullo-*X*, nullo-*Y* progeny with a paternal $2^{L}\cdot 2^{R}3^{L}$ chromosome were recovered out of 55 nullo-*X*, nullo-*Y* males. In the other sex chromosome classes, recovery of the paternally transmitted $2^{L}\cdot 2^{R}3^{L}$ was normal.

Autosomal Modification of Drive and Nondisjunction

These experiments provide information relative to a second issue: the modifying effect of different autosomes on nondisjunction and meiotic drive in $sc^{4}sc^{8}$ males. The raw data from the cross of $sc^{4}sc^{8}/y^{+}Y;TM2/St$ are presented, along with normal *X* controls, in Table

20. From the control it can be seen that no genotype is associated with particularly poor viability. The experimental cross is quite unremarkable. Nondisjunction and drive are both relatively high. There is no apparent tendency toward greater recovery of either *TM2* or its *St*-bearing homolog.

A comparison of sperm recovery from $sc^4sc^8/y^+Y;T(2;3)/St$ and $sc^4sc^8/y^+Y;TM2/St$ males is presented in Table 21. Nondisjunction is higher and chromosome recovery values lower in the *TM2*/*St* males. The difference in nondisjunction values is exaggerated by the inviability of nullo-*X*, nullo-*Y*;$2^L \cdot 2^R3^L$ sperm. Assuming frequencies of these sperm similar to their frequencies in the *X* and *Y* classes, nondisjunction in *T(2;3)* males is .45, still substantially lower than the .57 value given by the *TM2*/*St* males.

Since these males have different second and third chromosomes, it is unclear whether the differences in nondisjunction and drive should be attributed to the translocation, to the balancer, to the unmarked second chromosome, or to some combination. Ramel (1968) failed to find any "interchromosomal effect" of the Curly inversions on sc^4sc^8-induced drive or nondisjunction. It is possible that a third chromosome balancer would behave differently. In any case, this is a clear example of autosomal modification of meiotic drive.

Table 20 Results from Control Crosses

Progeny Phenotype	Sperm Genotype	Paternal Genotype	
		$\frac{sc^4 sc^8}{y^+Y}$ $\frac{TM2}{St}$	$\frac{y}{y^+Y}$ $\frac{TM2}{St}$
Females			
y bw St	X; St	69	132
y bw Ubx	X; TM2	71	159
y Cy St	X; St	64	162
y Cy Ubx	X; TM2	51	162
bw St	XY; St	5	---
bw Ubx	XY; TM2	0	---
Cy St	XY; St	5	---
Cy Ubx	XY; TM2	6	---
Males			
y bw St	St	101	---
y bw Ubx	TM2	77	---
y Cy St	St	72	---
y Cy Ubx	TM2	87	---
bw St	Y: St	25	144
bw Ubx	Y; TM2	40	128
Cy St	Y; St	20	146
Cy Ubx	Y; TM2	40	140

The females in both crosses were y/y;T(2;3)bw^{V4}/SM1, Cy; +

Table 21 Nondisjunction and Drive in sc^4sc^8 Males With and Without $T(2;3)bw^{V4}$

Paternal Genotype	Sperm Classes X	Sperm Classes XY	Sperm Classes 0	Sperm Classes Y	Nondisjunction 0:(X+0)	Recovery Ratios Y:X	Recovery Ratios XY:0
$sc^4sc^8/y^+Y;T(2;3)bw^{V4}/St$	1110	96	663	593	.37	.53	.14
$sc^4sc^8/y^+Y;TM2/St$	255	16	337	125	.57	.49	.05

The females in both crosses were y/y; $T(2;3)bw^{V4}/SM1$, Cy; +.

DISCUSSION

The main conclusion of this study is that sperm recovery in sc^4sc^8 males is inversely proportional to autosomal chromatin content. The ratio of autosomally deficient sperm to euploid sperm is twice as high in sc^4sc^8 as in controls. Similarly, the ratio of euploid to autosomally duplicated sperm is better than twice as high in sc^4sc^8. The simplest explanation of these results is that sc^4sc^8 alters sperm viability to favor recovery of sperm with less chromatin. One implication of this result is that recovery of *X*; autosomally euploid and of nullo-*X*, nullo-*Y*; autosomally euploid sperm from sc^4sc^8 males is less than perfect.

The results from this study are also relevant to the question of the role of pairing in the production of normal sperm. Following the suggestions of Baker and Carpenter (1972) and Peacock and Miklos (1973), sc^4sc^8 drive might result from mismatch of sex chromosome pairing partners. The extra unreacted pairing sites on the longer pairing partner act as "armed bombs" and destroy the sperm that contain them. This hypothesis can account for length dependence of sex chromosome sensitivity (discussed in Chapter 3) but does not explain the failure of free *X* duplications to improve *Y* chromosome recovery. It also does not account for the data presented in this chapter. If sc^4sc^8 acts by interfering with proper *X*-*Y* pairing, there is no reason to expect autosomal involvement at all. While a translocation between the second and third chromosomes might weaken local pairing and perhaps expose some autosomal pairing sites, why should this effect manifest itself only in the presence of sc^4sc^8? No hint of autosomal pairing difficulties can be seen in normal *X* males. These results imply that the *X* chromosome deficiency affects autosomes in some fashion not related to pairing.

The evidence for independence of sex chromosome and autosome sensitivity is interesting in light of the earlier discussion (Chapter 3) of competitive chromosome interactions. If $\underline{sc^4sc^8}$ causes a shortage of an essential chromosome processing material, then chromosomes must compete for adequate supplies of it. Whether competition takes place in the spermatocyte prior to anaphase or in spermatids after meiosis, one would expect that whenever both sex chromosomes succeed in garnering enough of the material, less would remain to be divided among the autosomes . Autosomally duplicated sperm would be less frequent among the XY class than among the X or Y classes. Similarly, the frequency of autosomally duplicated or even euploid sperm among the X or Y class should be lower than among the nullo-X, nullo-Y class. Autosome recovery frequencies should be conditional upon sex chromosome genotype and vice versa. This is not the case. Autosomal recovery frequencies are the same across all sex chromosome genotypes and sex chromosome recoveries are the same across all autosomal genotypes.

The absence of competition among chromosomes in $\underline{sc^4sc^8}$ males implies that sperm defects are not caused by shortage of a chromosome processing material. An alternative is that $\underline{sc^4sc^8}$ reduces the time available for a key meiotic process. The likelihood of a chromosome completing the step would be inversely proportional to its length. No competition would ensue because the scarce quantity, time, can not be sequestered.

Frequent references in this chapter and the previous one have been made to "chromosome sensitivity" as if it had been demonstrated that the $\underline{sc^4sc^8}$ deficiency actually alters the state of chromatin in some physical way. This has not been demonstrated. It has been shown that the recovery of sperm from sc^4sc^8 males depends inversely on their chromatin content, while sperm recovery from normal males is independent of chromatin content. This implies either that the chromatin has been altered by sc^4sc^8 to become sperm-lethal, or that some other aspect of sperm anatomy or physiology has been altered to render the sperm sensitive to perfectly normal chromatin. One example of the "insensitive sperm" type of explanation would be reduced motility. Perhaps sperm from sc^4sc^8 males are unusually sluggish. Such sperm might be less sluggish when carrying a lighter than normal nucleus and more sluggish when carrying extra nuclear weight.

Finally, it is interesting to compare these results with the results of investigations into the interactions between sex chromosomes and the *SD* autosomal meiotic drive systim. Sperm bearing the homolog of the *Sd* chromosome in heterozygous males frequently fail to function so that as many as 99% of the heterozygote's offspring inherit the *Sd* chromosome (Sandler, Hiraizumi and Sandler 1959; Hartl, Hiraizumi and Crow 1967; Tokuyasu, Peacock and Hardy 1977). Most of the Sd^+ survivors are females (Hiraizumi and Nakazima 1967). An attached-*XY* chromosome is recovered much better than a *Y* chromosome among Sd^+ offspring of attached-*XY*/*Y*; *Sd*/Sd^+ males. An even more pronounced effect is observed with attached-*XY*/*0*;*Sd*/Sd^+ males (Denell and Miklos 1971). It was concluded that different sex chromosomes can be ranked in order of recovery probability among Sd^+ offspring of *Sd* heterozygotes. The order, from best to worst, is attached-*XY*, *X*, *Y*, nullo-*X*, nullo-*Y*. The amount of sex chromatin affects the viability of these sperm; the more sex chromatin, the better the viability. It is curious that in both drive systems the viability of sperm should prove to depend dramatically on chromatin content, but in opposite directions -- positively in *SD*, negatively in sc^4sc^8.

CHAPTER 5

X HETEROCHROMATIC DUPLICATIONS AND MEIOTIC DRIVE

In *Drosophila melanogaster* males, normal *X*-*Y* pairing depends on interactions between discrete pairing sites (collochores) located in the *X* heterochromatin and in the centromeric regions of both arms of the Y (Cooper 1964). At metaphase, the *X*-*Y* bivalent appears to be held together by stringy material connecting one *X* site to one *Y* site although it is possible that prophase interactions involve more of the sites. Deficiency for substantial amounts of *X* heterochromatin causes frequent *X*-*Y* nondisjunction and dysfunction of both *Y* and *XY* sperm (Gershenson 1933; Sandler and Braver 1954; Peacock, Miklos, and Goodchild 1975). Several investigators have suggested that sperm dysfunction is a direct consequence of the pairing site deficiency and that normal *X*-*Y* pairing is essential not only to insure disjunction but also to permit proper chromosome processing for spermiogenesis (Baker and Carpenter 1972; Peacock and Miklos 1973). For a sex chromosome to be correctly processed, its pairing sites must interact with the pairing sites of its homolog during meiosis. Non-interacted pairing sites become gametic lethals.

This hypothesis accounts for the recovery disruption of the *X* chromosome from males carrying a heterochromatically deleted *X* (such as $\underline{In(1)sc^{4L}sc^{8R}}$ ($\underline{sc^{4}sc^{8}}$) and two *Y* chromosomes. In these males, the two *Y*'s pair and disjoin regularly from each other leaving the *X* without a pairing partner. Although it has but few pairing sites left, the deficient *X* evidently retains some pairing capacity since it can pair with a single *Y* (Cooper 1964; Peacock 1965). Its complete failure to pair in *XYY* males should, then, have negative consequences for *X*-bearing sperm viability. It does: *XY* sperm are recovered less than half as frequently as *Y* sperm (Sandler and Braver 1954).

If unreacted pairing sites are responsible for the skewed segregation ratios in these deficiency-*X* males, then other genotypes sharing this pairing site asymmetry but not deficient for *X* heterochromatin should also exhibit aberrant segregation. For example, an *X* or *Y* with double the normal dose of pairing sites should complete meiosis with unreacted pairing sites which would act as gametic lethals. The experiments described in this report test that prediction by examining the meiotic consequences of duplication for the *X* heterochromatin.

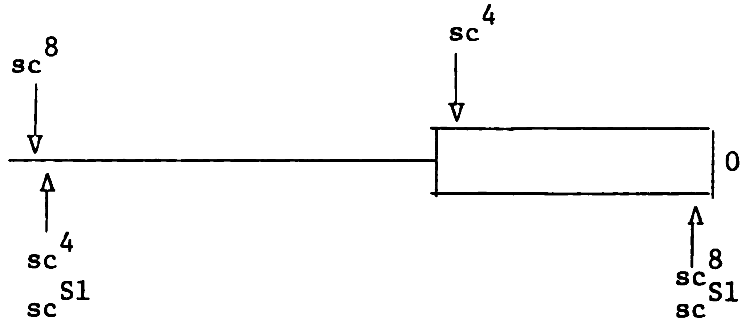
MATERIALS AND METHODS

Two *X* chromosomes duplicated for the pairing sites were selected for these experiments. Both have one dose of heterochromatin in its normal position adjacent to the centromere and a second dose near the tip. One, *In(1)sc*^{8L}*sc*^{4R} (*sc*^{8}*sc*^{4}) is the reciprocal product of the recombination event that generated *sc*^{4}*sc*^{8}, the heterochromatically deficient *X* that causes poor *Y* recovery (Gershenson 1933; Sandler and Braver 1954). The other, *In(1)sc*^{S1L}*sc*^{4R} (*sc*^{S1}*sc*^{4}), is of similar origin and structure. Figure 1 illustrates the structure and genesis of these chromosomes.

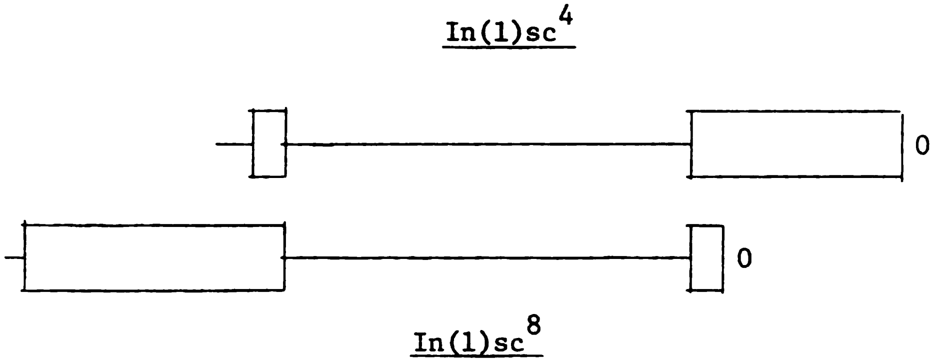
The euchromatic breaks in *In(1)sc*^{8} and *In(1)sc*^{4} are to the left and right, respectively, of the scute locus. The recombinant *sc*^{8}*sc*^{4} is deficient for that essential locus (Muller and Prokofyeva 1935) and is, therefore, inviable in males unless a scute duplication is present elsewhere in the genome. In one cross, the scute duplication was carried on the fourth chromosome; in the others, it was part of a free *X* duplication. Three different free *X* duplications were tested: *Dp(1;f)3*, *Dp(1;f)856*, and *Dp(1;f)1173*. The euchromatic breaks in *In(1)sc*^{S1} coincide, so males carrying *sc*^{S1}*sc*^{4} are viable without a duplication.

To test for meiotic drive, males carrying one of the duplicated *X*'s, a marked *Y* (*B*^{S}*Y*), and, in the case of *sc*^{8}*sc*^{4}, a scute duplication, were crossed to normal females and the recovery of the *X* was monitored. Two tests for zygotic lethality of *sc*^{8}*sc*^{4} were performed. The first was an egg hatch determination in a cross involving *sc*^{8}*sc*^{4} males. *sc*^{8}*sc*^{4}/*B*^{S}*Y*/*Dp(1;f)3* and control *Oregon R* males were placed singly in vials with one female each. Flies were transferred to fresh food every 12 hours and the

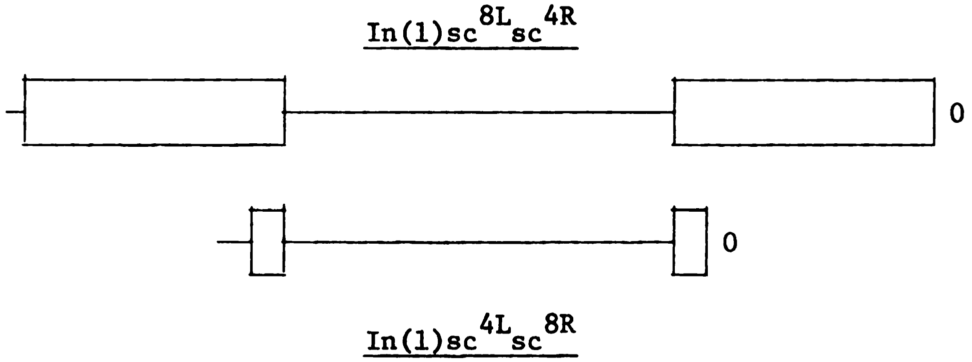
A. An *X* chromosome with scute inversion breakpoints represented.



B. Pairing and Exchange between *Ins(1)*sc^4 and sc^8



C. The Products of exchange between *Ins(1)*sc^4 and sc^8



The thin lines represent euchromatin and the wide areas represent heterochromatin.

Figure 5 Origin of Heterochromatically Duplicated and Deficient X's eggs in the old vials were counted. The egg hatch was the number of adults divided by the number of eggs. In the second experiment, the recovery of $\underline{sc^8sc^4}$ relative to a normal $\underline{X}$ was monitiored after transmission from a female. $\underline{sc^8sc^4}/\underline{y}$ females were crossed to $\underline{y}/\underline{B^SY}$ males.

RESULTS

Recovery of $sc^{S1}sc^{4}$

If duplication for *X* heterochromatic pairing sites causes gametic lethality, more sons than daughters should be recovered from males carrying $sc^{S1}sc^{4}$ and a normal *Y*. When males carrying $sc^{S1}sc^{4}$ and $B^{S}Y$ were crossed to *Oregon R* or to *y w* females (Table 22) the sex ratio was normal and there were no indications of other meiotic anomalies. Therefore, the *X* heterochromatic duplication in $sc^{S1}sc^{4}$ does not cause skewed *X*-*Y* segregation.

Recovery of $sc^{8}sc^{4}$

When males carrying $sc^{8}sc^{4}$, $B^{S}Y$, and one of several scute duplications were crossed to *y w* females, *X* chromosome recovery was poor: the sex ratio ranged from .45 to .63 (Table 23). Although a skewed sex ratio might indicate segregation distortion, it might also indicate post-fertilization lethality. The scute region deficiency in $sc^{8}sc^{4}$ could cause dominant lethality. One feature of the data in Table 23 supports the latter interpretation. In every cross, despite the poor recovery of $sc^{8}sc^{4}$ and $sc^{8}sc^{4}/Y$ sperm relative to their reciprocal products, the recovery of $sc^{8}sc^{4}/Dp$ sperm equals that of $B^{S}Y$ sperm. The scute duplication suppresses the lethality of $sc^{8}sc^{4}$. Since gamete phase lethality suppression is unknown, the most plausible interpretation is that female zygotes with one dose of the scute region frequently die. If this interpretation is correct, then the equal recovery of $sc^{8}sc^{4}/Dp$ sperm and $B^{S}Y$ sperm implies that $sc^{8}sc^{4}$ does not cause segregation distortion.

Three tests of this hypothesis were performed. In one, egg hatch measurements were made for crosses of $sc^{8}sc^{4}/B^{S}Y/Dp(1;f)3$ males and

Table 22 Recovery of $sc^{S1}sc^{4}$

	Genotype of Progeny		Sex Ratio
Maternal Genotype	$sc^{S1}sc^{4}/X$	$X/B^{S}Y$	Females:Males
y w/y w	2153	1903	1.13
+/+	2205	2060	1.07

$In(1)sc^{S1}sc^{4}/B^{S}Y$ males were crossed to the females listed in the first column.

Table 23 Recovery of $sc^{8}sc^{4}$ from Males Carrying Various Scute Duplications

	Sperm Classes						Sex Ratio
Paternal Genotype	X	$XB^{S}Y$	XDp	$B^{S}Y$	Dp	$B^{S}YDp$	Females:Males
$sc^{8}sc^{4}/B^{S}Y/Dp(1;f)3$	257	126	974	1081	1090	861	.45
$sc^{8}sc^{4}/B^{S}Y/Dp(1;f)856$	172	92	911	872	699	783	.50
$sc^{8}sc^{4}/B^{S}Y/Dp(1;f)1173$	137	164	900	998	1284	545	.43
$sc^{8}sc^{4}/B^{S}Y$; $Dp(1;f)sc^{H}/+$	128	---	765	722	----	699	.63

In all crosses, the females were y w/y w.

Oregon R controls to *y/y* females. The results indicated significant viability depression by sc^8sc^4. Egg hatch was 36% in the sc^8sc^4 cross and 61% in the control cross; the difference is statistically significant.

The second test was a measurement of sc^8sc^4 recovery following female transmission. sc^8sc^4/y females were crossed to y/B^SY males. sc^8sc^4 was recovered poorly: there were only 385 sc^8sc^4/y daughters compared to 1513 *y/y* daughters.

The third test was a determination of the efficacy of a female-transmitted scute duplication in rescuing a male-transmitted sc^8sc^4. Since sc^4sc^8 is the reciprocal crossover product of sc^8sc^4, it must be duplicated for the scute region. Therefore, sc^8sc^4/sc^4sc^8 females have two doses of scute and should exhibit normal viability. When $sc^8sc^4/B^SY/Dp(1;f)3$ males were crossed to $sc^4sc^8/sc^4sc^8/Y$ females, the recovery of sc^8sc^4/sc^4sc^8 daughters was normal. Thus sc^8sc^4 causes partial post-fertilization dominant lethality because of the scute region deficiency. It does not cause segregation distortion in males.

DISCUSSION

The major conclusion from these experiments is that despite the presence of extra pairing sites, the heterochromatically duplicated X chromosomes $\underline{sc^{8}sc^{4}}$ and $\underline{sc^{S1}sc^{4}}$ do not induce meiotic drive or other meiotic anomalies. This contradicts the notion that pairing site symmetry of X and Y chromosomes is necessary for proper meiotic chromosome processing. These results alone do not necessarily rule out the idea that pairing is required for chromosome processing. One might argue that the ability of X chromosomes carrying heterochromatin both distally and proximally to form intrachromosomal loops (Cooper 1964) would enable all the X pairing sites to participate in meiotic pairing. However, two other findings argue against the idea that pairing site interactions are crucial for chromosome processing. One is that addition of an X chromosome deficient for almost all the euchromatin but carrying a full dose of heterochromatin to a $\underline{sc^{4}sc^{8}}/\underline{Y}$ genotype does not mitigate the severity of Y chromosomal recovery disruption. The Y disjoins regularly from the free X duplication but is recovered less than half as frequently (Haemer 1978; chapter 1). Since the free duplication carries a full dose of heterochromatin and since it pairs regularly with the Y, one would expect normal pairing site interactions and normal Y recovery. Second, the probability of recovery of a sperm from a $\underline{sc^{4}sc^{8}}$ male is inversely proportional to the amount of chromatin it carries. Autosomal chromatin is as damaging to sperm viability as is sex chromatin (Chapters 3 and 4). It is difficult to see why the presence of unreacted Y chromosomal pairing sites should affect the recovery of autosomes.

An alternative interpretation of these data is that the absence of an X heterochromatic function from its normal position -- cis to the euchromatic genes -- is responsible for the skewed segregation ratios. in sc^4sc^8 males. Meiotic drive can not be an ordinary deficiency phenotype since the presence of *X* heterochromatin in the form of a free *X* duplication does not suppress it. Recovery disruptions are at least as severe in $sc^4sc^8/Y/Dp$ males as in sc^4sc^8/Y males. In both these males, and in $sc^4sc^8/Y/Y$ males, the *X* heterochromatin is either absent altogether or separated from the *X* euchromatin. Perhaps an intact *X* chromosome is a prerequisite for normal spermiogenesis. If so, then other types of rearrangements that violate the integrity of the *X* should disrupt spermiogenesis. Many translocations that separate the bulk of the euchromatin from the heterochromatin cause either meiotic drive or male sterility. Two *X;4* translocations with proximal euchromatic *X* breakpoints cause meiotic drive. Despite regular bivalent pairing and disjunction, the longer member of each bivalent (the *Y* and 4^PX^D) exhibits depressed recovery (Novitski and Sandler 1957; Zimmering 1960). Most translocations involving the *X* and one of the major autosomes (chromosomes *2* or *3*) cause sterility (Lifschytz and Lindsley 1972).

This sterility shares several features with the meiotic drive systems previously discussed. First, it affects only males. Second, it involves production of nonfunctional sperm. Third, it is dominant: addition of a duplication covering the region of the *X* breakpoint does not restore fertility. Fourth, male sterility, like meiotic drive, seems to result from separation of the bulk of the *X* euchromatic genes from a heterochromatic locus. Translocations with both breakpoints near the tips and translocations with the *X* breakpoint in the proximal part of the heterochromatin do not cause sterility. All other translocations, including those with *X* breaks in the distal part of the heterochromatin, cause sterility. Fifth, sex chromosome meiotic drive systems all cause partial sterility. $T(1;4)B^S$ (Novitski 1970) and $sc^4sc^8/Y/Y$ (Sandler and Braver 1954; Chapter 2) males are only weakly fertile. sc^4sc^8/Y males are moderately fertile but exhibit unusually early onset of sterility (Peacock and Miklos 1973). Sixth, some *X* heterochromatic deficiencies cause male sterility and others cause meiotic drive. Deficiencies encompassing both the heterochromatic bobbed locus and the proximal euchromatic suppressor of forked (*su(f)*) locus, are sterile even in the presence of a *Y* chromosome covering the deficient region (Lifschytz and Lindsley 1972). Deficiencies encompassing bobbed but not suppressor of forked, like sc^4sc^8, cause meiotic drive even in the presence of a duplication covering the deficiency (Gershenson 1933; Peacock and Miklos 1973; Yamamoto and Miklos 1977). There seems to be a complex locus or group of loci in the distal section of the *X* heterochromatin complete deficiencies for which cause male sterility and partial deficiencies for which cause meiotic drive. Finally, translocations between the *Y* and one of the major autosomes are fertile in males carrying a normal *X* but sterile in males carrying an *X* deficient for a substantial section of the basal heterochromatin (Lindsley, unpublished).

In light of the many similarities between sex chromosome meiotic drive and dominant chromosomal male sterility, it is proposed here that meiotic drive is a consequence of partial dominant chromosomal male sterility. Perhaps partially defective sperm are sensitive to the amount of chromatin they contain. Alternatively, sperm might malfunction in drive or in sterile genotypes because the chromosomes they contain are partially or completely defective. Either explanation is consistent with the observation that drive severity depends on chromatin content.

Lifschytz and Lindsley (1972) have proposed that the requirement for an intact *X* chromosome reflects a fundamental regulatory role of the *X* in spermatogenesis. They point out that a common feature of spermatogenesis in heterogametic organisms is early (premeiotic) *X* inactivation. (Lifschytz 1972; Lifschytz and Lindsley 1972). They also cite the fact that *X*-autosome translocations are male sterile in many organisms, including man. Perhaps severe disruptions of early *X* inactivation such as translocation of *X* genes to a major autosome or deletion of the entire regulatory locus cause complete sterility. Milder disruptions, such as translocation of *X* genes to the fourth chromosome (which may be supposed to be closer to the *X* in inactivation cycle than are the major autosomes) or deletion of part of the regulatory locus, cause meiotic drive.

CHAPTER 6

SUMMARY AND RECOMMENDATIONS

Summary

Several conclusions emerge from these studies. 1) The recovery of all chromosomes -- marked and unmarked *Y*'s, *X* chromosomes including euchromatic and heterochromatic deficiencies, and major autosomes -- is disrupted by the *X* heterochromatic deficiency, $\underline{sc^4sc^8}$. 2) The probability of recovery of a chromosome from a $\underline{sc^4sc^8}$ male is an inverse function of its length. The experimental discrimination is insufficient to decide whether this means that all chromatin is drive-sensitive or that there are many discrete response loci distributed along a chromosome. 3) If any interactions occur between autosomal and sex chromosomal recovery disruption they are weak ones. Autosomal and sex chromosomal recovery ratios are independent of each other. 4) Drive levels as measured by *X* chromosome recovery are independent of the amount of sex chromatin in the genome. 5) Heterochromatically duplicated *X* chromosomes do not induce meiotic drive, implying that unreacted pairing sites are not responsible for meiotic disruption in $\underline{sc^4sc^8}$ males. 6) Levels of drive and nondisjunction in $\underline{sc^4sc^8}$ males can be independently modified by *Y* chromosomes or autosomes.

These conclusions have several implications for understanding of the mechanism of sex chromosome meiotic drive. The length dependence effect could be explained by assuming that $\underline{sc^4sc^8}$ disrupts production of a chromosome processing material, causing a shortage and leading to competition among chromosomes. However, the lack of interaction between sex chromosomes and autosomes and the failure of additional sex chromatin to enhance drive argue against the notion that chromosomes in $\underline{sc^4sc^8}$ males must compete for a scarce resource. An alternative explanation of the length effect is that mispairing of unequal-sized homologs at meiosis I causes a failure to inactivate the unpaired stretch of the larger chromosome. This stretch is then an "armed bomb" which can destroy any sperm that carry it. This hypothesis implies that unpaired chromosomes like $\underline{sc^4sc^8}$ in three-sex-chromosome genotypes experience poor recovery because their bombs can not be defused. The recovery of an unpaired chromosome would be independent of the amount of sex chromatin in the genotype. This is what is observed. This hypothesis also accounts for the correlation between nondisjunction frequency and drive level since it postulates that mispairing of $\underline{X}$ and $\underline{Y}$ is the fundamental lesion which leads to both phenotypes. However, the armed bomb model fails to account for autosomal sensitivity to $\underline{sc^4sc^8}$-induced drive. It also predicts drive induction by heterochromatically duplicated $\underline{X}$'s, contrary to observation. Furthermore, the finding that regular $\underline{Y}$ chromosomal pairing with large $\underline{X}$ heterochromatic free duplications in $\underline{sc^4sc^8}$ males fails to improve $\underline{Y}$ recovery implies that the $\underline{X}$ defusing sites must be euchromatic. This seems far-fetched, given the consistent observation that $\underline{X}$-$\underline{Y}$ pairing is limited to the $\underline{X}$ heterochromatin.

Other, noncompetitive shortage models can be devised. For example, $\underline{sc^4sc^8}$ might reduce the time available for a key meiotic process. Longer chromosomes would be less likely to finish the step than shorter ones and would, more likely become gametic lethals. The evidence for autosomal sensitivity is consistent with this idea, but no critical test of it has been performed.

It is also quite possible that nothing at all is wrong with the post-meiotic chromosomes of $\underline{sc^4sc^8}$ males. It could be that $\underline{sc^4sc^8}$ damages some other aspect of sperm function in such a way as to leave sperm sensitive to the amount of chromatin in them. Since there is no evidence for defective chromatin, this is a possibility.

Since meiotic drive is caused by deficiency for X heterochromatin but not by duplication for it, it is reasonable to suppose that the meiotic disruptions are caused by the absence of some X heterochromatic function. However, addition of the X heterochromatin in the form of a free X duplication does not improve chromosome recovery, although it regularizes disjunction. The implication is that the X heterochromatin contains some function that must be present *cis* to the euchromatic genes for normal spermatogenesis to occur.

A number of observations are consistent with this view. One is the occurrence of meiotic drive in $\underline{T(1;4)B^S}$ males. In these males, most of the X euchromatin is separated from the basal heterochromatin and attached to a fourth chromosome centromere. The consequence is poor recovery of the Y and of the $\underline{4^PX^D}$ element, the two longer elements from each bivalent. Similar translocations between the X and other autosomes generally cause complete male sterility. The addition of a duplication covering the region of the X breakpoint does not rescue fertility. This dominance is what one would expect if the sterility is caused by separation of X genes from a *cis*-acting regulator. Lifschytz and Lindsley (1972) have proposed that precocious X inactivation, a common occurrence in male animal meiosis (Lifschytz 1972), is essential for normal spermatogenesis and male fertility, and that separating *X* genes from the *X* regulatory locus prevents early inactivation. The regulatory locus is evidently located in the base of the *X* judging from the dominant sterility of deficiencies encompassing both bobbed (in the heterochromatin) and suppressor of forked (*su(f)*) (in the proximal euchromatin near the euchromatin-heterochromatin border) (Lifschytz and Lindsley 1972).

It is suggested here that sex chromosome meiotic drive is a mild form of dominant chromosomal male sterility, and that *X* inactivation is interrupted in the drive genotypes to a lesser degree than in the sterile genotypes. Thus, $T(1;4)B^S$ would be understood as a milder case of the sterile *T(X;A)*'s. Perhaps the fourth chromosome is closer to the *X* in terms of inactivation cycle than are the other autosomes. The drive-inducing *X* heterochromatic deficiencies are similar in effect, but less disruptive, than the larger, male-sterilizing deficiencies. The implication is that the regulatory site is a large, complex locus or a repeated gene cluster, partial deficiencies of which induce varying degrees of sterility. With partial sterility, meiotic drive is observed either because the partly disturbed sperm have become unusually sensitive to chromatin content or because the chromatin has become mildly sperm-lethal. Another parallel between meiotic drive and male sterility is the weak fertility of many drive genotypes: $T(1;4)B^S$ (Novitski 1970), $sc^4sc^8/Y/Y$, and to lesser degrees, sc^4sc^8/Y and $sc^4sc^8/Y/Dp$. Finally, there is a dominant sterile interaciton between drive inducing *X* heterochromatic deficiencies and *T(Y;A)*'s (Lindsley, unpublished). The idea that sex chromosome meiotic drive and dominant chromosomal male sterility are closely related phenomenon is amenable to several experimental tests.

Recommendations for Future Research

The evidence for autosomal sensitivity to meiotic drive is so far based on only one experiment. It is not certain that some uncontrolled, extraneous factor is not responsible for the apparent sensitivity. It would be valuable to demonstrate autosomal sensitivity with other experimental set-ups. One experiment would be to construct sc^4sc^8 males heterozygous for a large, heterochromatic deficiency such as $Df(2)M\text{-}S2^{10}$. With appropriate markers the recovery of the deficiency and its homolog can be monitored. The expectation is that the deficiency should be recovered in excess of its homolog. A second solution would be to construct sc^4sc^8 males with two normal second chromosomes plus a free heterochromatic chromosome duplication. One such chromosome, Dp(2;f)1 (Lindsley and Grell 1968), has been in existence for some time. Several others have been constructed recently (Ganetzky, personal communication). The expectation is that a free second chromosome duplication should show reduced recovery in sc^4sc^8 males. Neither of these experiments is as sensitive as the $T(2;3)bw^{V4}$ experiment of Chapter 4, since in that experiment whole chromosomal arms were manipulated, whereas in this experiment only the dose of basal heterochromatin is altered. However, the impact of sc^4sc^8 is strong enough that it should be observed even in these less sensitive systems. With the deficiencies and duplications, interpretation of results is not complicated by possible effects of sc^4sc^8 on autosomal nondisjunction.

The autosomal system can be used for another look at the impact of additional sex chromatin on drive levels. The results in Chapter 3 indicated that *X* chromosome recovery is independent of the amount of sex chromatin in the genotype. It would be valuable to test the effect of sex chromatin amount on recovery of another, independently assorting chromosome pair. It is not obvious in advance which autosomal system (the translocation or the deficiencies and duplications) will prove most useful for these tests but trial and error will settle the point. One advantage of the deficiencies and duplications is that they permit a look at the reciprocal question, namely, what is the effect of changing the amount of autosomal chromatin on recovery of sex chromosomes?

Another way of measuring the impact of additional sex chromatin on drive levels is to compare frequencies of sperm developmental abnormalities between $sc^{4}sc^{8}/Y/Y$ and $sc^{4}sc^{8}/Y/Dp$ males in the electron microscope to ascertain what happens to the recovery of *Y* sperm when the relatively small free duplication is replaced as pairing partner by a second *Y*. According to the armed bomb model, the recovery of *Y* sperm should improve dramatically because the two *Y*'s should be able to defuse each other's bombs. *Relative* *Y* recovery does improve dramatically (Chapters 2 and 3) but this reveals nothing about *absolute* recovery of *Y* sperm. Under other, pairing-independent models of meiotic drive, absolute *Y* recovery should stay the same or get worse as a result of the substitution of a second *Y* for a free *X* duplication. Progeny counts can not answer this question because they measure only relative *Y* chromosome recovery. While the electron microscope can not reveal the genotype of abnormal sperm, it does permit an estimate of the average number of dysfunctional sperm per bundle. Under the armed bomb model, there should be fewer such sperm per bundle in $sc^{4}sc^{8}/Y/Y$ than in $sc^{4}sc^{8}/Y/Dp$ males. Under pairing-independent models, there should be more bad sperm per bundle in $sc^{4}sc^{8}/Y/Y$ than in $sc^4sc^8/Y/Dp$, since in the former genotype all sperm carry the relatively large and, therefore, drive-prone *Y* chromosome, while in the latter genotype half the sperm get the small free *X* duplication. Although the expected difference in mean dysfunctional sperm per bundle between the two genotypes is fairly large and in opposite directions under these two competing hypotheses, the results could be confounded by other factors. The biggest such factor would be autosomal recovery disruption. If the severity of autosomal recovery disruption depends strongly upon amount of sex chromatin in the genome, then it may be impossible to detect *Y*-specific effects on mean dysfunctional sperm per bundle. The experiments outlined above should reveal whether or not there is an effect of sex chromatin amount on autosomal recovery disruption.

Another reason for cytological examination of $sc^4sc^8/Y/Y$ and $sc^4sc^8/Y/Dp$ males is to look for post-meiotic *X* chromosome loss. *Y* chromosomes are certainly not lost (Cooper 1964) and there are genetic reasons (Chapter 1) for thinking that the *X* chromosome probably behaves like the *Y*. Post-meiotic cytology would be helpful in terms of confirming or refuting this genetic evidence.

Several investigators have suggested that meiotic drive in sc^4sc^8 males is caused by *X*-*Y* mispairing which in turn is due to the deletion of *X* chromosomal pairing sites (Baker and Carpenter 1972; Peacock and Miklos 1973). Although the results described above have not generally supported this model, there is still a body of evidence suggesting a close correlation between levels of nondisjunction and of drive (Zimmering 1963; Baker and Carpenter 1972; Peacock and Miklos 1973). Both drive and nondisjunction are deficiency phenotypes and both result from deletion of basal *X* heterochromatin. A deficiency mapping experiment should reveal whether or not the drive site(s) and the pairing sites are identical. Such an experiment would also clarify the relationship between dominant male sterility and dominant meiotic drive observed for different deficiencies in that region. It would test the suggestion that fertility is controlled by a tandemly repeated gene family, partial deficiencies for which induce varying degrees of male sterility. A large number of deficiencies for part of the basal heterochromatin are in existence or can be easily constructed including left-right combinations of scute and white-mottled inversions (Lindsley and Grell 1968), X-ray induced bobbed-lethal deficiencies (Lindsley, Edington and von Halle 1960), and the *su(f)* deficiencies implicated in male sterility (Lifschytz and Lindsley 1972). More deficiencies can be generated by X-ray mutagenesis as needed. By examining the phenotypes of these deficiencies with respect to fertility, nondisjunction, and sex chromosomal and autosomal recovery disruption, it should be possible to determine the locations of the pairing sites, drive sites, and fertility sites to determine to what degree these functions overlap.

A related question concerns the uniqueness of the *X* heterochromatic function concerned with insuring normal segregation ratios. Is it also found on the *Y* chromosome like other *X* heterochromatic functions--bobbed and the pairing sites? Or is it unique to the *X*? A way of answering this question is to replace the *X* heterochromatin with either Y^L or Y^S and test the resulting chromosomes for ability to induce drive. Some chromosomes appropriate for these experiments are already in existence, and others can be constructed as needed.

The suggestion that dominant chromosomal male sterility and sex chromosome meiotic drive are qualitatively similar rests on the numerous parallels between the phenotypes. It seems reasonable to test for other parallels. For example, su(f)-bb deficiencies are partly fertile in the presence of certain Y chromosomes. It would be valuable to know what happens in terms of nondisjunction and segregation ratios in these males. Meiotic drive and nondisjunction are subject to modification by a variety of genetic and environmental factors (Chapter 2). Do these modifiers also interact with fertility levels in the appropriate genotypes?

The above experiments are reasonable extensions of the work described in the previous chapters and would constitute the next few steps in the attempt to understand the genetics of sex chromosome meiotic drive. Two other experiments somewhat further afield from this work, but perhaps equally interesting, will be described briefly. One is a survey of T(1;4)'s to find out which of them induce drive. Very few have been tested so far. Two with breakpoints in 16D, including $T(1;4)B^S$, are drive inducers (Zimmering 1960). Another, $T(1;4)sc^H$, with a breakpoint near the tip is not (Chapter 5). It is interesting that T(X;A)'s with terminal X breakpoints tend to be fertile (at least when combined with terminal autosomal breakpoints) while those with central X breakpoints are all sterile no matter where the autosomal breakpoint falls (Lindsley and Lifschytz 1972). It would be valuable to know whether a similar pattern holds for drive-inducing and non-drive-inducing T(X;4)'s.

The second experiment is a reisolation and reexamination of Baker and Carpenter's (1972) male-specific X-linked meiotic mutants. All twenty of their EMS-induced mutants, isolated on the basis of causing high X-Y nondisjunction, were also found to cause skewed sex chromosome segregation ratios. In fact, they all mimicked sc^4sc^8. Two were mapped to the proximal X euchromatin. Before the others could be mapped, they all reverted. It would be ineresting to reexamine mutants of this phenotype in light of our current knowledge of sc^4sc^8. For example, do these mutants also induce autosomal drive? How do they behave in three-sex-chromosome genotypes? Do chromosomes show length dependent recovery probabilities in their presence? Another interesting aspect of these mutants is their high frequency. Baker and Carpenter had little trouble isolating twenty of them. It would be very interesting to know where they map. Are there a large number of *X* chromosomal functions involved in normal *X*-*Y* disjunction and segregation? Or are there one or a few hot spots for this type of mutation? The other interesting aspect of these mutants is their instability. It is very odd that all of them should revert in such a short time. Despite the difficluty of working with unstable mutations, the potential benefit in terms of understanding the central processes of spermatogenesis make them well worth a second look.

BIBLIOGRAPHY

BIBLIOGRAPHY

Baker, B.S. and A.T.C. Carpenter. Genetic analysis of sex chromosome meiotic mutants in *Drosophila melanogaster*. Genetics 71:255-286; 1972.

Brink, N.G. Protein synthesis during spermatogenesis in *Drosophila melanogaster*. Mutation Research 5: 192-194; 1968.

Cooper, K.W. Meiotic conjunctive elements not involving chiasmata. Proceedings of the National Academy of Sciences, USA 52: 1248-1255; 1964.

Das, C.C., B.P. Kaufmann and H. Gay. Autoradiographic evidence of synthesis of an arginine-rich histone during spermiogenesis in *Drosophila melanogaster*. Nature 204: 1008-1009; 1964.

Denell, R.E. and G.L.G. Miklos. The relationship between first and second chromosome segregation ratios from *Drosophila melanogaster* males bearing Segregation Distorter. Molecular and General Genetics 110: 167-177; 1971.

Erickson, J. Meiotic drive in Drosophila involving chromosome breakage. Genetics 51: 555-571; 1965.

Ganetzky, B. On the components of segregation distortion in *Drosophila melanogaster*. Genetics 86: 413-445; 1977.

Gershenson, S. Studies on the genetically inert region of the *X* chromosome of Drosophila. I. Behavior of an *X* chromosome deficient for a part of the inert region. Journal of Genetics 28: 297-312; 1933.

Glass, H.B. A study of dominant mosaic eye-color mutants in *Drosophila melanogaster*. II. Tests involving crossing-over and nondisjunction. Journal of Genetics 28: 69-112; 1933.

Glass, H.B. A study of factors influencing chromosome segregation in translocations of *Drosophila melanogaster*. University of Missouri Research Bulletin 231; 1935.

Gould-Somero, M. and L. Holland. The timing of RNA synthesis for spermiogenesis in organ cultures of *Drosophila melanogaster* testes. Wilhelm Roux' Archives 174: 133-148; 1974.

Haemer, J. Functions of Heterochromatin. Ph.D. dissertation, University of Washington; 1978.

Hardy, R.W. The influence of chromosome content on the size and shape of sperm heads in *Drosophila melanogaster* and the demonstration of chromosome loss during spermiogenesis. Genetics 79:231-264; 1975.

Hartl, D.L. and Y. Hiraizumi. Segregation Distortion. Ashburner, M. and E. Novitski, eds. The genetics and biology of Drosophila, volume 1B, London: Academic Press; 1976: 616-666.

Hartl, D.L., Y. Hiraizumi, and J.F. Crow. Evidence for sperm dysfunction as the mechanism of segregation distortion in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences USA 58: 2240-2245; 1967.

Hiraizumi, Y. and K. Nakazima. Deviant sex ratio associated with Segregation Distorter in *Drosophila melanogaster*. Genetics 55: 681-687; 1967.

Kettaneh, N.P. and D.L. Hartl. Histone transition during spermiogenesis is absent in Segregation Distorter males of *Drosophila melanogaster*. Science 193: 1020-1021; 1976.

Kettaneh, N.P. and D.L. Hartl. Ultrastructural analysis of spermiogenesis in Segregation Distorter males of *Drosophila melanogaster*: the homozygotes. Genetics 96: 665-683; 1980.

Lifschytz, E. *X* Chromosome inactivation: an essential feature of normal spermiogenesis in male heterogametic organisms. Beatty, R.A. and S. Gluecksohn-Waelsch, eds. Proceedings of the Edinburgh symposium on the genetics of the spermatozoon. Edinburgh: University of Edinburgh; 1972: 223-232.

Lifschytz, E. and D.L. Lindsley. The role of *X*-chromosome inactivation during spermatogenesis. Proceedings of the National Academy of Sciences USA 69: 182-186; 1972.

Lindsley, D.L. and E.H. Grell. Genetic variations of *Drosophila melanogaster*. Oak Ridge, Tennessee: Carnegie Institution of Washington Publication Number 627; 1968.

Lindsley, D.L. and E.H. Grell. Spermiogenesis without chromosomes in *Drosophila melanogaster*. Genetics, Supplement 61: 69-77; 1969.

Lindsley, D.L. and E. Lifschytz. The genetic control of spermatogenesis in Drosophila. Beatty, R.A. and S. Gluecksohn-Waelsch, eds. Proceedings of the Edinburgh symposium on the genetics of the spermatozoon. Edinburgh: University of Edinburgh; 1972: 203-222.

Lindsley, D.L. and L. Sandler. The meiotic behavior of grossly deleted *X* chromosomes in *Drosophila melanogaster*. Genetics 43: 547-563; 1958.

Lindsley, D.L., C.W. Edington, and E.S. vonHalle. Sex-linked recessive lethals in Drosophila whose expression is suppressed by the *Y* chromosome. Genetics 45: 1649-1670; 1960.

Miklos, G.L.G., A.F. Yanders, and W.J. Peacock. Multiple meiotic drive systems in the *Drosophila melanogaster* male. Genetics 72: 105-115; 1972.

Monesi, V. Synthetic activities during spermatogenesis in the mouse. Experimental Cell Research 39: 197-224; 1965.

Moore, G.P.M. DNA-dependent RMA synthesis in fixed cells during spermatogenesis in mouse. Experimental Cell Research 68: 462-465; 1971.

Muller, H.J. and A.A. Prokofyeva. The individual gene in relation to the chromomere and the chromosome. Proceedings of the National Academy of Sciences USA 21: 16-26; 1935.

Muller, H.J. and F. Settles. The nonfunctioning of the genes in spermatozoa. Z. Indukt. Abstamm.-Vererbungsl. 43: 285-312; 1927.

Novitski, E. The concept of gamete dysfunction. Drosophila Information Service 45: 87-88; 1970.

Novitski, E. and I. Sandler. Are all products of spermatogenesis regularly functional? Proceedings of the National Academy of Sciences USA 43: 318-324; 1957.

Olivieri, G. and A. Olivieri. Autoradiographic study of nucleic acid synthesis during spermatogenesis in *Drosophila melanogaster*. Mutation Research 2: 366-380; 1965.

Peacock, W.J. Nonrandom segregation of chromosomes in Drosophila males. Genetics 51: 573-583; 1965.

Peacock, W.J. and G.L.G. Miklos. Meiotic drive in Drosophila: new interpretations of the Segregation Distorter and sex chromosome systems. Advances in Genetics 17: 361-409; 1973.

Peacock, W.J., G.L.G. Miklos, and D.J. Goodchild. Sex chromosome meiotic drive systems in *Drosophila melanogaster*. I. Abnormal spermatid development in males with a heterochromatin deficient *X* chromosome (sc^4sc^8). Genetics 79: 613-634; 1975.

Peacock, W.J., K.T. Tokuyasu, and R.W. Hardy. Spermiogenesis and meiotic drive in Drosophila. Beatty, R.A. and S. Gluecksohn-Waelsch, eds. Proceedings of the Edinburgh symposium on the genetics of the spermatozoon. Edinburgh: University of Edinburgh; 1972: 247-268.

Ramel, C. The effect of the curly inversions on meiosis in *Drosophila melanogaster*. II. Interchromosomal effects on males carrying heterochroatin deficient *X* chromosome. Hereditas 60: 211-222; 1968.

Roberts, P.A. The genetics of chromosome aberration. Ashburner, M. and E. Novitski, eds. The genetics and biology of Drosophila, volume 1A. London: Academic Press; 1976: 68-184.

Sandler, L. and G. Braver. The meiotic loss of unpaired chromosomes in *Drosophila melanogaster*. Genetics 39: 365-377; 1954.

Sandler, L. and A.T.C. Carpenter. A note on the chromosomal site of action of *SD* in *Drosophila melanogaster*. Beatty, R.A. and S. Gluecksohn-Waelsch, eds. Proceedings of the Edinburgh symposium on the genetics of the spermatozoon. Edinburgh: University of Edinburgh; 1972: 233-246.

Sandler, L. and Y. Hiraizumi. Meiotic drive in natural populations of *Drosophila melanogaster*. V. On the nature of the *SD* region. Genetics 45: 1671-1679; 1972.

Sandler, L., Y. Hiraizumi, and I. Sandler. Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of Segregation-Distorter. Genetics 44: 233-250; 1959.

Spofford, J.B. Position effect variegation. Ashburner, M. and E. Novitski, eds. The genetics and biology of Drosophila, volume 1C. London: Academic Press; 1976: 955.

Tokuyasu, K.T., W.J. Peacock, and R.W. Hardy. Dynamics of spermiogenesis in Drosophila melanogaster. VII. Effects of Segregation-Distorter (*Sd*) chromosomes. Journal of Ultrastructure Research 58: 96-107; 1977.

Yamamoto, M. and G.L.G. Miklos. Genetic dissection of heterochromatin in Droshophila. The role of basal *X* heterochromatin in meiotic sex chromosome behavior. Chromosoma 60: 283-296; 1977.

Zimmering, S. Modification of abnormal gametic ratios. Science 130: 1426; 1959.

Zimmering, S. Modification of abnormal gametic ratios in Drosophila. I. Evidence for an influence of *Y* chromosomes and major autosomes on gametic ratios from Bar-Stone translocation males. Genetics 45: 1253-1268; 1960.

Zimmering, S. The effect of temperature on meiotic loss of the *Y* chromosome in male Drosophila. Genetics 48: 133-138; 1963.

Zimmering, S. Genetic and cytogenetic aspects of altered segregation phenomena in Drosophila. Ashburner, M. and E. Novitski, eds. The Genetics and Biology of Drosophila, volume 1B. London: Academic Press; 1976: 569-615.

Zimmering, S. and R.E. Green. Temperature dependent transmission rate of a univalent *X* chromosome in the male *Drosophila melanogaster*. Canadian Journal of Genetics and Cytology 7: 453-456; 1965.

Zimmering, S. and M. Perlman. Modification of abnormal gametic ratios in Drosophila. III. Probable time of the A-type effect in Bar-Stone translocation males. Canadian Journal of Genetics and Cytology 4: 333-336; 1962.

Zimmering, S., L. Sandler, and B. Nicoletti. Mechanisms of meiotic drive. Annual Review of Genetics 4: 409-436; 1970.