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Crossing over in a T(1;4) Translocation in *Drosophila melanogaster*

By

John David Osborne 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Masters of Science

In

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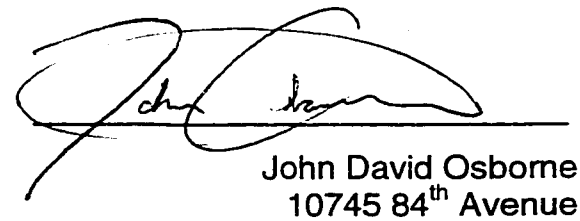

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Crossing over in a T(1;4) Translocation in *Drosophila melanogaster* by John David Osborne in partial fulfillment of the requirements for the degree of Masters of Science in Molecular Biology and Genetics.

Dr. John Locke

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Dr. Mike Russell

Dr. Mike Walter

Oct 12th /1998

To the peons of science, because they do all the work.

Abstract

Chromosome 4 of *Drosophila melanogaster* is unlike the other autosomes in that it does not normally undergo crossing-over in female meiosis (Bridges, C.B. 1921). The reason for this phenomenon is still not known. The current hypothesis postulates that the small length of chromosome 4 may allow a "centromere effect" to cover its entire length (L. Sandler and P. Szauter, 1978). I have tested this and other hypotheses by measuring the rate of crossing-over on the distal portion of the X and the distal portion of chromosome 4 in a reciprocal X-4 translocation. Results indicate that the "centromere effect" is indeed responsible for the lack of crossing over on chromosome 4. Furthermore these results argue against the copolymer sequence $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ as a requirement for crossing over.

Acknowledgement

First and foremost I would like to thank John Locke who was more than I had hoped for in a supervisor. He was constant source of guidance and support throughout my project, and his advice (including numerous thesis corrections) was invaluable. More than just a supervisor, he was a friend and never discouraged my interests in politics, writing and computing science even when they cut into my research time.

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Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

bp	base pair
cM	centimorgan
DEB	diepoxybutane
DNA	deoxyribonucleic acid
kb	kilobase
M	molar
mg	milligram
mM	millimolar
mRNA	messenger RNA
ng	nanograms
NOR	nucleolar organizing region
PCR	polymerase chain reaction
SC	synaptonemal complex
µg	micrograms
µM	micromolar
⊗	genetic cross symbol

Chapter 1.

Introduction

1.1 Recombination in Genetic Systems

1.1.1 Recombination

Recombination has been defined as "the process of creating progeny with combinations of genes or alleles other than those occurring in the parents" (King and Stansfield, 1990). Along with outcrossing, it is a key aspect of sexual reproduction that produces genetic diversity and allows for repair of damaged DNA. The two major mechanisms responsible for recombination in eukaryotes are independent assortment among different chromosome pairs and crossing over within a chromosome pair. The focus of my thesis is on the second aspect of recombination, crossing over, or more specifically, with the lack of crossing over on one chromosome, chromosome 4 of *Drosophila melanogaster.*

1.1.2 Crossing over

Crossing over is a fundamental process in eukaryotes. It normally involves the exchange of genetic material between two homologous chromosomes. Crossing over is a key mechanism in the generation of genetic diversity. Without crossing over (and assuming there is no mutation) only the two parental types of chromosomes would be produced in diploid organisms. This would drastically limit the genetic diversity of chromosomes within the entire species. In addition, crossing over maintains sequence variability on chromosomes by reducing the impact of both Muller's Ratchet (the "degeneration" of a non-recombining chromosome from linkage disequilibrium; Muller, 1964) and through any selective sweeps (Charlesworth et al, 1995, Aquadro, 1997) that may occur. Such sweeps may drive a diverse population of chromosomes to homogeneity, if a single chromosome is strongly selected for. In addition Muller's Ratchet "degeneration" prevents such non-recombining species from removing the mutational load (even in the presence of intense selection) leading to the accumulation of deleterious sequences by drift and selective noise. Crossing over is also important in ensuring the proper segregation of these chromosomes (Darlington, 1932).

Furthermore, the role of crossing over is not restricted just to creating and ensuring recombination in meiosis. Crossing over is also used in the repair of damaged DNA, by allowing one homologue to be used as a template for repairing the damaged chromosome (for a review see Lewin, 1997). Thus, crossing over is an essential process in eukaryotes and the mechanisms and their control are of general interest and significance.

1.1.3 Role of Crossing over in Chromosome Segregation

Crossing over has long been thought to ensure that homologues segregate properly at meiosis (Darlington, 1932). Since then most evidence indicates that chiasmata are the physical manifestation of exchange and support a model in which chiasmata are necessary and sufficient to ensure disjunction (Beadle, 1932; Jones, 1971; reviewed in Hawley, 1988). Crossing over can be seen as chiasmata during prophase I and early metaphase I. During prophase I chiasmata form at one or more regions along homologous chromosomes and then migrate to the ends of the chromosome in a process known as terminalization. In eukaryotes chiasmata facilitate segregation of homologous chromosomes during meiosis by stabilizing the bivalent on the metaphase plate. Without this exchange, the likelihood of aneuploidy and unbalanced gametes is increased. For instance in humans if chromosome 21 fails to undergo meiotic crossing over, it is much more likely to produce disomic gametes, resulting in Down's Syndrome. Warren et al (1987) examined the frequency of DNA polymorphisms on the long arm of chromosome 21, in families with a single Down's syndrome child. They found that recombination between loci on chromosome 21 that had undergone non-disjunction, was significantly reduced relative to both the affected family and in control families. Baker et al (1976) found a similar lack of recombination on non-disjunction chromosomes in *D. melanogaster*.

1.1.4 Evolutionary Implications of Crossing over on Chromosomes

Crossing over is an important process for ensuring the fitness of chromosomes (Muller, 1964; Rice, 1994). Rice demonstrated this experimentally by measuring the fitness after 35 generations of two types of *D. melanogaster* chromosomes. The population in which crossing over was prohibited (by balancer chromosomes) had a lower fitness than the control line where crossing over was permitted. This difference was attributed to crossing over removing harmful mutations from the chromosome. According to Rice, lack of crossing over on a chromosome "interferes with the process of purifying selection and thereby causes chronic accumulation of small effect, deleterious mutations by a variety of mechanisms". While this selection pressure may allow for the accumulation of mutations that decrease the fitness of the flies, this lack of crossing over will still lead to a reduction in the number of different haplotypes that are possible in a population. For example the examination of a 1.1 kb fragment of the ci^D gene on chromosome 4 of *Drosophila melanogaster* provides evidence for lack of genetic diversity on chromosome 4 (Berry et al, 1991). A sequence comparison of this fragment in 10 lines of *D. melanogaster* and 9 lines in *D. simulans* revealed only a single polymorphism in one of the *D. simulans* lines. This difference is significant ($P < 0.001$) when compared to the alcohol dehydrogenase locus, where similar data is available. In *D. melanogaster* the alcohol dehydrogenase gene has 21 polymorphisms versus the 0 polymorphisms of the ci^D gene fragment. This indicates a marked lack of sequence diversity on this fragment of chromosome 4, and likely the entire chromosome.

Another possible implication of the lack of crossing over, is a possible buildup of repeated sequences. Satellite DNA (which consists of tandem arrays of repeated sequences) is often found in heterochromatin (Peacock et al, 1977). Since some heterochromatic regions do not undergo crossing over (Baker, 1958) it is possible that lack of crossing over may lead to "heterochromatization" of a chromosome. This appears true in the case of the neo-Y chromosome of *Drosophila miranda*, a chromosome that was formed by a translocation of one of the autosomes to the Y chromosome (Steinemann, 1982). This neo-Y chromosome contains numerous copies of a transposable element called TRAM, while the former homologue of the neo-Y contains none (Steinemann and Steinemann, 1997). Thus it is possible that chromosome degeneration and "heterochromatization" is driven by an accumulation of mobile elements which can not be removed due to the effects of Muller's Ratchet.

Conversely it may be that pre-existing heterochromatin on a chromosome may prevent crossing over. Tempting as it is to speculate, there is as yet no evidence whether the relationship between "heterochromatization" and lack of crossing over on a chromosome is even one of cause and effect. In conclusion, crossing over certainly plays a role in chromosome evolution, affecting both genetic diversity and fitness.

1.1.5 Crossing over and the Genetic Map

Crossing over has a practical significance in genetics; it has allowed the construction of genetic maps, an important tool for geneticists, even today. *D. melanogaster* was the first organism in which a genetic map was constructed (Sturtevant, 1913). The genetic distance between two loci is calculated by dividing the recombinant progeny for two markers by the total number of progeny and multiplying by 100. This gives a measure of the recombinant frequency which is an approximation of the genetic distance usually expressed in genetic map units termed centiMorgans (cM) of how close two gene loci are on the same chromosome. The smaller the number, the closer the two genes are on the genetic map. Loci further apart than 50cM on the same linkage group or located on different linkage groups are equally likely to have their alleles segregate together or apart. This is because chromosome segregation from the metaphase plate is random with respect to the poles of the cell; thus chromosomes have an equal chance of going to either pole.

1.1.6 The Centromere Effect and the Genetic Map

The genetic map defines distance in map units along a chromosome. While map distance does increase with an increase in the size of the chromosome, it is not a direct reflection of the physical length of the chromosome. Eukaryotic chromosomes of substantially different size may have the same total map distance. Furthermore along any given chromosome the map distance relative to the physical distance can vary substantially. There is a reduction of crossing over (and thus a decrease in map distance) in the region close to the centromere in many, (if not all) eukaryotes. There is also to a lesser extent a similar reduction of crossing over near the telomere in some species

(such as *Drosophila*) although other species (such as humans) actually show an *increase* in the frequency of crossing over at the telomeres.

The non-random distribution of crossing over with less crossing over near the centromere is known as the "centromere effect" (Beadle 1932). Beadle examined crossing over between chromosome 9 of maize, and its teosinte homolog. In testcrosses he observed little if any crossing over between the y_{g2} and w_x genes. These genes are close to the centromere, on the short arm of chromosome 9. A similar lack of crossing over has been found near the centromere in many other species, including *Drosophila*.

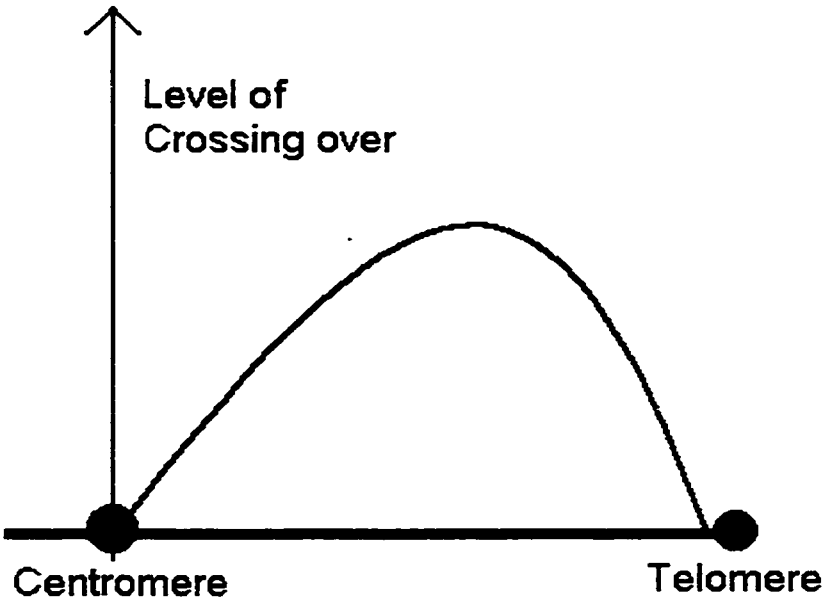


Figure 1.1. Graph of Centromere Effect

This diagram shows the distribution of crossover frequency along a hypothetical eukaryotic chromosome. The frequency of crossing over is reduced at the centromere and telomere. The centromere effect varies from organism to organism, and between chromosome arms of the same organism and is usually more severe near the centromere than the telomere.

1.2 Molecular Biology of Crossing Over

1.2.1 Homologous Chromosome Recognition

The molecular biology of meiotic recombination is gradually becoming understood. The very first stage of meiotic recombination is the recognition and subsequent conjunction of homologous chromosomes to produce a bivalent. Unfortunately, chromosome recognition has not yet been as well as elucidated as other aspects of meiotic recombination. The most obvious mechanism by which homologous chromosomes may recognize one another is through the complementarity of strands of a DNA duplex. Whether the random diffusion of DNA within the nucleus is sufficient to provide the motive force for chromosome conjunction is still not known. One of the reasons that a simple homology search may be insufficient to account for chromosome recognition is the trouble that two homologous pieces of DNA would have in recognizing one another. Since there are massive amounts of non-homologous DNA interfering with easy recognition, particularly in the genomes of higher eukaryotes it seems unlikely that such a haphazard method could efficiently bring the homologous sequences together. Furthermore this ignores the condensed state of chromosomes, which would make such interactions unlikely. Pairing sites are likely to be required to improve the efficiency of the eventual homology search.

In addition to simple diffusion, other more elaborate recognition systems have been proposed in order to explain how condensed chromosomes can pair. Zickler (1984) has even suggested that the chromosomes may already be paired. More recently, it has been argued (Haber, 1998) that different organisms use different methods of chromosome recognition and pairing.

1.2.2 Chromosome Pairing and Initiation of Meiotic Recombination

Although chromosome pairing has been used interchangeably with the term synapsis, it is more often used as a general term to describe the whole process of alignment, from chromosome recognition to synaptonemal complex formation. Synapsis on the other hand describes the close alignment of homologous chromosomes and begins with the first appearance of the synaptonemal complex.

The two organisms where much of the research on chromosome pairing and pairing has been done are *Saccharomyces cerevisiae* and *Drosophila melanogaster*. Recent results suggest that the initiation of recombination and the subsequent pairing that occurs are very different processes in these organisms, and they reflect the relative difference in the complexity of their respective genomes (Haber, 1998). In *Saccharomyces* there is good evidence that double stranded breaks initiate recombination and are required for synapsis (Roeder, 1995). In *Drosophila* specific pairing sites along the chromosomes align so that synapsis can occur, which itself is a requirement for recombination.

1.2.3 Double-strand Breaks May Be Necessary For Recombination in Yeast

Several lines of recent evidence from yeast indicates that double-strand breaks are required for the initiation of recombination (reviewed in Roeder, 1995; Kleckner, 1996). Firstly, double-strand breaks precede the formation of the synaptonemal complex (SC) and any recombination. In addition more convincing evidence comes from the *rad50* mutant in *S. cerevisiae*. In *S. cerevisiae* double-strand breaks create flush ends which are then converted to long 3' single stranded ends. If these flush ends are not converted (such as in *rad50* mutants) no recombination occurs. Thus, the required conversion of double stranded breaks to long 3' single stranded ends suggests that double strand breaks are necessary for recombination. Further evidence comes from a deletion of the Spo11 gene, encoding an endonuclease responsible for the creation of meiotic double stranded breaks. Mutations in Spo11 eliminate both recombination and SC formation. Finally, double stranded breaks have been detected in yeast in leptotene, where they are found at specific sites which have a high frequency of recombination. The frequency of recombination from these sites declines in a polar manner in one or both directions, suggesting that a recombination event is spreading from the initial double stranded break. Taken together, these observations strongly indicate that recombination may be initiated from double stranded breaks.

1.2.4 Synapsis Initiation and the Synaptonemal Complex in Yeast

The *rad50* mutant also provides evidence that double-stranded breaks are required to produce an SC (Alani et al, 1990; Lewin, 1997). In *S. cerevisiae* the formation of the axial element occurs in leptotene, about the same time that double stranded breaks in DNA first appear. These axial filaments are normally converted to an SC in late leptotene and zygotene. The SC then persists until the formation of recombinant molecules, and disassociates in diplotene. However in *rad50* mutants, the conversion of this axial filament to an SC does not occur and no SC is produced.

Furthermore a properly formed SC is not even required for recombination, as some mutants which have an abnormal SC can still undergo recombination. Even better evidence is provided by a deletion of the *ZIP1* gene (Sym et al, 1993). This deletion completely eliminates the formation of the SC, but recombination still occurs. However all known mutants that fail to undergo recombination also fail to form an SC. Thus the SC appears to be the result of recombination, rather than a structure which is required in order for recombination to occur.

Thus the current model for the initiation of recombination in yeast is one in which a double stranded break is turned into a loose end, which engages in a homology search at a "gene by gene" level (Kleckner et al, 1991). The relatively small size of the *S. cerevisiae* genome facilitates the search, which would be more difficult in the larger genome of a higher eukaryote like *D. melanogaster.* Once the double stranded break has found its "match" recombination begins, and subsequently recombination dependent formation of the SC commences. This initiation of SC formation is now known to be dependent on the function of

the *ZIP2* gene, as mutants of this gene pair properly, but do not form an SC (Chua and Roeder, 1998).

1.2.5. Pairing and Chromosome Alignment in *Drosophila*

Pairing sites in *D. melanogaster* are hypothesized to be the limited number of locations where the homologous chromosomes initially pair to form the synaptonemal complex (SC). Evidence for pairing sites comes from the examination of crossover suppression in translocation heterozygotes (Hawley, 1980). Hawley examined meiotic exchange in females heterozygous for numerous T(1;4) and T(1;Y) translocations, with breakpoints at various places along the X chromosome (chromosome 1 is the X chromosome *in D. melanogaster*). He found that crossing over is suppressed in translocation heterozygotes near the breakpoint. This suppression extended to specific intervals distal to the breakpoint. Five such intervals along the X chromosome were deduced. Hawley suggested that these intervals are bounded by putative pairing sites, which must be intact and paired with their partner on their homolog in order to ensure that crossing over can occur within the paired interval.

Specific cytological evidence of pairing sites still has not been found in *D. melanogaster* and "there is as yet no unambiguous evidence for specific pairing sites of SC initiation (Giroux, 1988). However evidence for pairing sites has been reported in *Caenorhabditis elegans* (McKim et al, 1993, reviewed in Zetka and Rose, 1995). This is consistent with the idea that higher eukaryotes with greater genetic complexity require pairing sites in order for chromosomal alignment to occur. However, regardless of whether specific pairing sites exist in *Drosophila* or in any other organism, there is still no evidence that pairing sites determine the location of a recombination event as appears to be the case with double stranded breaks in yeast.

1.2.7 Synapsis in *D. melanogaster*

D. melanogaster females produce a SC, even in heterochromatic regions. However the structure of the SC along the X chromosome in females is morphologically different between heterochromatic and euchromatic regions (Carpenter, 1975). This correlates with the presence or absence of crossing over in those regions. The cause of this morphological difference between euchromatic and heterochromatic SC is not known, but one possibility would be sequence differences between DNA found in heterochromatin versus euchromatic regions. Although there are sequence differences between heterochromatic and euchromatic regions, no sequences have yet been identified as being responsible for the production of their respective characteristic synaptonemal complexes.

The story with *Drosophila* males is completely different. *D. melanogaster* males do not produce a SC (Hawley, 1988), and do not undergo crossing over (Morgan, 1912). Disjunction in males may be mediated through an entirely different mechanism, at least for the sex chromosomes. Thin threads that form between the nucleolar organizing region (NOR) on the sex chromosomes have been called collochores (Cooper, 1964). However the inability to find these

threads in *D. simulans* at its rRNA genes suggests that these thin threads may just be remnants of the nucleolar organizing region (Ault and Rieder, 1994).

1.2.8 Fine Structure of the Synaptonemal Complex

Once the paired homologous chromosomes are approximately 300nm apart, a SC begins to forms in a process termed synapsis (Giroux, 1988). Synapsis starts when each chromosome condenses around an axial element. After axial element formation the two homologous chromosomes align. Although the SC brings the homologous chromosomes just over 200nm from each other, this is still too distant for recombination. It is likely that nodes or recombination nodules (as they are termed in *Drosophila*) are the sites of recombination because they form after production of the SC and are the only visible links between the paired chromosomes, but this role for recombination nodules has yet to be demonstrated. After alignment of the homologous chromosomes the SC begins to form and is now composed of 3 components (Figure 1.2). The axial element of each chromosome is now called a lateral element and the two lateral elements are separated by a dense central element. The chromatin lies on the other side of the lateral element. The frequency and pattern of these sites along a chromosome vary from organism to organism, and meiocyte to meiocyte.

1.2.9 Control of crossing over and the Synaptonemal Complex

Although a normal SC is not required for recombination as once thought, it may have a role in determining where crossing over can occur. In this role, the SC may inhibit rather than promote crossing over as suggested by the phenotype of *Zip1*, a yeast mutant. The *ZIP1* gene product is a structural component of the central region of the SC and in these mutants crossover interference is abolished (Sym and Roeder, 1994; 1995). The exact role that the SC plays in creating or limiting crossovers is not known. The most obvious possibility is that the spreading of the SC from the initial site of the recombination directly prevents the formation of new crossovers, thereby mediating crossover interference.

Although evidence from *zip1* mutants clearly indicates that the *S. cerevisiae* SC plays a role in controlling crossover interference, it is not clear whether the SC plays any role in controlling the site and frequency of crossing over along the chromosomes of higher eukaryotes. As mentioned previously the structure of the SC in heterochromatic regions differs from that of the SC in euchromatic regions in both *S. cerevisiae* and *D. melanogaster*. Although the *ZIP1* gene product has no known role in crossover placement along a chromosome arm, the present evidence does not rule out the possibility that some other component of the SC may have this role. One such component may be the product of the *Tam1* gene, which localizes to the telomeres of *S. cerevisiae* chromosomes (Chua and Roeder, 1997). Sister chromatids in Tam1 mutants separate early and cross over interference is reduced. Unlike all other mutants in *S. cerevisiae* that affect interference, the frequency of crossing over in Tam1 mutants is unaffected. This strongly suggests a role for Tam1 and the SC in the control of the distribution of crossing over.

In planets, *Allium ursinum* (Loidl, 1987) there is a consistent lack of a SC in the centromeric region. Therefore at least in *A. ursinum*, the SC can not be responsible for the inhibition of crossing over near the centromere. Certainly given that a normal SC is not required for recombination (and is in fact thought of as a consequence of recombination in *S. cerevisiae)*, the molecular mechanism of crossover placement along a chromosome is probably more complicated than SC positioning.

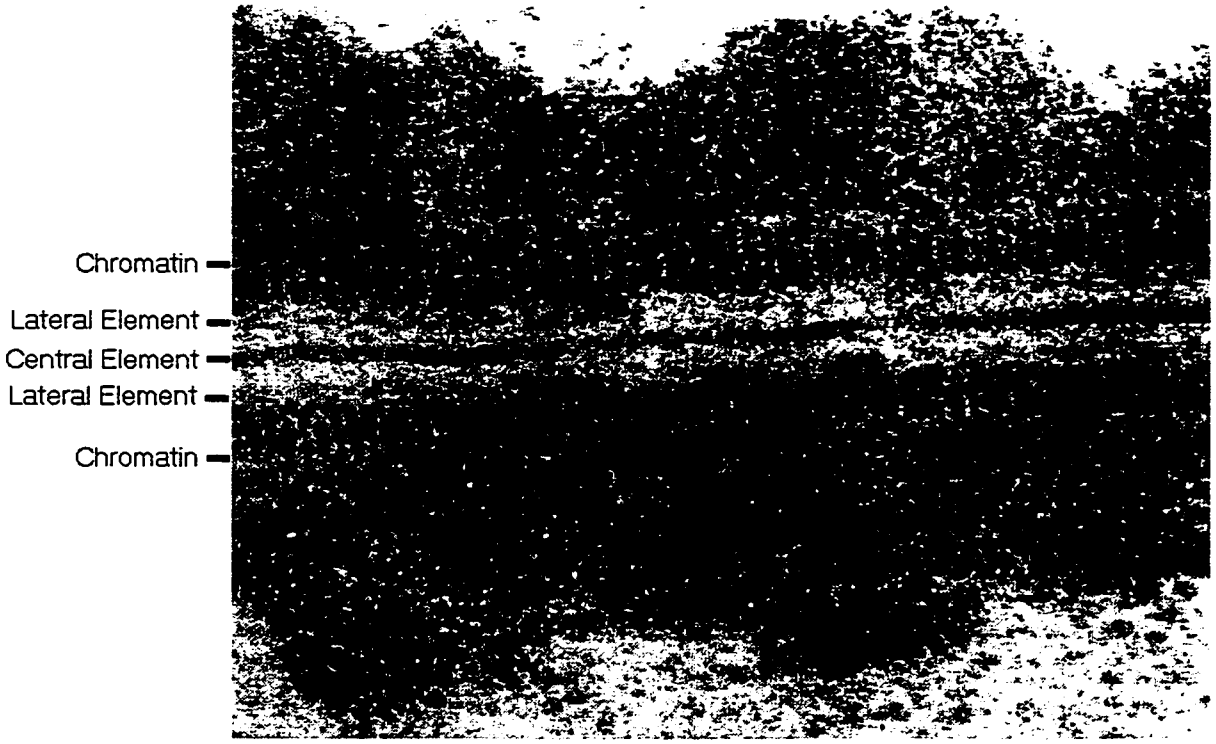


Figure 1.2. Synapsis in *Neotellia*

Picture of the synaptonemal complex in *Neotellia* taken by M. Westergaard and D. Von. Wettstein from Lewin (1992).

1.3 Crossing over in *D. melanogaster*

1.3.1 Absence of crossing over in male *Drosophila*

Despite the purported benefits of crossing over, Morgan noted a lack of meiotic exchange in Drosophila wild type males (Morgan 1912). Wild type males in normal conditions produce cross over chromosomes with a frequency of ≤0.001% (Hannah-Alava 1968). In the absence of crossing over, recombinants are possible only through independent assortment of the chromosomes.

The homologous autosomes of males do not produce a synaptonemal complex and do not form chiasmata. They can be induced to produce putative crossover chromosomes through heat treatment (Whittinghill, 1955) but it is not clear whether this is actual crossing over or the more recently discovered phenomena of hybrid dysgenesis.

Given the importance of crossing over in ensuring the proper segregation of homologous chromosomes, it is of interest to note that chromosomes in male *Drosophila* segregate properly. In both males and females disjunction of the sex chromosomes appears to be mediated by specific chromosomal sites known as collochores (Cooper, 1964), which is the name given to thin threads that form between the nucleolar organizing regions (NOR) on the sex chromosomes. Cooper believed these collochores provided cytological evidence for pairing sites along the X chromosome. This is consistent with older experiments (Painter and Muller, 1932) which showed that deletions of X heterochromatin prevent normal disjunction of the sex chromosomes in males.

More recent experiments (McKee and Lindsley, 1987) also suggest collochore formation appears to be dependent on the basal heterochromatin of the X chromosome and an actual sequence responsible for X-Y pairing has now been purported in *D. melanogaster* (Merill et al, 1992). It is found in both the X chromosome heterochromatin and near the Y centromere. This sequence is a 240 bp repeat in the non-transcribed intergenic spacer (IGS) region of the rDNA gene (Ren et al, 1997). This sequence was identified by its ability to partially restore X-Y pairing capacity for an X chromosome deficient in heterochromatic sequences. An increase in number of these repeats further increases the restoration of X-Y pairing. Thus promoter-containing ribosomal DNA fragments are capable of functioning in *D. melanogaster* males as pairing sites.

While collochores mediate disjunction for sex chromosomes, they do not mediate disjunction of the autosomes in males (Yamamoto, 1979). Instead general homology is probably responsible to ensure the proper disjunction of the autosomes in males (McKee et al, 1993).

1.3.2 Segregation of achiasmatic chromosomes

Given the importance of crossing over in ensuring the proper segregation of chromosomes, it is worthwhile to consider the fate of non-crossover chromosomes. In female *Drosophila*, crossing over only occurs on chromosomes X, 2 and 3; normally no crossing over occurs on chromosome 4.

Despite this absence of chiasmata to stabilize chromosome 4 along the metaphase plate, chromosome 4 segregates properly in more than 99.9% of meioses. This is because *D. melanogaster* has a process termed the distributive pairing system (Grell, 1976), to ensure the proper segregation of chromosomes that do not undergo exchange. The distributive system according to Hawley et al (1993) is actually two distributive systems, the homologous and heterologous. The heterologous distributive pairing system tries to explain the segregation of non-homologous chromosomes that do not undergo exchange. Size is postulated to be important in the segregation process since small X chromosome duplications induce non-disjunction of chromosome 4 only when the duplications are similar in size to chromosome 4 (Hawley et al, 1993). There is also regular segregation of attached-X and attached-4 chromosomes to separate poles presumably because of the size difference. Thus the heterologous distributive system uses the gross difference in shape and size between chromosome 4 and any non-exchange X-chromosomes to ensure the proper segregation of these chromosomes.

The homologous distributive system uses sequence homology to segregate chromosomes. According to Hawley, heterochromatic regions mediate this segregation. Evidence for this is that there is a higher level of segregation of two normal chromosome 4's from a Dp(1;4), if there is a greater amount of chromosome 4 heterochromatin on the duplication chromosome. Thus the greater amount of heterochromatin on the duplication chromosome, the greater the disruption of normal segregation. It is the homologous distributive system, which is postulated to be responsible for the normal segregation of chromosome 4 (Hawley et al, 1993).

If such a system for segregating achiasmatic chromosomes were real, it would relieve some of the selection pressure on chromosome 4 to undergo exchange, as an exchange would no longer be needed to improve segregation. Conversely it may be the case that the lack of meiotic exchange on chromosome 4 may been the instigator for the evolution of a system which would ensure the proper exchange of homologous chromosomes which do not undergo crossing over.

Cytological evidence also supports the distinction between heterologous and homologous achiasmatic segregation (Dernberg et al, 1996). The heterochromatin of homologous chromosomes remains associated throughout prophase until metaphase I, irrespective of whether the chromosomes undergo crossing over. This is not the case with non-homologous chromosomes, which do not pair at all prior to disjunction. In neither case do euchromatic regions pair, thus supporting the model of Hawley (1992) that heterochromatin is important in homologous chromosome segregation.

Thus, it appears that *Drosophila* has evolved two methods to ensure the proper segregation of chromosomes. The heterologous system uses the size and shape of chromosomes to ensure proper segregation of chromosomes whereas the homologous system relies on homology between chromosomes for proper segregation. These "systems" can be genetically separated. Each "system" has its own set of genes required for proper function, the only known

similarity between the two systems is the requirement for the *nod* gene product (Zhang et al, 1990). Otherwise they are completely independent in terms of gene products required in order to function and their effect on chromosome segregation (Hawley et al, 1993).

Although heterochromatin is important for the proper segregation of homologous chromosomes, it can not account for this process in *Drosophila* males, which have proper segregation of homologous autosomes without any requirement for heterochromatic homology. Rather, the sites for pairing (and presumably proper segregation) are found distributed throughout the euchromatin of the autosomes, not the heterochromatin in males (McKee et al, 1993).

1.4 Chromosome 4 of *Drosophila melanogaster*

1.4.1 Structure of Chromosome 4

Estimates from measurements of mitotic chromosomes have placed the size of chromosome 4 at 0.2-0.3 um in length, or about 4% of the *D. melanogaster* genome (Hochman, 1976). More recently, Locke and McDermid 1993) used pulsed field gel electrophoresis to estimate the size of chromosome 4 at 5.2 megabases (Mbp). Most of this (~4 Mbp) is heterochromatic, as chromosome 4 contains only about 50 polytene chromosome bands, which probably represents under half of the chromosome (See Figure 1.2). Thus Hochman has estimated that the euchromatic region of chromosome 4 represents only about 1% of total euchromatin (Hochman, 1976).

Chromosome 4 is acrocentric, with a small left arm that has been occasionally reported in chromosome squashes. Its existence has been confirmed by the existence of translocations to the left arm (Roberts, 1972). To date, no genes have been localized to the left arm of chromosome 4.

About 80% of chromosome 4 is composed probably of simple, tandem repeated sequence and appears as α-heterochromatin located in the under-replicated chromocenter of polytene chromosomes (Locke and McDermid, 1993, Lohe et al, 1993). Furthermore, the banded region of chromosome 4 has unusual properties that differentiate it from all of the other chromosomes in *Drosophila melanogaster.* First, Miklos et al (1988) observed that repetitive sequences found in the β-heterochromatin of other chromosomes are dispersed along the entire length of chromosome 4. This repetitive DNA sequence family has been termed the Dr.D family. Conversely, other repetitive DNA sequences such as and $(C)_n$ and $(CA)_n$ have been found in all euchromatic regions, with the exception of chromosome 4 euchromatin (Pardue et al, 1987). Furthermore a heterochromatic binding protein HP1 binds to α-heterochromatin, β-heterochromatin as well as several sites along chromosome 4 (James and Elgin, 1986). All of this suggests that in some way chromosome 4 is "heterochromatic-like" since it contains a heterochromatic protein and is clearly different from other euchromatin (Miklos, 1988).

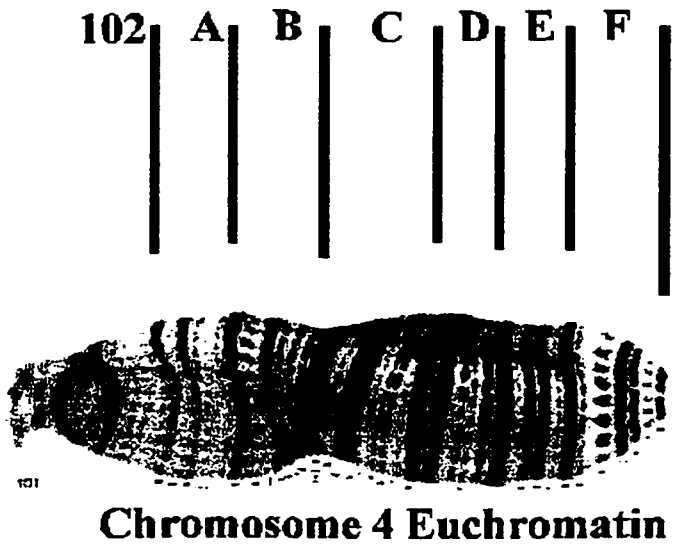


Figure 1.3. Cytology of Chromosome 4

Polytene chromosome squash of wild type chromosome 4 euchromatin (Sorsa, 1988).

1.4.2 *D. melanogaster* chromosome 4 lacks crossing over

Chromosome 4 normally undergoes little, if any crossing over in diploid wild type females. Sturtevant estimated that the number of natural crossovers ever detected in females up to 1951 was probably less than 5 (Sturtevant, 1951). No reliable numbers have ever been produced for map distances on chromosome 4 from wild type flies, although Bridges (1921) calculated the *bent* to *eyeless* distance as 0.14 map units. However no data was presented and it is possible that these crossovers were in fact non-disjunction events.

1.4.3 Crossing over on Chromosome 4 Homologs in other *Drosophila* species

Other species of *Drosophila* possess chromosome 4 homologues, which have often been termed "dot" chromosomes since they appear as dots on metaphase spreads of mitotic chromosomes. One such homologue is chromosome 6 of *Drosophila virilis*, a species that has been estimated to have diverged from *D. melanogaster* over 60 million years ago (Beverly and Wilson, 1984). In *D. virilis* crossing over occurs on chromosome 6. Chino and Kikkawa (1932) found 1.06% crossing over between the *D. virilis* genes *glossy* and *gap*. Nearly equal proportions of double recessives and wild type flies were found, implying that the recombinant progeny were not a result of non-disjunction. However it should also be noted that the overall frequency of crossing over in *D. virilis* is higher than in *D. melanogaster*. Although the physical size of both the X chromosome and chromosome 4 in *D. melanogaster* is about the same as their equivalents in *D. virilis* (Chino and Kikkawa, 1932), the genetic map is not. In *D. virilis* the X chromosome is 182 cM (Chino, 1929) whereas it is 66 cM in *D. melanogaster* (Morgan, Bridges and Schultz, 1931). Thus the lack of crossing over on *D. melanogaster* chromosome 4 may simply be the result of a lower rate of overall crossing over as suggested by Chino and Kikkawa (1932).

Whether crossing over occurs on the dot chromosomes of *D.simulans* and *D. mauritiana* is not known. Unlike *D. virilis*, these species have diverged from *D. melanogaster* much more recently, perhaps as recently as 0.6 mya (Hey and Kliman, 1993). Thus it would seem unlikely that crossing over would occur in these species after such a recent divergence. However if crossing over does occur, *D. mauritiana* is a more likely suspect than *D. simulans*. This is because the genetic map of *D. mauritiana* is 1.8 times the size of *D. melanogaster*, and does not have a significant centromere effect (True et al, 1996). These genetic attributes may be sufficient to allow crossing over to occur on chromosome 4. Crossing over is much less likely in *D.simulans* with its smaller genetic map, greater centromere effect and sequence similarity with *D. melanogaster* (Pardue et al, 1987).

All of this indicates that dot chromosomes are (or were) capable of crossing over, but for whatever reason chromosome 4 of *D. melanogaster* does not. Whether this is because chromosome 4 lacks some element or system that would allow crossing over, or some other factor is preventing crossing over on chromosome 4 is not clear. Experiments that lend support to either of these models as will be discussed in section 1.7.

1.5 Generation of Artificial Exchange on Chromosome 4

1.5.1 Crossing over in triploids

Meiotic recombination on chromosome 4 can be induced in triploid *Drosophila melanogaster* that are disomic for chromosome 4. Sturtevant (1951) used "diplo-IV triploid females" to map five loci (*ci*, *bt*, *gv*, *ey* and *sv*) on chromosome 4.

Why crossing over occurred in these "diplo-IV triploid" females is still unknown. One possible explanation has been offered by Hochman (1976), who suggested "if the paired centromeres normally block crossing over in 4, perhaps one member of the pair is occasionally "peeled away" by an unpaired X or large autosome". The most likely chromosome to "peel away" chromosome 4 would probably be the X chromosome, because the X chromosome and chromosome 4 have a tendency to remain associated (Dernberg et al, 1996). It should be kept in mind that there is no evidence as to how this type of association could alleviate the centromere effect, or even if the centromere effect requires paired centromeres to operate.

1.5.2 Crossing over after oocyte heat treatment

Chromosome 4 exchange has also been observed in diploids by heat treatment of the female at the time of oocyte formation (Grell, 1971). This was done by taking a population of time synchronized females heterozygous for chromosome 4 markers, and then subjecting to heat treatment (32°C or 35°C) for various time periods during development. This heat treatment was most effective in inducing females to produce progeny with a recombinant chromosome 4 when the females were heat-treated during the period of oocyte DNA synthesis. Oocyte formation begins at 126 hours and the most effective time point was between 132 and 156 hours, for the production of exchanges. However the observed crossing over may have been due to heat induced mutagenesis rather than a normal exchange. This is possible since "anomalous crossover products" were observed. This includes events like frequent allele conversions from spa^{pol} to *spa*, which is more suggestive of mutagenesis than crossing over. It should also be noted that heat treatment has been known to reduce the centromere effect (Stern, 1926). Thus the possibility remains that such heat treatment may indeed induce legitimate crossing over on chromosome 4 in diploid females.

1.5.3 The affect of meiotic mutants in crossing over in D. melanogaster

Meiotic mutations can induce crossing over on chromosome 4. Sandler and Szauter (1978) measured crossing over on chromosome 4 in *mei-9, mei-S282*, *mei-218* and *mei-352* mutant backgrounds. With the exception of *mei-9,* all of these strains allowed crossing over on chromosome 4. These mutants reduced the overall frequency of crossing over by selectively reducing distal, more than proximal, euchromatic exchanges near the centromere (Baker et al,

1976). This converts the normal biased (centromere effect) distribution of crossing over to one that is more uniform over the chromosome's length. Since these mutants eliminate the normal regional differences in crossing over frequency, the absence of crossing over on chromosome 4 could be accounted for by the wild type distribution of crossing over along the chromosome.

The mechanism by which these meiotic mutants affect chromosome 4 crossing over is not known. These mutants appear to be defective in some feature of crossing over that dictates the normal distribution of exchange. They (impair normal crossing over) reduce the number of exchange nodules (Carpenter, 1979) and most of the exchange nodules that do form in *mei-218* are morphologically abnormal. These meiotic mutants also display a high frequency of meiotic loss and non-disjunction. This is probably due to the absence of chiasmata that are required to segregate chromosomes properly at the first meiotic division where the extra non-disjunction reduced by these mutants occurs (Carpenter and Sandler, 1974). These mutants all appear to affect recombination generally, that is on all chromosomes rather than specifically on chromosome 4. One may propose from this that crossing over is somehow "prevented" on chromosome 4, and not that chromosome 4 is inherently incapable of crossing over.

1.5.4 The effect of balancer chromosomes on chromosome 4 crossing over

"Balancer" chromosomes in *D. melanogaster* often contain multiple inversions and cause a reduced frequency of crossing over in females heterozygous for such balancer chromosomes. This reduction is more than can be accounted before by the production of non-viable gametes, which reduces the number of flies in the crossover classes, and may be the result of steric interference in chromosome pairing. An increase in crossing over on the other chromosomes is associated with this reduction in crossover frequency on the balancer chromosome and its homologue. This increase however, is insufficient to affect crossing over on chromosome 4. Curry (reported in Bridges, 1935) was unable to detect crossing over on chromosome 4, despite using five heterozygous inversions on the other three chromosomes. It seems that chromosome 4 cannot be "forced" to undergo crossing over under these conditions.

1.6 Models for the absence of crossing over on Chromosome 4

1.6.1 Two Possible Models to Explain Lack of Crossing over on Chromosome 4

Two kinds of models have been proposed to account for the lack of crossing over on chromosome 4, involving position or sequence. The "position" model postulates that chromosome 4 loci do not undergo crossing over because these X loci are so close to the centromere that the centromere effect extends to cover the entire chromosome. The alternative "sequence" models suggest that

the base sequence composition of chromosome 4 DNA is distinct from the other chromosomes (more repetitive and heterochromatic) and like such sequences on other chromosomes, it does not undergo crossing over.

1.6.2 The Centromere Effect in Drosophila

As in some other eukaryotic chromosomes, the distribution of chiasmata along a typical *Drosophila* chromosome arm is subject to the centromere effect (Offerman and Muller, 1932). This non-random distribution is reflected in the genetic map, derived from the frequency of crossing over along the chromosome. As in some other eukaryotes there is little genetic exchange near the centromere, and map distances relative to the physical distance of the chromosome are either short or non-existent near the centromere. Thus it is not surprising that there are virtually no chiasmata observed in the proximal heterochromatin compared to the distal euchromatin (Carpenter, 1975).

One of the original investigators of the centromere effect in *Drosophila melanogaster* was Mather (1939), compared standard map distances between X chromosome markers to map distance in four different X chromosome inversion lines. He noted that there was an increase in exchange for proximal markers of the X chromosome when they were moved distally by an inversion. Furthermore there was also a corresponding decrease in exchange between distal markers when they were moved proximally. Both these increases and decreases were significant, often doubling or halving the former map distance.

Unfortunately Mather could not distinguish between an increase in map distance due to an increase in crossing over in euchromatin, heterochromatin or both. This was because the region between the markers in these inversions contained both euchromatin and heterochromatin and the breakpoints did not clearly delineate these two types of sequence. Regardless, his experiment demonstrated that meiotic exchange for proximal markers is increased when they are moved away from a *Drosophila* centromere.

1.6.3 Centromere Effect on Heterochromatin versus Euchromatin

Originally the term centromere effect referred simply to the lack of crossing over near the centromere (Beadle, 1932). At that time it was not clear whether the centromere effect was the result of the centromere, or whether the inability of sequences to undergo crossing over near the centromere was the result of the heterochromatin found associated with the centromere. As it turns out the very low rate of genetic exchange observed within the centric heterochromatin is not simply due to the centromere *per se* (Baker 1958). Baker observed that centric heterochromatin does not undergo recombination when moved to distal locations by chromosomal aberrations. He used the *D. virilis* homozygous reciprocal translocation $T(3;5)pe^{m51}$ to measure crossing over between pe^{m51} and *sv*, two genes which almost completely delineate the translocated centric heterochromatin. That is to say that there is either very little or no euchromatic sequences between these genes and the centric heterochromatin, and any crossovers observed, should take place within the centric heterochromatin.

When the testcross results were compiled, only three crossovers were detected to give a total of 0.16 map units between the two genes. However, Baker suspected that some or all of these crossovers may have been due to exchange within the small amount of euchromatin between the breakpoint and *sv*. However given the large size of the centric heterochromatin in the region, the occurrence of crossovers does not weaken his argument. If crossing over were to occur in the centric heterochromatin the frequency of such an occurrence is so much lower that it would be accurate to say that the ability of centric heterochromatin (even when removed from the centromeric region) to cross over is fundamentally different from that of euchromatin.

All of this implies that absence of exchange near the centromere seems to be an inherent property (a sequence specific effect) for centric heterochromatin whereas reduction of crossing over for euchromatin seems to be an acquired property. Thus it is strictly the reduction or abolition of crossing over in euchromatin located proximally to a centromere which is now termed the centromere effect.

1.6.4 Centric Heterochromatin and the Centromere Effect

Is the "centromere effect" is really due to the effect of the centromere on euchromatin, or a result of a spreading of heterochromatin from centric heterochromatin to euchromatic sequences? This spreading would then prevent the formation of chiasmata in euchromatin. There is little evidence to support the "spreading" model of crossing over suppression. Centric heterochromatin removed from the centromere in *T(3;5)pe*^{m51} seemed to have little or no power to reduce crossing over proximally to the translocation (Baker 1958). Strangely the map distance for distal chromosome 5 markers still adjacent to the now centrally located centric heterochromatin was markedly reduced in map distance, by over 40% for one region. This result is the exact opposite of the centromere effect! There is no easy explanation as to why the centric heterochromatin would now have a more powerful effect on these markers when moved from the centromere, and no other studies on the ability of centric heterochromatin to reduce crossing over when moved away from the centromere have been reported.

1.6.5 Evidence for the "Position" Model

The small length of chromosome 4 may allow a centromere effect (whether due to the centromere itself or to centromeric heterochromatin) to extend and cover the entire gene-containing region of the chromosome. The best evidence for this are the aforementioned meiotic mutations (*mei-S282*, *mei-218* and *mei-352*) which allow crossing over on chromosome 4. They act to alter the distribution of crossing over, creating a more equitable distribution of crossing over along the chromosomes. This allows regions near the centromere to show elevated levels of crossing over relative to wild type. Thus the lack of crossing over on chromosome 4 implies that these regional constraints "account

for the absence of recombination on chromosome 4 in wild type" (Sandler and Szauter, 1978).

Although these meiotic mutants are capable of allowing crossing over on euchromatic sequences near the centromere of other chromosomes, they are not capable of inducing exchange in centric heterochromatin. Carpenter and Baker (1982) examined the effect of eight such meiotic mutants, including those that allow crossing over on chromosome 4. None of them allowed crossing over to occur on the centric heterochromatin of the X chromosome. This suggests that these two systems of crossover suppression are under separate genetic control. The first system is under the control of meiotic mutants like *mei-S282*, *mei-218* and *mei-352*, and affects only euchromatin. This is distinct from the lack of crossing over in centric heterochromatic sequences because crossing over for these sequences is unaffected by these meiotic mutations. Whether this is due to an intrinsic inability of heterochromatin to undergoing crossover, or is the result of other factors is not clear. What is clear is that because chromosome 4 is affected by these meiotic mutants, it should be placed in the first system. Further evidence for this model comes from a comparison between the size of chromosome 4 and portions of the other chromosomes about the same size as chromosome 4 near their respective centromere. Based on the distances in Table 1.1, (compiled from Lindsey and Zimm, 1992) , it is possible that the centromere effect could cover all of chromosome 4 provided the strength of the centromere effect is roughly equivalent for each chromosome in *D. melanogaster*. However these cytological distances do not consider the size of the centric heterochromatin, which alters the distance of these chromosome 4 sized regions from their respective centromeres. They give only the relative distance away from the centric heterochromatin for each chromosome arm.

Table 1.1. Estimates of the Genetic Distance at Chromosome Centromeres

Chromosome Arm	Genetic Distance	Cytological Distance	Genetic Map Position	Map Distance
X	*bb* to *su(f)*	20F to 20A	65.9-66.0	0.1
2L	*cta* to E(SD)	40A to 40F	54.8-55	0.2
2R	*l(2)41Ab* to *sxc*	41A to 42A	55.1-55.2	0.1
3L	*CkIIa* to *l(3)80fj*	80A to 80F	47.0-47.0	0
3R	*Dsk* to *U1snRNA82Eb*	81F to 82E	47.1-47.1	0

While this suggests that the centromere effect may be responsible for the lack of crossing over on chromosome 4, it is by no means conclusive. While meiotic mutants may modify the wild type distribution of crossing over along a chromosome arm and allow crossing over to occur on chromosome 4, it does not imply that chromosome 4 is simply centromere affected euchromatin. Although these mutants relieve the centromere effect, it can not be determined if that removal was complete. There is no way which to determine whether full crossing over ability has been restored to the "natural" level of the euchromatin of other

autosomes. It is possible that chromosome 4 does not fit neatly into the centric heterochromatin/euchromatin dichotomy, only that a limited aspect of cross over suppression is relieved in this genetic background. With this model, chromosome 4 may have originally been unable to undergo crossing over because of the centromere effect, but the subsequent accumulation or loss of the appropriate repeated sequences may now have rendered this chromosome crossing over deficient.

From the centromere effect diagram (Figure 1.1) there also appears to be a "telomere effect", which is seen on other chromosomes and affects crossing over in a manner analogous to the "centromere effect" but from the telomere. A telomere effect could also be responsible for reduced recombination on chromosome 4. Both the centromere effect and the telomere effect act with a position dependent, sequence independent manner. However the telomere effect in *Drosophila* is unlikely to be responsible for the lack of crossing over on chromosome 4 since other chromosome ends of similar cytological distance to chromosome 4 do show crossing over and have a measurable genetic map distance (Table 1.2, compiled from Lindsey and Zimm,1992).

Table 1.2. Estimates of the Genetic Distance at Chromosome Telomeres

Chromosome Arm	Genetic Distance	Cytological distance	Genetic Map Position	Map Distance
X	*y* to *dor*	1A to 2A	0.0 to 0.4	0.4
2L	*bhe* to *shr*	21A to 22A	0.0 to 2.3	2.3
2R	*dat* to *tipD*	60B to 60F	107-110	~3.0
3L	*ve* to *Aprt*	61A to 62B	0.2 to 1.5	1.3
3R	*ld* to *l(3)SG71*	99C-F to 100C-F?	102-110.9	~9.0
4	*spa* to *ci*	102F to 102A	0.0 to 0.0	0

1.6.6 Evidence for the "Sequence" Model

As discussed in section 1.4, the euchromatin-associated sequence of chromosome 4 differs from that of the other chromosomes in *D. melanogaster*. It is not simply that chromosome 4 has its own characteristic sequences, but that it has repetitive sequences in common with heterochromatic regions of other chromosomes; regions that do not undergo crossing over. One such sequence is a repetitive sequence called Dr.D, which is found predominantly in β-heterochromatin (Miklos, 1988). β-heterochromatin is found between euchromatin and centric or α-heterochromatin (Heitz, 1934). Although β-heterochromatin is intermediate between euchromatin and α-heterochromatin in cytological terms, it is similar to euchromatin in terms of transcriptional activity (Lakhotia and Jacob, 1974). β-heterochromatin differs from euchromatin at the sequence level in harboring dispersed repetitive DNA, including both simple repeats as well as mobile element families such as Dr.D (Miklos et al, 1990). Dr. D is dispersed throughout the entire banded region of polytene chromosome 4 (Miklos, 1988). Thus it may be the case that chromosome 4 sequences are a target for β-heterochromatin formation. Protein HP1 is already known to bind

specifically to both α and β-heterochromatin and to certain sequences along chromosome 4 (James and Elgin, 1986). This protein or others like it may then interfere with the formation of functional recombination nodules. Therefore it is possible that these dispersed β-heterochromatic sequences are responsible for the lack of crossing over on chromosome 4.

An indication that repetitive sequences play a role in the control of crossing over comes from evolutionary conserved sequences such as $(dC\text{-}dA)_n\cdot(dG\text{-}dT)_n$. These sequences hybridize to the euchromatic arms of all chromosomes, with the exception of chromosome 4 euchromatin (Pardue et al, 1987, Lownhaupt et al, 1989). This distribution exactly matches the distribution of crossing over in *D. melanogaster*, and this association between hybridization and lack of crossing over holds true for different species of *Drosophila*. While CA/GT hybridization does not occur on the dot chromosomes of *D. melanogaster*, *D. simulans*, *D. pseduoobscura* and *D. miranda* it does occur on the dot chromosome of *D. virilis*. *D. virilis* is also the only known species of Drosophila to have crossing over on its dot chromosome. It is possible that these CA/GT repeats may be required for crossing over and their absence may preclude crossing over on a chromosome. In addition, these sequences may determine the general level of crossing over on a chromosome. In the case of *D. virilis* these repeats are much more abundant than in *D. melanogaster*, which is consistent with the roughly 2.8 times greater rate of crossing over found in *D. virilis* (Chino and Kikkawa, 1932). Thus, it has been speculated (Lownhaupt et al, 1989) that "meiotic recombination in heterochromatic regions is due to a lack of "recombinogenic" repeat sequences".

1.7 Testing the Models

1.7.1 The use of Translocations in testing the models

In general terms, the lack of crossing over on chromosome 4 has been speculated to be due to either centromere effects (position dependent model) or to specific sequences along chromosome 4 (sequence dependent model). The position of chromosome 4, relative to its centromere, can be changed through chromosome rearrangements. A translocation that moves chromosome 4 sequences away from the chromosome 4 centromere offers an opportunity to separate the potential effects of position from the sequence dependent effects of chromosome 4 on crossing over. Markers in the gene rich banded region on chromosome 4 can be used to assay any change in crossing over.

T(1;4)w^{m5} is a homozygous viable reciprocal translocation that exchanges all the chromosome 4 euchromatin with the tip of the X chromosome (Muller, 1930). A schematic diagram of *T(1;4)w*^{m5} is shown in Figure 1.4. The distal portion of chromosome 4 euchromatin (called 4^D) includes a small amount of centric heterochromatin and the entire gene-containing region. The distal tip of the X chromosome (X^D) is translocated just proximal to the *white* gene, and includes band 3C2 to the tip of the X chromosome. The two resulting chromosomes consist of 4^P+X^D and X^P+4^D (See Figure 1.4).

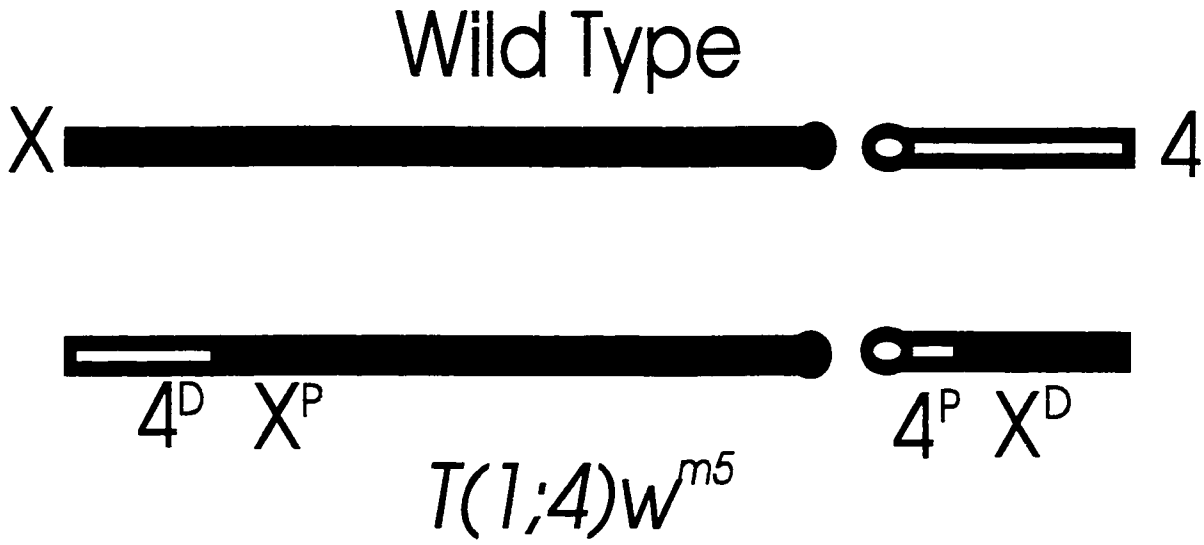


Figure 1.4. Schematic diagram of wild type and the *T(1;4)w*^{m5} translocation

On this diagram white regions represent chromosome 4 and black regions represent X chromosome sequences. Centromeres are shown by ellipses. The small translocation chromosome $4^{P}X^{D}$ is approximately 3X larger than chromosome 4. (Hawley et al, 1993)

The *white*$^+$ gene (at 3C2), which has been transposed to a new position on chromosome 4^P + X^D is a visible marker for this translocation chromosome. The 4^PX^D chromosome shows typical position effect variegation (PEV) of the *white*$^+$ locus. This *white*$^+$ gene is now subject to spreading effects of the adjacent heterochromatin and variegates, hence the "white mottled" (w^m) phenotype.

The small 4^PX^D translocation chromosome has been estimated from metaphase chromosome preparations to be three times the size of a normal chromosome 4 (Hawley et al, 1992). This is surprising given that the translocated X chromosome euchromatin is approximately equivalent in cytological size to the translocated euchromatin of chromosome 4. One explanation given for this apparent discrepancy is that *T(1;4)w*^{m5} may actually be derived from *In(1)w*^{m4}, which then underwent an almost perfect re-inversion before being translocated to chromosome 4 (Hawley et al, 1992). This would explain the large size of 4^PX^D chromosome as a result of X chromosome centric heterochromatin that was inverted distally to the *white* gene and the translocation breakpoint. However there is no direct evidence for *T(1;4)w*^{m5} being a triple chromosome aberration.

Regardless of the origin of the *T(1;4)w*^{m5} translocation, it allows two separate questions to be asked; (1) can chromosome 4 sequences undergo crossing over when separated from the chromosome 4 centromere and (2) will distal X chromosome sequences still undergo crossing over when moved into the proximity of the chromosome 4 centromere. Thus the *T(1;4)w*^{m5} translocation allows me to determine whether the sequence or position of chromosome 4 is responsible for the normal inability of this chromosome to undergo crossing over.

1.7.2 Can crossing over occur on 4^D?

If the absence of crossing over on chromosome 4 is due to the centromere effect, the movement of chromosome 4 to the tip of the X chromosome should remove such an effect and permit crossing over. If we find crossing over on 4^D, it would imply that the position of chromosome 4 genes relative to the centromere is responsible for the lack of recombination while the unique sequences of chromosome 4 are not.

If we look for but do not find crossing over on 4^D it would imply the centromere effect is not responsible for the lack of crossing over. Sequences of chromosome 4 would instead be implicated in the lack of crossing over on chromosome 4. This result would not be able to discriminate between such sequences distributed along chromosome 4 or localized at one (or more) sites such as the centric heterochromatin. It also would not exclude a telomere effect, as the translocation includes the chromosome 4 telomere. A telomere effect may be unlikely though, since the telomere effect is not very strong on the other chromosomes of *Drosophila melanogaster* (see Table 1.2).

1.7.3 Is crossing over inhibited on X^D?

Crossing over normally does occur within the region defined by X^D when it is at its normal position on the X chromosome. If crossing over can occur on X^D

when it is relocated next to the chromosome 4 centromere, it would imply that the placement next to the chromosome 4 centromere is not sufficient to repress crossing over. This outcome would be consistent with the centromere effect NOT being responsible for the normal lack of crossing over on chromosome 4, and would imply the sequences of chromosome 4 could be responsible for the normal lack of crossing over on chromosome 4. Alternatively if crossing over were not detectable on X^D it would imply the centromere IS able to repress crossing over and that the sequences of chromosome 4 need not be responsible for the lack of crossing over on this chromosome.

1.7.4 Implications of Results

Understanding of the control of crossing over is important because the process of crossing over itself is fundamental to genetics. Crossing over is involved in the ancient processes of DNA repair and meiotic recombination. Both of these genetic systems use crossing over, thus any impairment could lead to anything from diseases like cancer to disorders like Down's syndrome. Understanding crossing over should provide insight into these processes, as well as facilitate understanding of chromosome evolution.

Chromosome 4 is a good model as the highly repetitive nature of chromosome 4 sequences (Lohe et al, 1993) makes it more similar than other *Drosophila* chromosomes to the chromosomes of higher eukaryotes. Furthermore, as the exception to the rule of crossing over on the autosomes of higher eukaryotes, chromosome 4 deserves attention.

1.8 Summary of Experimental Outline

The translocation $T(1;4)w^{m5}$ moves the banded region of chromosome 4 from its centromere proximal location. If crossing over occurs between chromosome 4 marker, it implies that the proximity to the chromosome 4 centromere is responsible for the lack of crossing over. If this is the case, X^D which is now close to the chromosome 4 centromere should no longer be able to undergo crossing over. However if no crossing over occurs between chromosome 4 markers yet crossing over still occurs between X chromosome markers it suggests that the sequences on chromosome 4 are responsible for the lack of crossing over. Absence of crossing over on both X^D and 4^D would suggest that both the centromere effect and chromosome 4 sequences could be responsible for the lack of crossing over on normal chromosome 4. By these two models, crossing over on both X^D and 4^D should not occur.

Chapter 2.

Materials and Methods

2.1 Stock Lists

Table 2.1 Stock List

Stock Name	Genotype	Lab Book Name (if different)	Source
85	$M(4)101^{57g}/ci^{D}$		Ken Tartof
104	$T(1;4)w^{m5}$; ci^{D}		John Locke
167	$y\ w^{[67c23(2)]}$		Ross Hodgetts
307	$y\ w$; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{N}$		John Osborne, via 167 and 641
312	$T(1;4)w^{m5}$; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{N}$		John Osborne, via 307 and 104
313	$In(1)w^{m4};w^{m4}$;mwh red e su(KD343)/TM3; $M(4)101^{57g}/ci^{D}$		Scott Hanna
641	ci^{1} gv1 $ey^{R}\ sv^{N}$		Indiana University Stock Centre
w^{JO1};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)\ w^{JO1}$; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	w^{A4}	John Osborne
w^{JO2};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)\ w^{JO2}$; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	w^{A8}	John Osborne
w^{JO3};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)\ w^{JO3}$; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	w^{A2}	John Osborne
Y^{JO1};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)w^{m5}\ y^{JO1}$; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	y^{A1}	John Osborne
Y^{JO2};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)w^{m5}\ y^{JO2}$; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	y^{A7}	John Osborne
Ey^{JO1};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)w^{m5}$; ey^{JO1}; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	ey^{A3}	John Osborne
Ey^{JO2};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)w^{m5}$; ey^{JO2}; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	ey^{A5}	John Osborne
Ey^{JO3};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)w^{m5}$; ey^{JO3}; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	ey^{A27}	John Osborne
Sv^{JO1};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)w^{m5}$; sv^{JO1}; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	sv^{A2}	John Osborne
Sv^{JO2};$ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)w^{m5}$; sv^{JO2}; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	sv^{B48}	John Osborne
Sv^{JO3};$ci^{1}gv^{1}\ ey^{R}\ sv^{n}$	$T(1;4)w^{m5}$; sv^{JO3}; $ci^{1}\ gv^{1}\ ey^{R}\ sv^{n}$	sv^{B9}	John Osborne
w^{JO1} ; ci^{D}	$T(1;4)w^{m5},w^{JO1}$; ci^{D}		John Osborne
Y^{JO1} ; ci^{D}	$T(1;4)w^{m5}\ y^{JO1}$; ci^{D}		John Osborne
Y^{JO2} ; ci^{1}	$T(1;4)w^{m5}\ y^{JO2}$; ci^{D}		John Osborne
Ey^{JO1}; ci^{D}	$T(1;4)w^{m5}$; ey^{JO1} ; ci^{D}		John Osborne
Ey^{JO2}; ci^{D}	$T(1;4)w^{m5}$; ey^{JO2} ; ci^{D}		John Osborne
Sv^{JO1}; ci^{D}	$T(1;4)w^{m5}$; sv^{JO1} ; ci^{D}		John Osborne

* lethal is rescued by TM3 (results not shown)

2.2 Media and Culture Conditions of Drosophila Stocks

All *Drosophila melanogaster* stocks were grown at room temperature (approximately 22°C) in vials or bottles. A single litre of fly media contained: 10g agar, 100g sucrose, 100g Brewer's yeast, 100mg chloranphenicol, 10ml propionic acid, 4.3g NaH_2PO_4 and 2.7g Na_2HPO_4 (Nash and Bell, 1968).

2.3 Cytology

Salivary gland chromosome squashes were prepared using a modification of the technique described in Ashburner's *Drosophila* A Laboratory Manual (1989), Protocol 18. Third-instar larvae were dissected in one or two drops of 45% acetic acid on a clean microscope slide. With a pair of fine forceps most or all of the associated fat body was removed from the salivary glands. Salivary glands were then transferred to a fresh slide containing one drop of aceto-orcein consisting of 1% acetic acid in orcein and 1% acetic acid in carmine, mixed in a 1:1 ratio. The salivary glands were then covered by a cover slip, and the excess liquid was blotted away with a tissue. A blunt pencil tip was then applied to the top of the cover slip and the chromosomes were spread apart by moving the pencil in a spiral pattern along the top of the cover slip. A tissue (Kimwipe™) was then placed over the cover slip and pressure was applied to spread and flatten the salivary gland polytene chromosomes. After blotting again, the slides were ready for observation. Polytene chromosomes were observed with a Zeiss AxioPhot Photomicroscope at 1000X magnification.

2.4 DEB Mutagenesis

T(1;4) w^{m5}, w^{m5} /ci^1 gv^1 ey^R sv^n males were starved for ~20 hrs in vials containing 1% agarose. This starvation induces feeding when given sucrose with DEB. Approximately 50 males were used per vial. They were then transferred to 50ml plastic screwcap tubes with tissue soaked in mutagenizing solution (2ml of 1% sucrose and 5mm DEB). They were fed this mutagenizing solution for 14-20hrs. The mutagenized males were then transferred to a vial of food containing virgin homozygous *y w;* ci^1 gv^1 ey^R sv^n females (Stock #307 above). Female progeny were examined for mutations. Putative mutants were backcrossed to the aforementioned homozygous tester strain to confirm the mutation and generate a stock.

2.5 Photography

Photographs of the mutant animals were taken with Kodak Gold (ASA 100) 35mm film on a Zeiss DR Stereomicroscope. Photographs of mutants were taken at 20X power for the *yellow* mutant, 40X power for the *eyeless* and *shaven* mutant, and 80X power for the *white* mutant.

2.6 Extractions of Genomic DNA

Extractions of fly genomic DNA were carried out using a modified version of an unpublished protocol by Greg Gloor and William Engels in DIN (Drosophila Information Newsletter). Squishing buffer (SB) consisted of 10 mM Tris-Cl pH8.2, 1 mM EDTA, 25 mM NaCl, and 200 µg/ml Proteinase K. Proteinase K was added fresh daily from a frozen 20 mg/ml stock solution. Two flies (the male and female line progenitors) were placed in a single 0.5 ml Eppendorf tube and mashed for 5-15 seconds using the pipette tip of a 200 µl Pipetman. Some of the 100 µl of SB in the tip was released as the fly was mashed, and the rest was subsequently expelled after the maceration period. The fly remnants were then digested by Proteinase K at either 37°C for approximately 20-25 minutes or left at room temperature for approximately 25-30 minutes. The Proteinase K was then heat inactivated in a water bath of approximately 95°C for 1-2 minutes.

2.7 Polymerase Chain Reaction Conditions

2.7.1 Reaction Conditions

All PCR amplifications were run on a RoboCycler™ (Stratagene) with the following cycle times (unless otherwise mentioned). The first cycle consisted of a 2 minute 95°C denaturing period, followed by 1 minute 30 second annealing period at 60°C and then 3 minutes of a 73°C extension time. Cycles 2-29 consisted of 1 minute of 94°C denaturing, a 1 minute and 15 second annealing period at 60°C and a 1 minute extension period at the temperature of 73°C.

Each reaction tube consisted of 1 µl of DNA from the genomic DNA extraction, 3 µl of 10X PCR mix, 1.5µl of 10mM stock solution of each of the 2 primers (Bioservices, University of Alberta), 0.5 µl of Taq polymerase (Bioservices, University of Alberta), and 22.5 µl of distilled water. Light mineral oil was then added to bring the volume to approximately 60 µl. 10X PCR mix consisted of 500 mM Tris-Cl pH8.2, 15 mM of $MgCl_2$, 500 mM of KCl, 0.01% gelatin and 2 mM of each dNTP's all dissolved in distilled water.

Agarose gel electrophoresis was carried out in gels of approximately 1.0-% agarose concentration. The running buffer used was TAE made from a 50X TAE Stock Solution (50X= 242g of Tris base, 57.1 ml of glacial acetic acid, 100 ml 0.5 M EDTA pH 8.0 and distilled water to 1 litre).

2.7.2 Primer List

Table 2.2. Sequence of Primers from 5' to 3'

X	GATGCTTCAATCTGCAGC
D	TCTGACAGTGTGGGAAAATT
G	TAATGTTGAGCTGCAATTTC
U	TAATACTTCTACACGTTTCA
JS1	ATACATATGTTTCATTACGG
JS2	ATGCGTTTGTATGTATATTG
JS3	TACTCAGTTCAAATCTTGTG

Chapter 3 - Results I

Creation of Drosophila Mutations and Stocks

3.1 Screen for New Markers Mutations on *T(1;4)w^{m5}*

3.1.1 Generating Mutant alleles on t(1;4)w^{m5}

The first step towards assessing the frequency of crossing over was to generate mutant alleles on the T(1;4) translocation pair. Since crossing over measures recombination between two genes, at least one easily visible mutation must be produced in each of 4 genes to measure crossing over on both distal arms of the translocation. On the X chromosome, the genes *white* and *yellow* were targeted for mutagenesis, and on chromosome 4 the genes *eyeless* (*ey*) and *shaven* (*sv*) were used. The chromosome 4 gene *cubitus interruptus* (*ci*) was not used because the "Dubinin Effect" (Dubinin and Sidorov, 1934) will produce false positives. (The Dubinin effect arises when a chromosome 4 translocation is heterozygous with a *ci* allele and results in a *ci* phenotype.) The *grooveless* mutation was available but difficult to score accurately. To help guard against the possibility that any single mutant may somehow produce atypical results, I decided to make and use two mutants per locus. This guards against the possibility of any second site mutations which may affect crossing over.

Figure 3.1 shows the crossing scheme to generate mutants on the *T(1;4)w^{m5}* translocation. The first step is to use DEB to treat males hemizygous for the translocation, then cross these males to recessive tester females (*y w ; ci^{1} gv^{1} ey^{R} sv^{n}*). These females are homozygous. Female progeny were scored (male flies were not scored as they do not carry the mutagenized translocation) for the presence of mutations in *y*, *w*, *ey* or *sv*. Putative mutants were then crossed to tester males (*y w ; ci^{1} gv^{1} ey^{R} sv^{n}*) to verify that the putative mutation was heritable. DEB was selected as a mutagen because it was accessible in the lab, easy to work with and a reliable mutagen (Reardon et al, 1986).

3.1.2 Results of Mutagenesis for y, w, ey, and sv

43,334 female progeny from the DEB mutagenesis were examined for new mutations in the *white*, *yellow*, *eyeless* and *shaven* genes. All male progeny were ignored because they lack the translocation chromosome. This total is also the equivalent of 43,334 chromosomes, from which 11 independent mutants were recovered: 3 *eyeless*, 3 *shaven*, 3 *white* and 2 *yellow* (Table 3.1). Of those 11 mutants, 8 (two per locii) were chosen to serve as markers for measuring crossing over on *T(1;4)w^{m5}*.

Each putative mutant was crossed to *y w ; ci^{1} gv^{1} ey^{R} sv^{n}* males to confirm a heritable mutant phenotype. Mutant males from each line produced by this cross were then crossed to the parental translocation *T(1;4)w^{m5}; ci^{1} gv^{1} ey^{R}*

Figure 3.1. DEB Mutagenesis Screen

Mutagenized males are testcrossed to homozygous recessive females. Female progeny are then examined for putative *ey*, *sv*, *y* and *w* mutations. Male progeny do not contain the translocation and are not scored.

DEB Mutagenesis Screen

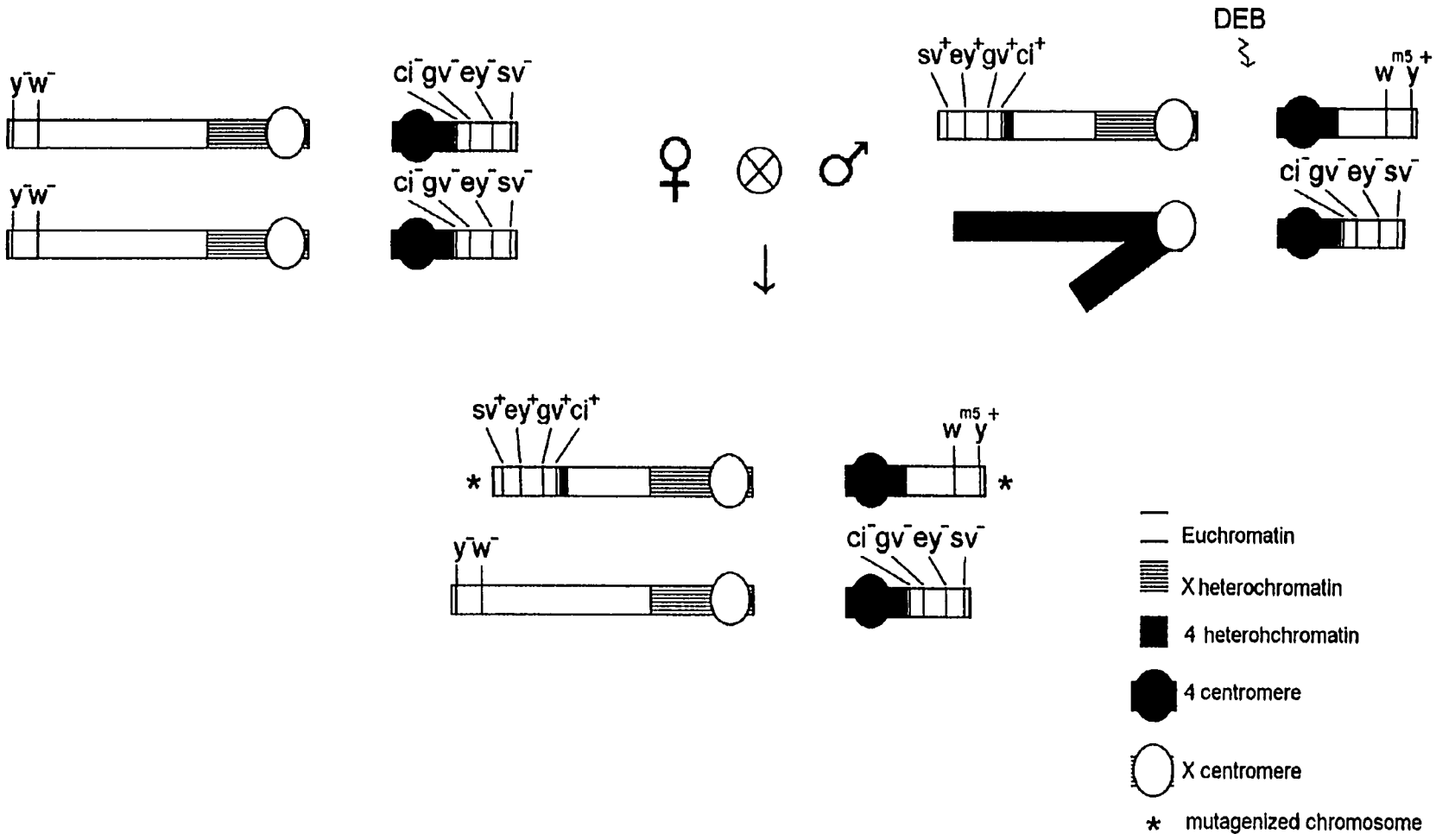
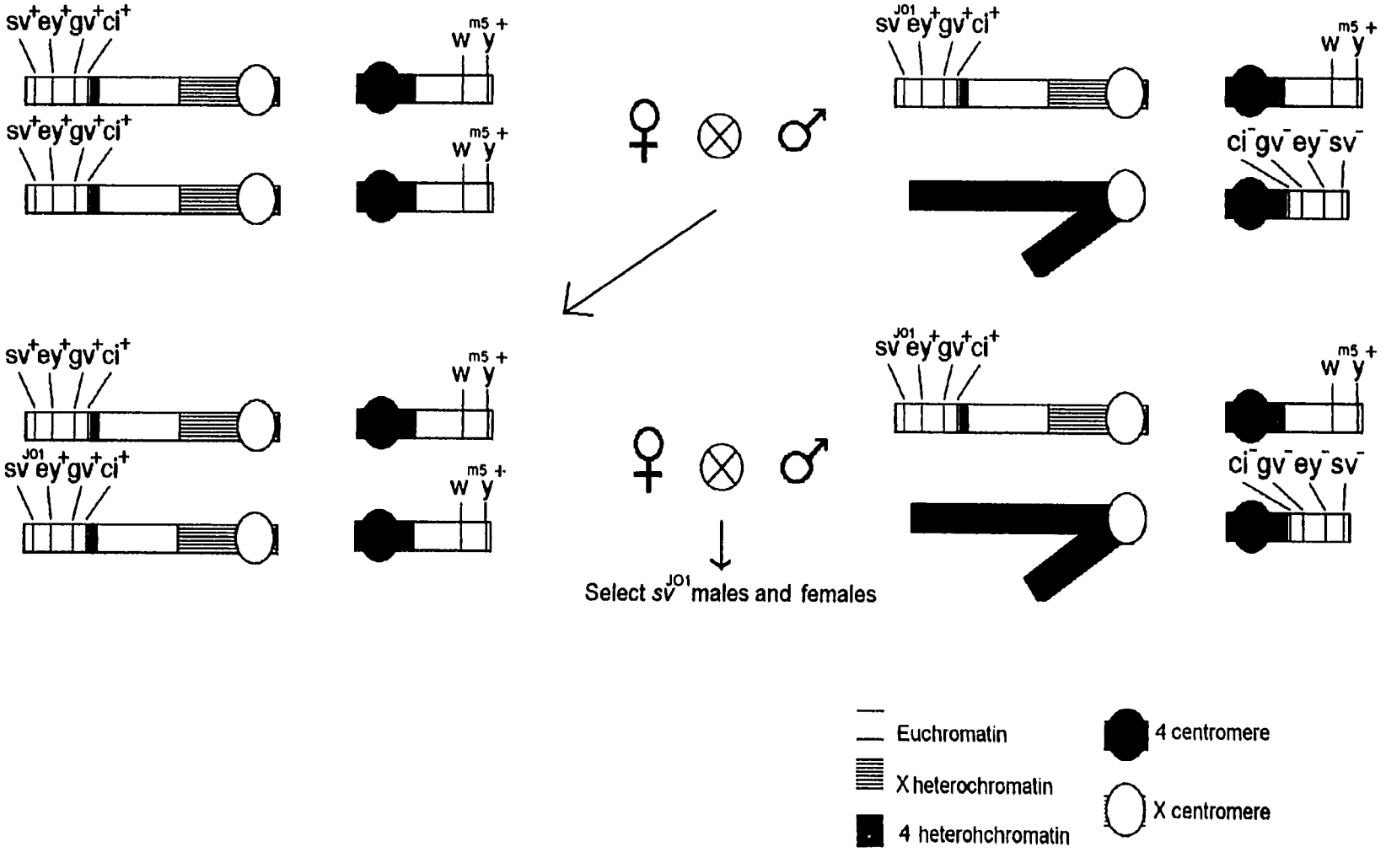


Figure 3.2. Creating Mutant Stock

Mutants were crossed to the wild type parental translocation. To generate the mutant stock, the mutant phenotype was then selected for.

Creating Mutant Stock



sv^{n}. The appropriate mutant phenotype was selected until a stock was produced. A diagram of this cross is shown in Figure 3.2. Doing this not only removes the $y\,w$ chromosome and creates a mutant stock, but it can be used to cross off some other potential deleterious mutations that may have been induced by the mutagenesis. This technique was used successfully to remove a recessive lethal in the mutant line sv^{JO2}. However similar crosses were unsuccessful in separating the w^{JO2} allele and the w^{JO3} allele from their respective recessive lethal mutations.

Table 3.1. Mutagenesis Results

Generated Alleles			
white	***yellow***	***Eyeless***	***Shaven***
w^{JO1}	y^{JO1}	ey^{JO1}	sv^{JO1}
w^{JO2}	y^{JO2}	ey^{JO2}	sv^{JO2}
w^{JO3}		ey^{JO3}	sv^{JO3}

3.1.3 Failure to create viable w^{JO2} and w^{JO3} mutant stocks

The mutagenesis produced three new *white* mutants, two of which (w^{JO2} and w^{JO3}) also carried X-linked recessive lethal mutations. These lethal mutations prevent a mutant stock from being created because the translocation-carrying males are not viable. To solve this problem, females from both lines were crossed to a stock containing the TM3 balancer chromosome. The TM3 balancer chromosome contains a small duplication of the X chromosome on the third chromosome, which covers the breakpoint region in $T(1;4)w^{m5}$. It was suspected that the failure to cross off the linked lethal mutation was because it is near the translocation breakpoint. Thus TM3 might rescue the lethal phenotype.

The w^{JO2} (but not w^{JO3}) allele was rescued by TM3. Unfortunately, the stock produced from this cross was not healthy and could not be maintained. This was probably because of the combination of translocations, inversions, duplications and recessive lethal genes in this stock. Thus, a balanced stock of w^{JO2} was never produced, and the mutation has been maintained by selection each generation.

3.2 Phenotypic Characterization of New Mutants

3.2.1 Photographs of mutants

Mutant lines generated from the DEB mutagenesis show full penetrance of their phenotypes. Examples of typical flies of each of the four genotypes are provided in Figure 3.3.

3.2.2 Cytology of mutant chromosomes

Salivary gland polytene chromosome squashes from all mutant lines used in the crosses were examined to identify possible visible chromosomal aberrations that might interfere with crossing over. For each mutant line, 3 slides

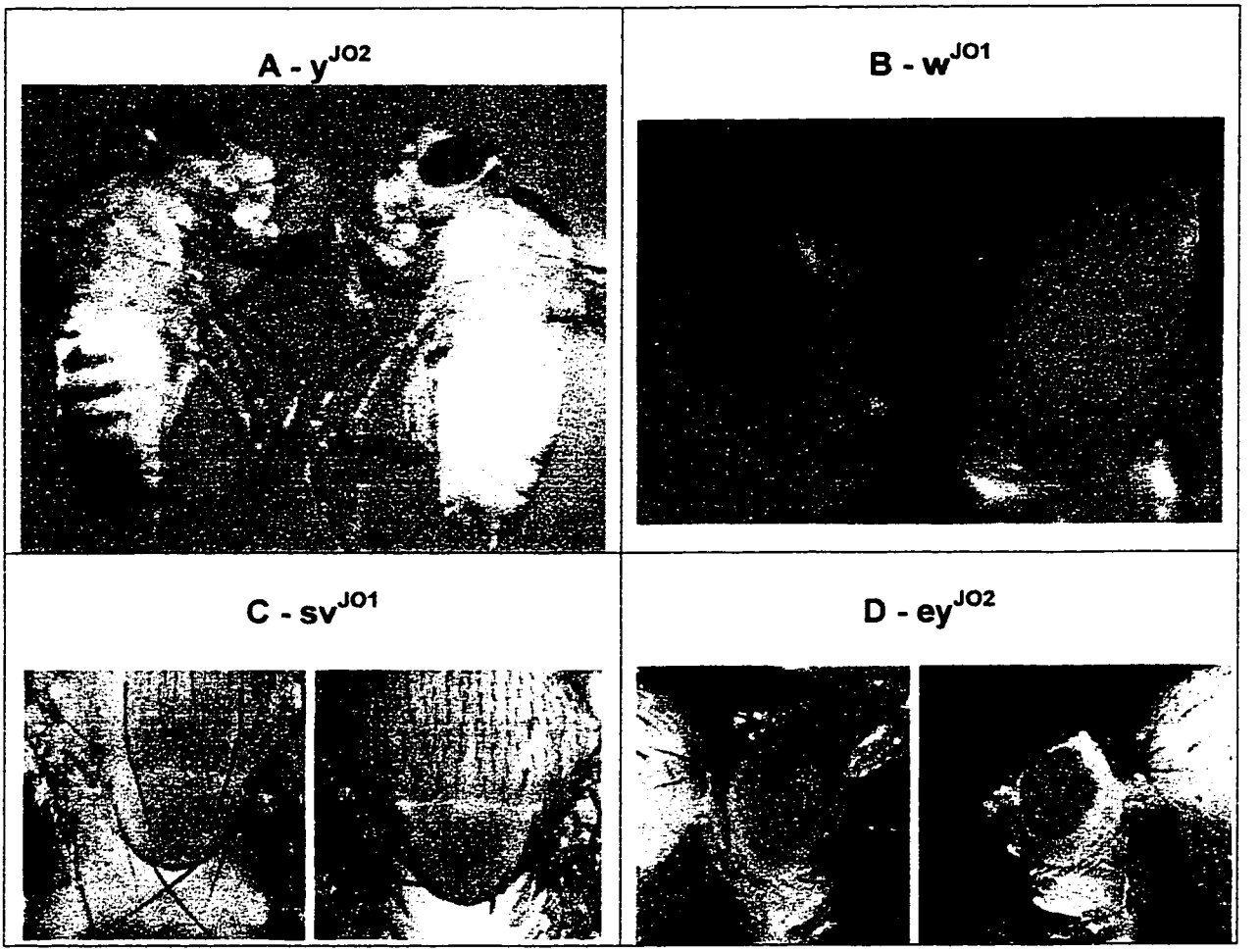


Figure 3.3. Mutant Phenotypes from DEB Mutagenesis

Phenotype of mutants produced from the DEB mutagenesis.
(A) Mutant y^{JO2} body color on the right, with parental strain on the left
(B) Mutant w^{JO1} eye on the right, with parental w^{m5} eye phenotype on the left
(C) Mutant sv^{JO1} bristles on the right, wild type bristles on the left
(D) Mutant ey^{JO2} on the right showing moderate expression, parental strain on the left

were prepared and at least five nuclei from each slide were examined.

Since DEB is known to induce deletions (Reardon et al, 1986; Shukla and Auerbach,1980), the region near each mutation was closely examined for the presence of any visible deletions or rearrangement. In addition, the determination of whether a mutant line had a deletion was not made in saiivary glands homozygous for the mutant translocation. Instead they were first crossed *to T(1;4)w^{m5};ci^{1} gv^{1} ey^{R} sv^{n}* which contains the parental translocation. This has the advantage of making potential deletions more readily detectable, since they should form a visible deletion loop if sufficiently large.

No new deletions or aberrations were found in any mutant. Furthermore a constriction distal to the white gene, which has been suggested to represent inverted X chromosome heterochromatin (Hawley et al, 1993) was not observed. The photographs in Figure 3.4 show polytene chromosomes from each of the 8 lines used in the crosses.

3.3 Assay for loss of *ci^{1} gv^{1} ey^{R} sv^{n}*

3.3.1 Problems with mutant lines containing the multiply marked chromosome 4

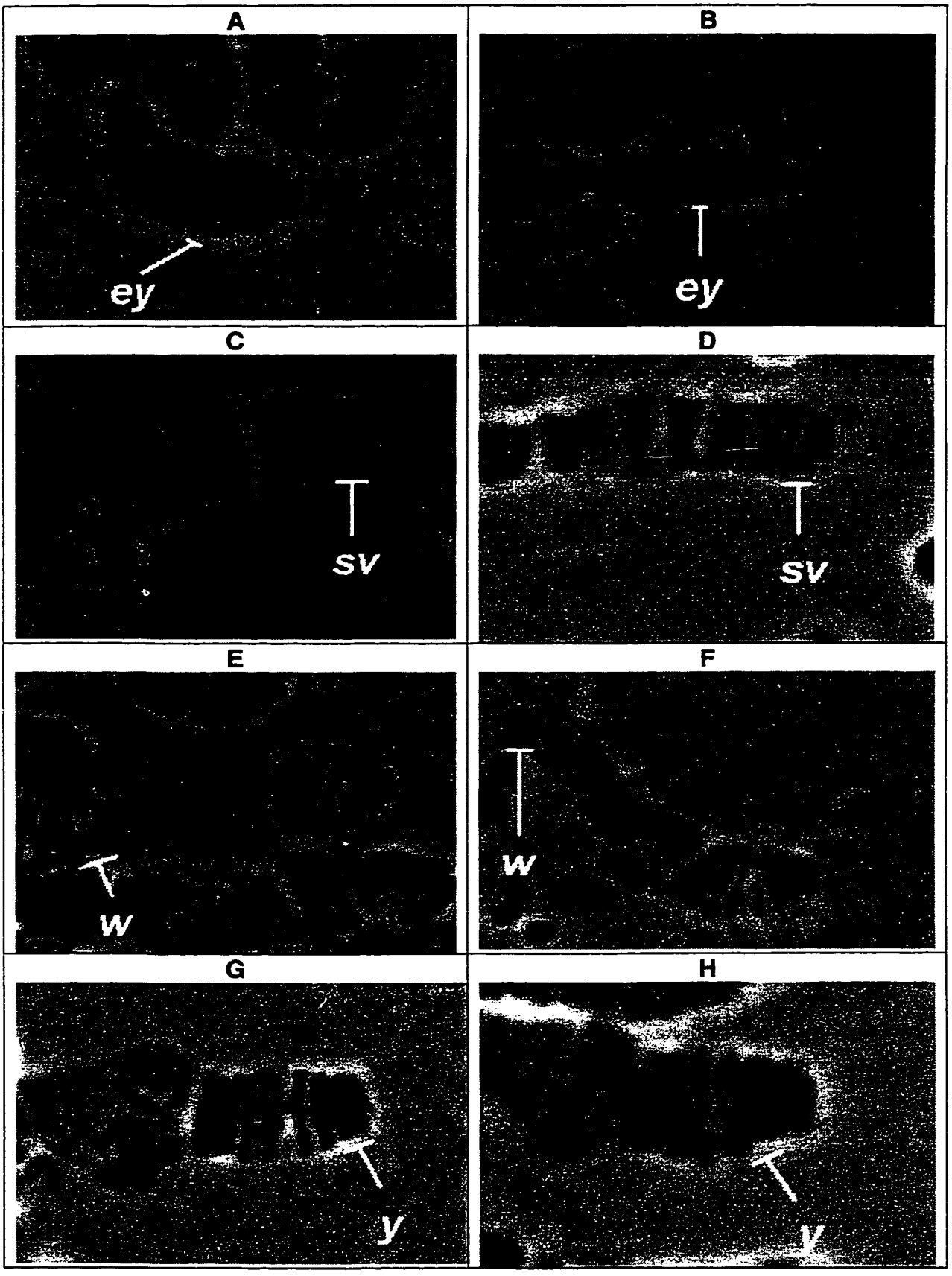
The mutant lines each contained the multiply marked (*ci^{1} gv^{1} ey^{R} sv^{n}*) chromosome 4. This chromosome created two problems in assaying crossing over on the translocation. Firstly, because chromosome 4 is approximately one third the size of the $4^{P}X^{D}$ chromosome it will cause non-disjunction of the $4^{P}X^{D}$ chromosomes in flies trisomic for chromosome 4 during meiosis. This is because the "heterologous" distributive system fails to distinguish between these chromosomes. Chromosome 4 will go to one pole and the two small translocation chromosomes will go to the other pole. This will produce wild type progeny that appear to be recombinant for X chromosome markers but are not. This frequency of non-disjunction is so severe, that approximately 20 percent of all progeny from such a cross show non-disjunction (results not shown). Such a high frequency makes testing of all such false positives impractical so the extra chromosome must be removed from the cross.

The second problem with this extra chromosome is that it might somehow interfere with crossing over between the translocation chromosomes and thus invalidate the test. Even if it does not directly interfere with them, its presence would still be problematic. Sturtevant was able to achieve crossing over between non-translocated chromosome 4's in *Drosophila melanogaster* diploid for chromosome 4, but triploid for all other chromosomes (Sturtevant, 1951). To avoid these problems the recessive multiply marked chromosome 4 was replaced by a dominantly marked and easily scored *ci^{D}* chromosome.

By replacing the recessive *ci^{1}* containing multiply marked chromosome with a dominantly marked *ci^{D}* chromosome any extra chromosome can be readily selected against. This ensured that no trisomic-4 females would be used as parents to determine the frequency of crossing over. In addition, such a procedure ensures the genetic constitution of the female is diploid and that there is no interference from an extra chromosome 4.

Figure 3.4. Polytene Squashes of Mutants

Chromosome squashes were prepared by crossing each mutant line to the parental (non-mutagenized) strain to produce females heterozygous for the mutation, but homozygous for the translocation. Thus each mutant line is heterozygous with the parental *T(1;4)w*^{m5} translocation.
(A) ey^{JO1}
(B) ey^{JO2}
(C) sv^{JO1}
(D) sv^{JO2}
(E) w^{JO1}
(F) w^{JO2}
(G) y^{JO1}
(H) y^{JO2}


A
ey
B
ey
C
sv
D
sv
E
w
F
w
G
y
H
y

3.3.2 Replacing ci^1 gv^1 ey^R sv^n with ci^D in the mutant lines

The chromosome chosen to replace the ci^1 gv^1 ey^R sv^n multiply marked chromosome was a chromosome marked with ci^D. It was chosen for its dominant visible marker and good viability. The lines were previously in a multiply marked background, so new crosses were done to replace the multiply marked chromosome with a chromosome 4 containing ci^D as its only marker. This crossing scheme is outlined in Figure 3.5.

Lines with a ci^D background were made by crossing virgin females of the appropriate $T(1;4)w^{m5}$ mutant line to M^{57g}/ci^D males, and then selecting for ci^D males. These males were then crossed to females with the ci^+ phenotype of the appropriate translocation line to generate a ci^D stock for each of the mutant translocation lines. Unfortunately this crossing scheme has the disadvantage that some of the females with the ci^+ phenotype may also be carrying a multiply marked chromosome. This is possible because ci^1 does not show full penetrance in $T(1;4)w^{m5}$ females carrying two copies of the ci^+ translocation in addition to ci1. The "Dubinin Effect" does not function completely in this genetic background. Thus this crossing scheme will produce some lines which contain the multiply marked chromosome 4 in addition to ci^D. To solve this problem, I used PCR to identify those lines with the hidden ci^1 gv^1 ey^R sv^n chromosome.

3.3.3 A PCR assay to identify the ci^1 in four mutant lines

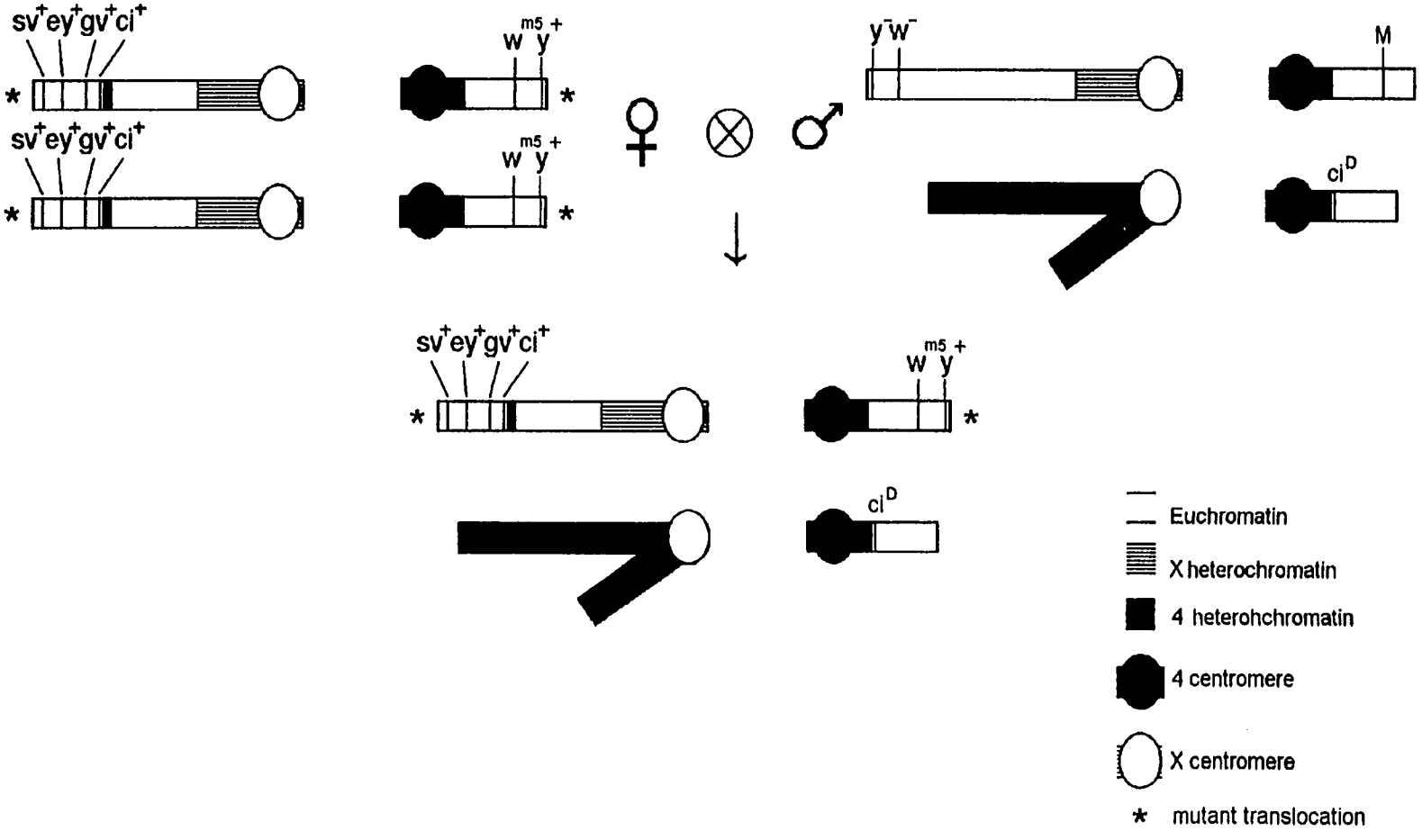
As discussed above, one of the problems with the crosses to stock the mutant translocations in a ci^D background is that these lines may also contain the ci^1 multiply marked chromosome in addition to the ci^D chromosome. Since this chromosome contains only recessive markers, visible markers could not determine its presence or absence. Thus there was no visual way to ensure that a heterozygous female in which the level of meiotic crossing over was being measured, was really diploid for chromosome 4. Thus many of these supposedly diploid females might have actually contained a genetically "hidden" extra chromosome 4. Trisomy 4 is quite common in $T(1;4)w^{m5}$ stocks in that at least half of the female flies in the ci^1 stocks of the mutant lines displayed the ci^1 (results not shown). This is because a non-translocated chromosome 4 is essential for male viability. Fortunately, in $T(1;4)w^{m5};ci^D$ stocks, this problem was not too serious because females trisomic with an extra ci^D marked chromosome ($T(1;4)w^{m5}/T(1;4)w^{m5};ci^D$)4 can be readily distinguished from both diploid females or females trisomic for the ci^1 chromosome but otherwise diploid. While diploid females carrying the ci^1 chromosome may have either a ci^1 or a ci^+ phenotype, females trisomic for ci^D always displayed the ci^D phenotype. Even if a mutant stock had both a ci^D chromosome 4 and a multiply marked chromosome 4 "floating" in it, females with the ci^D phenotype could be taken from this stock with the certainty that they did not contain the multiply marked chromosome.

This was not the case with males. Males that displayed the ci^D phenotype may actually been trisomic for chromosome 4, and possess the ci^1 multiply marked chromosome 4. Thus at least four lines (two each for X chromosome

Figure 3.5. Adding ci^D to Mutant Lines

Males carrying ci^D marked chromosome 4 (Stock #85) are crossed to females from each mutant line containing the translocation. Females selected for this cross are phenotypically ci^+. This decreases the odds that the female carries the multiply marked chromosome 4. ci^D males from this cross are then backcrossed to phenotypically ci^+ females in pairs to generate a line for testing by PCR that is hopefully multiply marked chromosome 4 free.

Adding ci^D to Mutant Lines



markers and chromosome 4 markers) needed to be tested by PCR before multiply marked chromosome free males could be taken from them. These males could then be used to produce females that are diploid for chromosome 4 in order to test for meiotic crossing over.

3.3.4 Production of *ci*^{1} free lines

The four lines containing *ci*^{D} but free from the multiply marked chromosome were produced in the same fashion as the four lines not screened for *ci*^{1}. The only difference is that the *ci*^{1} free lines were set up as pair matings, and the two parents were used for PCR. The *ci*^{D} mutant translocation lines *ey*^{JO1}, *ey*^{JO2}, *y*^{JO1} and *y*^{JO2} were all set up in this manner, and the male and female line progenitors were subsequently screened by PCR. Thus all of the *y* and *ey* lines were tested by PCR to ensure that they did not contain the *ci*^{1} containing multiply marked chromosome. Males from these ci^{1} free lines were crossed to females of *w* and *sv* lines.

3.3.5 Identifying the location of the *gypsy* insert in the *ci*^{1} allele

A restriction map of the *ci* region (with primers indicated) is shown in Figure 3.6. Primers were constructed in order to create an assay for the *ci*^{1} marked chromosome as described in section 3.3.6. The *ci*^{1} allele is known to contain a single *gypsy* insert found within a 1.6kb *Nsi* I restriction fragment (Slusarski et al, 1995). In order to use this insert to assay for the *ci*^{1}-containing multiply marked chromosome it was necessary to locate the insert with more accuracy. PCR was performed using primers X and U (see Figure 3.7) on genomic *ci*^{1} DNA. The production of the expected 671bp product in this reaction indicates that the *gypsy* insert is not between primer X and primer U. Thus this result further delineates the *gypsy* element to a 554bp fragment within the *Nsi* I fragment, between primer U and the *Nsi* I restriction site at base pair 1123. Reducing the location of the insert from a 1.6kb region to a 554bp region made the construction of a PCR assay for the presence of the *ci*^{1} containing multiply marked chromosome possible.

3.3.6 Creating an assay to screen for *ci*^{1} in the multiply marked chromosome

To assay for *ci*^{1} in the multiply marked chromosome, I constructed primers JS1, JS2, and JS3 using sequence information already obtained from the *ci* region (Ahmed and Podemski, 1997).

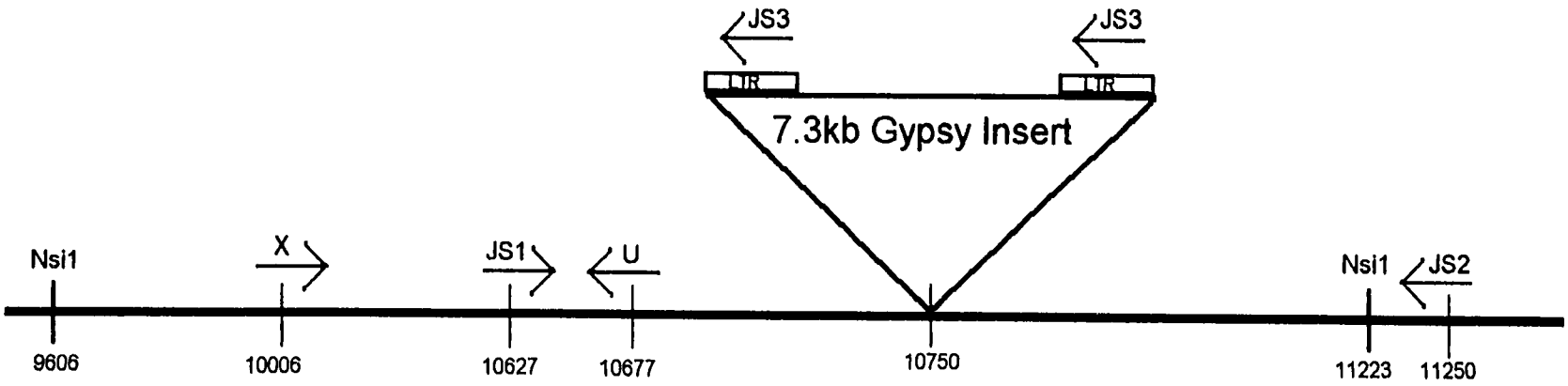
JS1 and JS2 are flanking primers on either side of the *gypsy* insert and are located 584 base pairs apart (Figure 3.6). These primers will produce an approximately 0.6kb band when there is a wild type copy of the *ci* locus and no product in flies homozygous for the *ci*^{1} allele. The insertion of the 7.3 kb *gypsy* transposable element between these two primers makes amplification of a JS1/JS2 product unattainable when using only a one-minute extension period with these reaction conditions.

The sequence of JS3 is complementary to a 20 base pair stretch of the *gypsy* LTR. While the sequence of the *gypsy* mobile element was known

Figure 3.6. Primer Map of the *ci* region

Region surrounding the 7.3kb *gypsy* insert responsible for the ci^1 phenotype. PCR primers are named over an arrow, which serves to indicate the orientation of the primer (arrowhead is 3' end). Position is indicated by a base pair number on the bottom which corresponds to GenBank sequence #U66884.

Primer Map of the *ci* Region



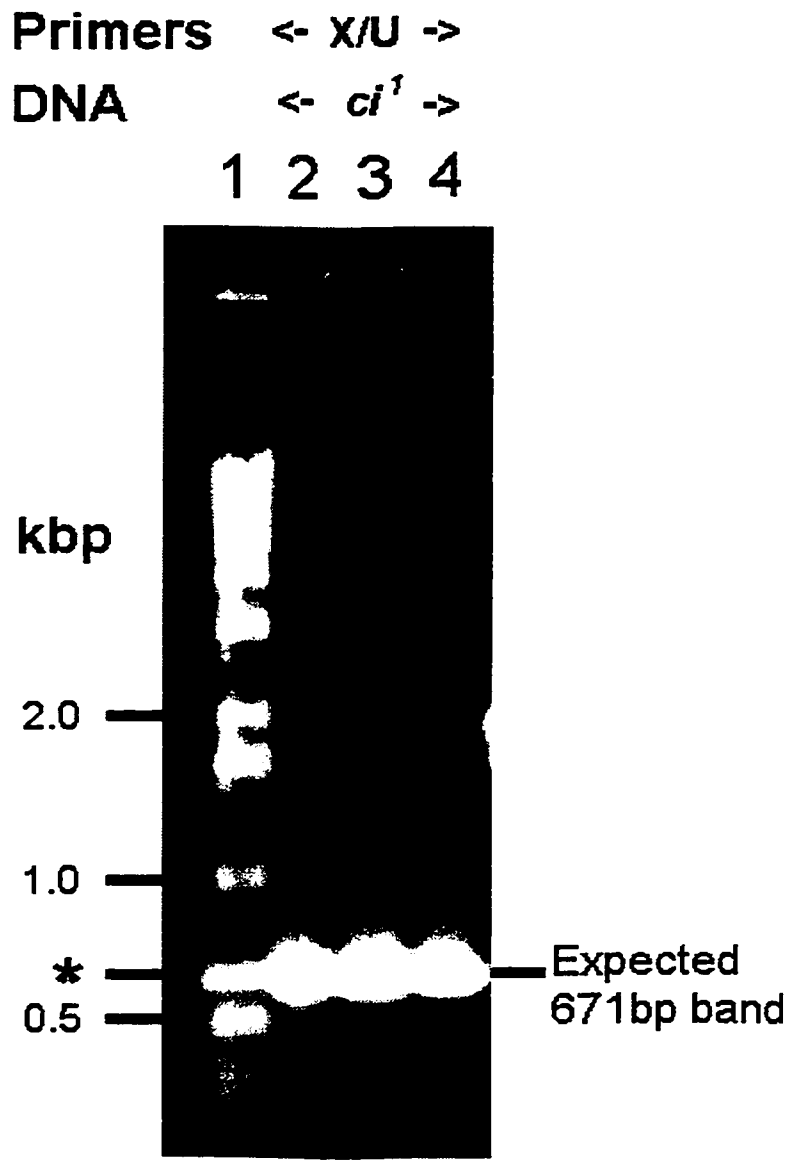


Figure 3.7. Relative Location of the *ci*^{1} Insert

Lane 1 is the 1 kb ladder, lanes 2-4 are all amplification of a 671 bp band using primers X and U on a *ci*^{1} genomic template (strain #641). Production of 671 bp band indicates the insert is not between these primers and lies to the right of primer U. If the *gypsy* insert were between primer X and primer U the distance between the primers would be increased by the size of the gypsy insert (7.3 kb) and amplification could not occur with the 1 minute extension time used in this PCR reaction. The asterisk indicates an overflow band in the ladder from lane 2.

(Freund and Meselson,1984). and a primer complementary to the gypsy LTR could be constructed, the orientation of the gypsy element in ci^1 was not known. However since only JS1, and not JS2, produces a PCR product in conjunction with JS3 this indicates that the insert is in the orientation as shown in Figure 3.6. (reverse orientation).

The size of the band produced by primers JS1/JS3 is approximately 340 as shown in Figure 3.8. This 340 bp product consists of *gypsy* LTR sequence to the left of JS3 and genomic DNA between the leftmost LTR and JS1. Therefore the point of insertion is approximately 123 bp to the right of JS1, as shown in Figure 3.6.

This assay for the ci^1 allele can now be used to screen for the presence of the multiply marked chromosome in mutant fly stocks. Single male and female pairs were mated to produce ci^D stocks free of the multiply marked chromosome. To confirm the loss of the multiply marked chromosome the two flies used to establish these lines can be screened for the presence of a *gypsy* insert which indicates the presence of the unwanted chromosome. If either parent produced a 0.3kb band with primers JS1 and JS3, then they have the unwanted chromosome. Failure to produce such a band (with the proper controls) indicates that these flies, and thus the stock, are ci^1 free and thus lack the multiply marked chromosome.

3.4 Results of PCR assay for ci^1 and the multiply marked chromosome.

3.4.1 Description of the PCR Assay

Both a positive and negative control were used. The negative control consisted of a sample containing male and female DNA from the parent translocation stock ($T(1;4)w^{m5};ci^D$) which lacks the ci^1 mutation. The positive control consisted of a sample containing DNA from a male of the parent translocation stock and a female homozygous for the translocation but trisomic for chromosome 4. The female thus has a genotype of $T(1;4)w^{m5}$; ci^1 gv^1 ey^R sv^n with two copies of the reciprocal translocation and one copy of the multiply marked 4 containing ci^1. So a positive result (production of the 340bp JS1/JS3 product) requires that only one of the two parent flies need have a single ci^1 containing multiply marked chromosome.

In addition to running a single reaction consisting of just the JS1 and JS3 primers, an additional reaction with primers JS1 and JS2 was run for a positive genomic DNA control. This was done to detect the possibility that the failure to produce a band in the JS1/JS3 reaction merely indicated the procedure failed to work correctly. Only lines with samples that produced the correct 0.6kb band in the JS1/JS2 reaction, failed to produce a band in the JS1/JS3 reaction, and for which both controls worked were considered to have passed the assay and were free of ci^1 and thus the multiply marked chromosome.

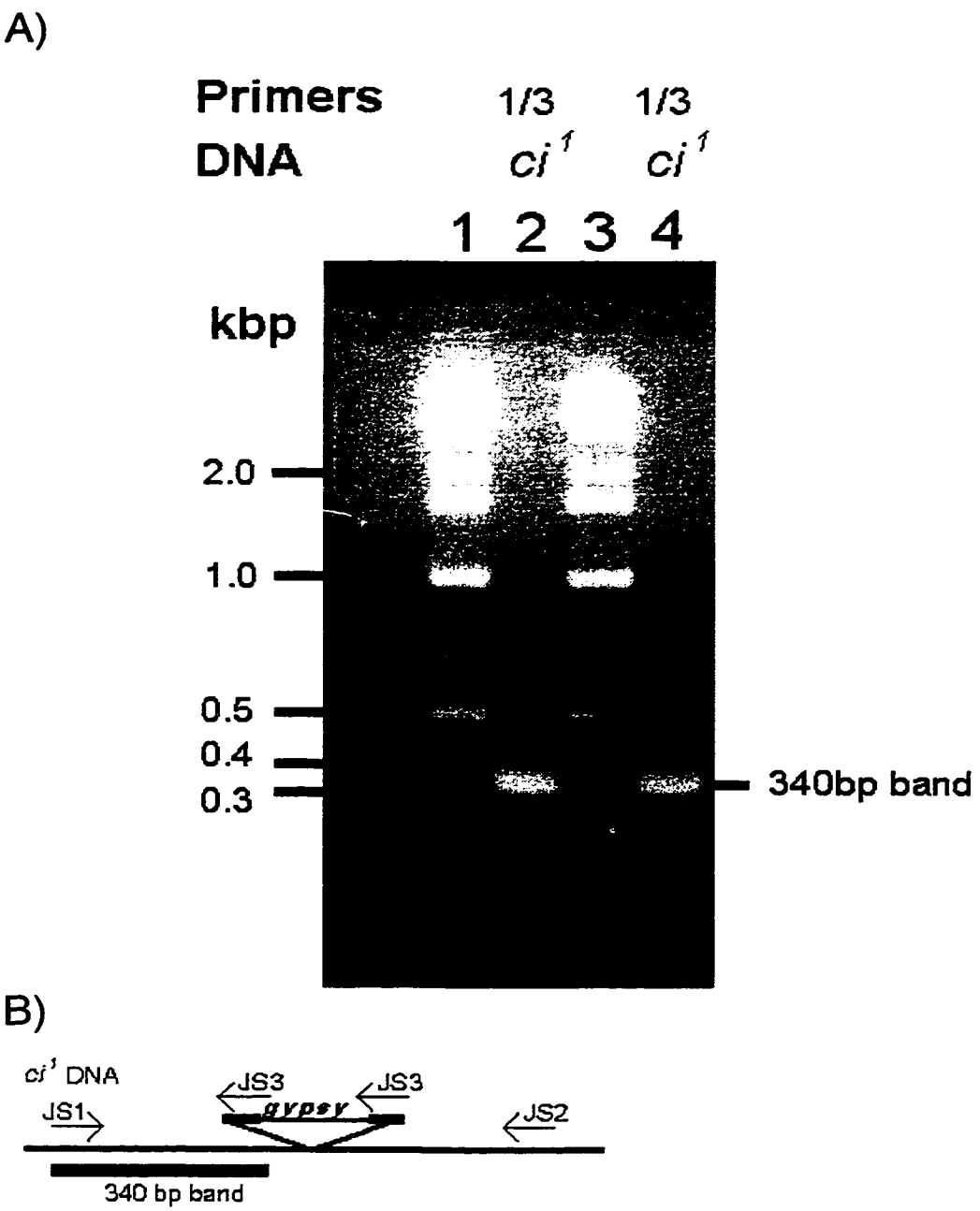


Figure 3.8. Further Location of *ci*[1] Insert

A) Gel separation of PCR products. Lanes 1 and 3 contain marker (1kb ladder), lanes 2 and 4 are the products of PCR amplification of *ci*[1] DNA and primers JS1 and JS3. The presence of the 340 bp band indicates that the JS3 sequence in the *gypsy* LTR is 340 bp to the right of JS1.

B) Diagram showing the relationship of the PCR products to the *gypsy* element in *ci*[1].

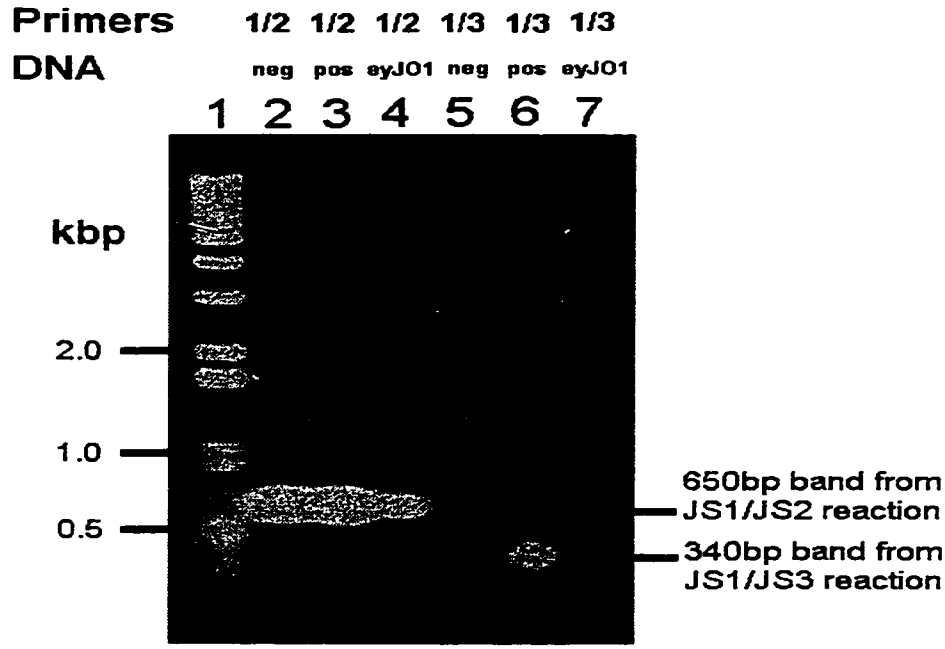
3.4.2 PCR Results

The lines ey^{JO1}, ey^{JO2}, y^{JO1}, y^{JO2} and w^{JO1} were all tested and a stock was created with ci^{D} and without contamination from the ci^{1} containing multiply marked chromosome (Figure 3.9-3.12). While it was not necessary to make a stock from w^{JO1} in ci^{D} (as $w^{JO1}ci^{D}$ females can readily be distinguished from both ci^{1} and ci^{+} females) the absence of the multiply marked chromosome in this stock allowed flies to be collected without the need for scoring.

3.5 Stock Creation Summary

Of the eleven different mutants from the DEB mutagenesis, it was my intent to use eight for testing crossing over on $T(1;4)w^{m5}$ (to produce convincing results that would not rely upon any single mutant). Unfortunately, a mutant stock of w^{JO2} could not be assayed because it contained a recessive lethal that inseparable from the desired *white* marker. Attempts to rescue the lethal phenotype with the TM3 balancer chromosome did not produce a viable stock. In the end, only seven different mutants were available to test for crossing over on $T(1;4)w^{m5}$.

A)



B)

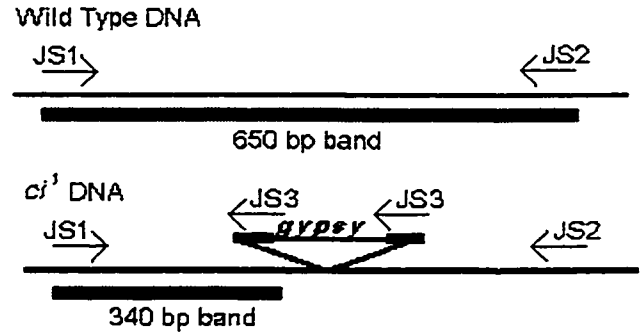


Figure 3.9. PCR Products amplified from ey^{JO1} DNA

A) Gel separation of PCR products. Lanes 2-4 are reactions with primers JS1 and JS2, lanes 5-7 are reactions with primers JS1 and JS3 and lane 1 is 1kb ladder. DNA is as follows: Lane 2, negative control (#104 DNA); lane 3, positive control (#104 and #312 DNA); lane 4, ey^{JO1} DNA; 5-7 as per lane 2-4. The presence of the 650bp band (from JS1/JS2 primers) in lane 4, and the absence of a band in lane 7 indicates that ey^{JO1} is free of the multiply marked chromosome.

B) Diagram showing the relationship of the PCR products to the *gypsy* element in ci^1.

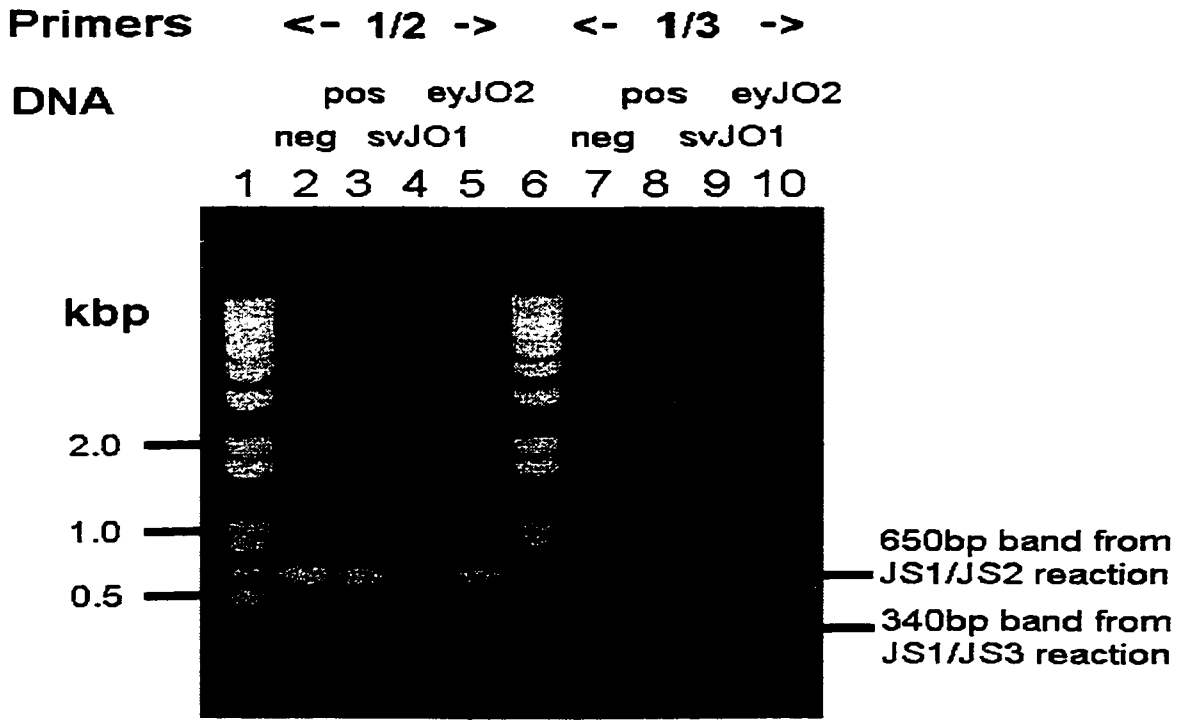


Figure 3.10. PCR Products amplified from ey^{JO2} and sv^{JO1} DNA

Gel separation of PCR products. Lanes 2-5 are reactions with primers JS1 and JS2, lanes 7-10 are reactions with primers JS1 and JS3 and lane 1 and 6 are 1kb ladder. DNA is as follows: Lane 2, negative control (#104 DNA); lane 3, positive control (#104 and #312 DNA); lane 4, sv^{JO1} DNA; lane 5, ey^{JO2} DNA; lane 7-10 as per lane 2-5. The presence of the 650bp band (from JS1/JS2 primers) in lane 5, and the absence of a band in lane 10 indicates that ey^{JO2} is free of the multiply marked chromosome, unlike this sv^{JO1} line. See Figure 3.9(B) for primer map.

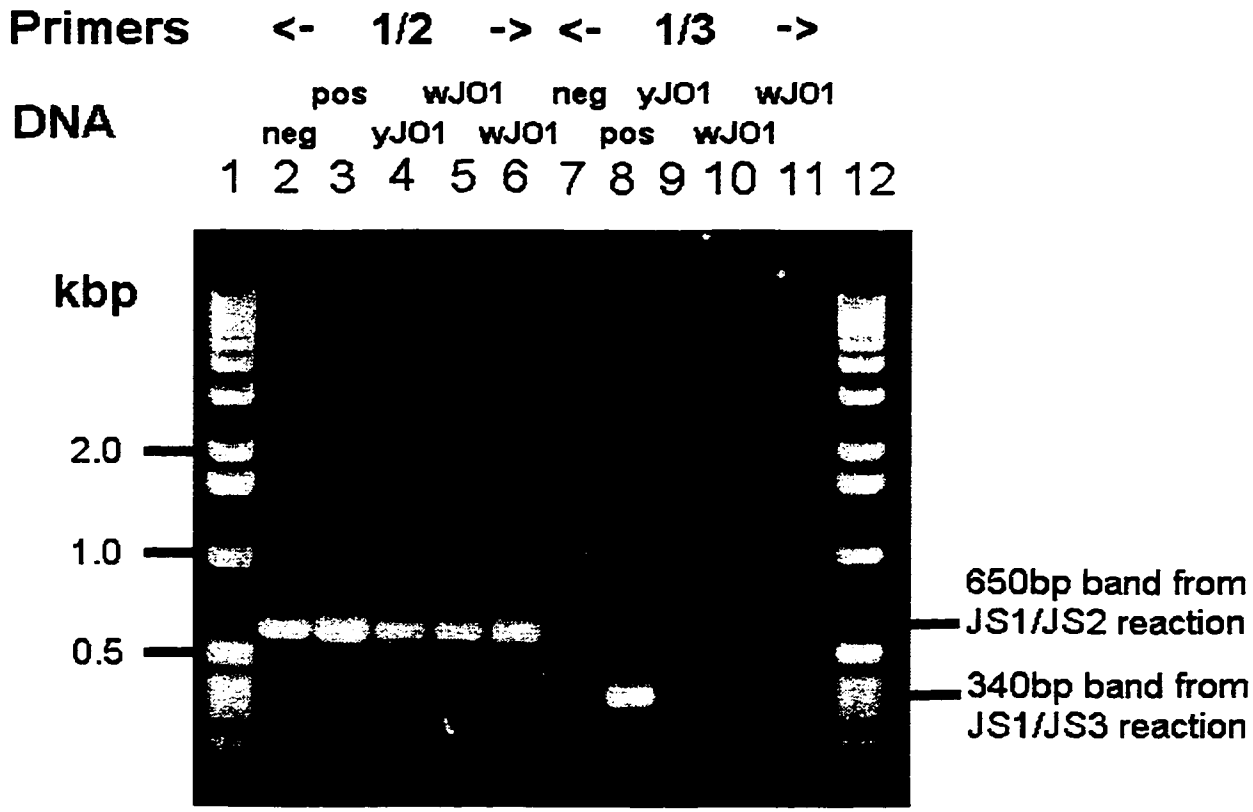


Figure 3.11. PCR Products amplified from y^{JO1} and w^{JO1} DNA

Gel separation of PCR products. Lanes 2-6 are reactions with primers JS1 and JS2, lanes 7-11 are reactions with primers JS1 and JS3 and lanes 1 and 12 contain 1kb ladder. DNA is as follows: Lane 2, negative control (#104 DNA); lane 3, positive control (#104 and #312 DNA); lane 4, y^{JO1} DNA; lane 5-6, w^{JO1} DNA; lane 7-11 as per lane 2-6. The presence of the 650bp band (from JS1/JS2 primers) in lane 5 and the absence of a band in lane 9-11 indicate that these lines are free of the multiply marked chromosome. See Figure 3.9(B) for primer map.

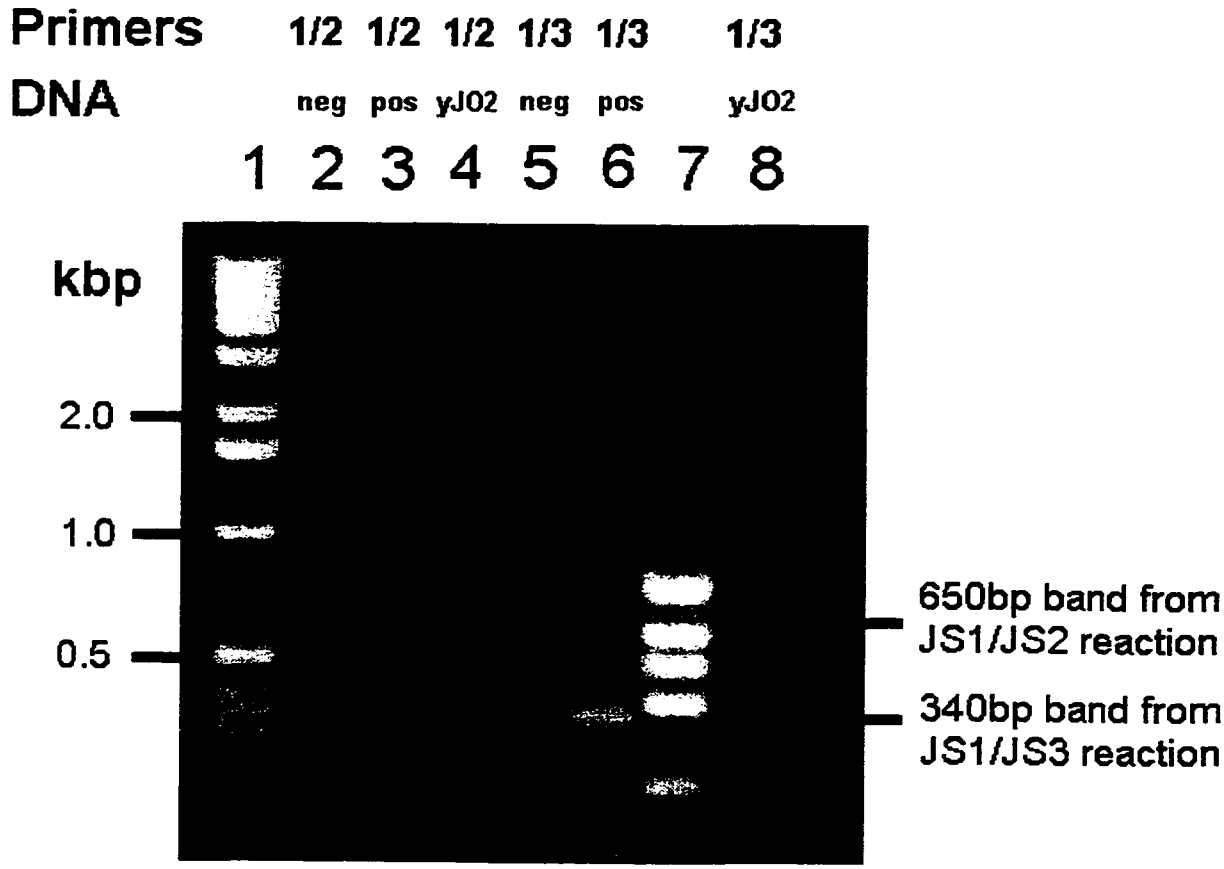


Figure 3.12. PCR Products amplified from y^{JO2} DNA

Gel separation of PCR products. Lanes #2-#4 are reactions with primers JS1 and JS2, lanes 5, 6 and 8 are reactions with primers JS1 and JS3. Lanes 1 and 6 are both ladders. Lane 1 is a 1kb ladder and lane 7 is a modified pUC19 plasmid cut with *Hinf*1. Non-ladder DNA is as follows: Lane 2, negative control (#104 DNA); lane 3, positive control (#104 and #312 DNA); lane 4, y^{JO2}; lane 5, 6, and lane 8 as per lane 2, 3, and 4. The presence of the 650bp band (from JS1/JS2 primers) in lane 4, and the absence of a band in lane 8 indicate that this line is free of the multiply marked chromosome. See Figure 3.9(B) for primer map.

Chapter 4 - Results II

A Test for Crossing over in *T(1;4)w^{m5}*

4.1 Crossing Scheme to Test for Crossing over on *T(1;4)w^{m5}*

4.1.1 Cross Outline

To determine the level of crossing over on the *T(1;4)w^{m5}* translocation, a standard testcross was used. An outline of this cross is shown in Figure 4.1. Male and female flies containing mutations at different loci were crossed to produce females homozygous for the translocation, but heterozygous (in repulsion) for the markers on the translocation. All fathers used to produce these females came from lines in which no *ci^{1}* was present, thus guaranteeing that these females were diploid for all chromosomes. Further proof of this is that no non-disjunction was detected in any the X chromosome crosses, which would have been the case had the heterozygous female been triploid for chromosome 4.

Figure 4.1 is accurate for all crosses with the exception of those involving *w^{A8}*. In those lines, some of the virgins used may have contained the *ci^{1}* chromosome in addition to the genotype shown below. The example shows a hypothetical cross between a *shaven* and an *eyeless* mutant.

4.2 Cross Results

Table 4.1. Crossing over on the X distal region of *T(1;4)w^{m5}*

	Progeny				
	Recombinant		Parental		
Cross	**w^{-}y^{-}**	**w^{m5}y^{+}**	**w^{m5}y^{-}**	**w^{-}y^{+}**	**Total**
w^{JO1};*ci^{D}* ⊗y^{JO1};ci^{D}	0	0	1471	1420	2891
w^{JO1};*ci^{D}* ⊗y^{JO2};ci^{D}	0	0	1387	1361	2748

Figure 4.1. Testing for Crossing Over

Male and female flies containing a different mutation on the same chromosome were crossed to produce females heterozygous for the markers on the translocation, but homozygous for the translocation. All males used to produce these females came from lines in which no ci^1 was present, thus guaranteeing that these females were diploid for all chromosomes.

Testing for Crossing Over

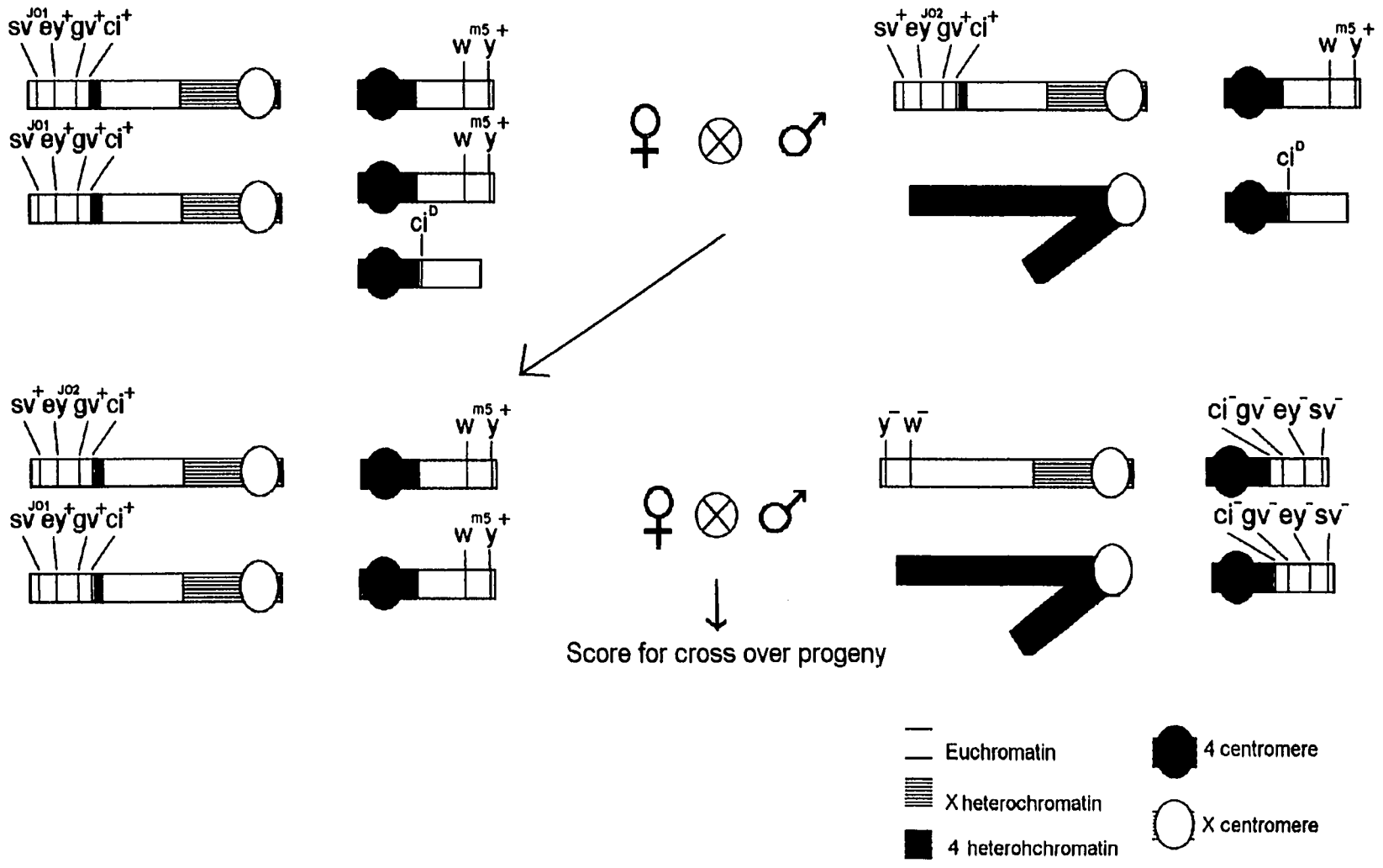


Table 4.2. Crossing over on the 4 distal region of *T(1;4)w^{m5}*

	Progeny				
	Recombinant		Parental		
Cross	$ey^{-}sv^{-}$	$ey^{+}sv^{+}$	$ey^{+}sv^{-}$	$ey^{-}sv^{+}$	Total
$ey^{JO1};ci^{D}$ $\otimes sv^{JO1}$	3	2(3)	1431	1413	2849
$ey^{JO2};ci^{D}$ $\otimes sv^{JO1}$	0	2(4)	2269	2916	5187
$ey^{JO1};ci^{D}$ $\otimes sv^{JO2}$	0	0	0	0	0
$ey^{JO2};ci^{D}$ $\otimes sv^{JO2}$	0	0(0)	769	814	1583

Numbers in brackets indicate putative recombinants that died before their genotype could be determined.

4.3 Confirmation of Crossover Progeny

Putative recombinant progeny were testcrossed to recessive males and females as appropriate. This step was important in dealing with putative wild type recombinants that may have been the result of a less than complete penetrance. Unfortunately, not every putative recombinant progeny survived to produce offspring. Seven such putative recombinants died before they could be properly tested. This is problematic because some of these could be legitimate recombinants and therefore the frequency of recombination on chromosome 4 may in fact be higher than the results indicate, since potential recombinants died before producing any offspring. This loss can be compensated for by multiplying the number of dead potential recombinants by the chance of them being recombinant. This chance is the total number of recombinant progeny found, divided by the total number of putative recombinant progeny tested.

Thus 18.2% of the seven putative recombinants which died before their genotype could be confirmed as likely to have been recombinant, as only 4 of 22 putative wild type recombinants were actually recombinant. Thus the true number of recombinants is increased by 1.27 (18.18% x 7) flies and the non-recombinant category by 5.73 flies.

4.4 Map Distance Calculations

The purpose of this work was to detect the presence or absence of crossing over on this translocation chromosome. Since there were recombinants it was possible to calculate the map distance so as to compare results with previous experiments that measured the level of crossing over between genes on chromosome 4.

Table 4.3. Map Distance Between Markers on *T(1;4)w^{m5}*

Cross	Crossover Progeny	Total Progeny	Map Distance (cM)
$w^{JO1};cl^{D} \otimes y^{JO1};cl^{D}$	0	2891	0
$w^{JO1};cl^{D} \otimes y^{JO2};cl^{D}$	0	2748	0
$ey^{JO1};ci^{D} \otimes sv^{JO1}$	5	2849	0.2
$ey^{JO2};cl^{D} \otimes sv^{JO1}$	2	5187	0.04
$ey^{JO2};cl^{D} \otimes sv^{JO2}$	0	1583	0
y-w total	0	5639	0
ey-sv total	7	9619	0.07
ey-sv total (corrected)	8.27	9624.73	0.09

Although a calculation for map distance between *yellow* and *white* on *T(1;4)w^{m5}* yields 0 *cM*, we can not say that crossing over does not occur between these two genes only that in this experiment no crossovers were detected. Crossing over may be in fact be occurring, but at some very low level. An estimate for the upper limit of the map distance between these genes can be calculated as follows:

$$\text{Map Distance} = 1 - [\, e^{(\ln(0.05) \,/\, \text{Number of Parental Progeny})} \,]$$

This formula determines, for a given number of parental progeny an exact map distance at which there is only a 5% chance to see that number of parental progeny. Thus this formula is a variant of:

$$0.05=(1 - \text{Map Distance})^{(\text{Number of Parental Progeny})}$$
$$\ln(0.05)=\text{Number of Parental Progeny} * \ln(1 - \text{Map Distance})$$
$$\ln(0.05) \,/\, \text{Number of Parental Progeny} = \ln(1 - \text{Map Distance})$$
$$1 - \text{Map Distance} = e^{(\ln(0.05)/\text{Number of Parental Progeny})}$$
$$\text{Map Distance} = 1 - [\, e^{(\ln(0.05) \,/\, \text{Number of Parental Progeny})} \,] \times 100\ cM$$

Table 4.4. Maximum Theoretical Map Distance Between *y* and *w* in *T(1;4)w^{m5}*

Cross	Parental Progeny Scored	Maximum Map Distance in *cM*
$w^{JO1};ci^{D}$ $\otimes y^{JO1};ci^{D}$	2891	0.1036
$w^{JO1};ci^{D}$ $\otimes y^{JO2};ci^{D}$	2748	0.1090
Total	5639	0.05311

4.5 Problem: contamination of ey^{JO1}; ci^{D}

On July 15, 1998 I noticed that the ey^{JO1}; ci^{D} stock either had become contaminated or had picked up modifiers of chromosome 4 genes. Few flies in this stock displayed the ey^{JO1} phenotype, and the majority of both female and male flies had a ci^{+} phenotype. The ci^{D} chromosome originally present in this stock appeared to have been lost or modified by another locus for majority of the stock. This had gone unnoticed because of the long lag time between crossing this stock with sv^{JO1}, and the more recent crosses with the sv^{JO2}. The probable cause of this problem was a mutation at a second site, as outcrossing often revives the eyeless phenotype. Flies of this stock were extremely unhealthy and the recessive lethal ci^{D} locus was under tough selective pressure. This is consistent with the results from an ey^{JO1} to sv^{JO2} cross. Although progeny from this cross did show the ey^{JO1} phenotype (which hereto had been lost in the original stock) approximately one quarter of the flies showing a wild type recombinant phenotype. The ey^{JO1} to sv^{JO2} crosses were subsequently aborted. Thus only 3 of the 4 chromosome 4 crosses can be done for this thesis.

4.5 Summary of Results

Table 4.5. Summary of Results

Genotype	*y-w* crossing over	*ey-sv* crossing over
Wild Type	Yes	No
T(1;4)w^{m5}	No	Yes

4.6. Conclusion

The production of crossover progeny on the translocated chromosome 4 sequences clearly demonstrated that the centromere effect is responsible for the lack of crossing over on chromosome 4. Consistent with this result was the absence of cross over progeny on translocated X chromosome sequences,

indicating that these X chromosome sequences are unable to undergo crossing over when moved adjacent to the chromosome 4 centromere.

Chapter 5

Discussion

5.1 General Implications of Results

5.1.1 Effect of Results on Models Tested

The centromere effect model for the lack of crossing over on chromosome 4 is supported by these results. The observation of crossing over on the translocated distal chromosome 4 sequences strongly suggests that it is the position of chromosome 4 relative to its centromere that is responsible for the lack of crossing over on this chromosome. Although the map distance between *ey* and *sv* was small, the mere fact that crossing over is detectable on this translocation chromosome but not on a normal 4 is enough to dismiss the notion that chromosome 4 lacks some sequence criteria necessary for crossing over.

One possible criticism of this conclusion is that possible "recombinogenic" sequences like $(dC\text{-}dA)_n\text{-}(dG\text{-}dT)_n$ may be able to operate at a distance. Thus the crossing over on the translocated chromosome 4 sequences is simply the result of the close proximity to recombinogenic sequences on the X chromosome, rather than the removal of distal chromosome 4 sequences from the chromosome 4 centromere. However, there are two major problems with this argument. First there is no evidence that such sequences would be able to operate over such large distances, as the *ey* gene is probably at least 500kb from X chromosome sequences. The distribution of $(dC\text{-}dA)_n\text{-}(dG\text{-}dT)_n$ repeats on the X chromosome (with the exception of cytogenetic region 13-17) is very closely clustered (Pardue et al, 1987). Even cytogenetic region 13-17 does not appear to have gaps of the same size or greater than chromosome 4 that lack the $(dC\text{-}dA)_n\text{-}(dG\text{-}dT)_n$ "recombinogenic" sequence. This suggests that if these sequences operate they need to be close to their target sequence.

Furthermore even if such recombinogenic sequences could operate over such a distance, this is not consistent with the results obtained for crossing over between *y* and *w*. If recombinogenic sequences have the ability to bestow crossing over, then they should have allowed crossing over on the translocated distal portion of the X chromosome, since they were included as part of the translocation. This was not the case, and so the centromere effect is the model that best explains the lack of crossing over on chromosome 4 and the results described in this research.

5.1.2 Remaining Functions for Copolymer Repeats

While the results of these experiments indicate that these repeats are not required for crossing over, they do not rule out these sequences from having a role in crossing over. The discrepancy between the physical map and the genetic map such that the ratio of kb to cM changes along the chromosome arm in *Drosophila* reveals a large variation in the frequency of crossing over along the chromosome arm, and not just the centromere effect. Other regional high points

and low points in the frequency of crossing over are present on all the chromosome arms (see the Chapter on Mapping and Exchange in Ashburner, 1989). The wild type functions of the previously discussed meiotic mutants are necessary for creating this regional distribution of crossing over, since their products eliminate this wild type distribution. The $(dC\text{-}dA)_n$-$(dG\text{-}dT)_n$ repeats or some other sequence may be the target for action by the products of these meiotic mutants (Szauter, 1984). If this is the case, the results of this experiment indicate that these products must only act on these sequences to control the frequency of crossing over, not its initiation.

There is however one problem with this idea. If these sequences do regulate the level of crossing over on adjacent sequences, then it is not possible for chromosome 4 to lack these sequences and be regulated by meiotic mutant products (Sandler and Szauter,1978). Since crossing over on chromosome 4 takes place in the genetic background of meiotic mutants, these $(dC\text{-}dA)_n$-$(dG\text{-}dT)_n$ repeats or similar sequences can not be involved with the system of crossing over distribution regulation produced by meiotic mutants.

Thus it is simpler to postulate that $(dC\text{-}dA)_n$-$(dG\text{-}dT)_n$ repeats have nothing to do with of crossing over in *Drosophila*. However, I believe that it is likely that the wild type product of the meiotic mutant genes do need some target sequence in order to control the distribution of crossing over. Without this sequence requirement it is hard to explain the regional high and low points of crossing over in *Drosophila*, since the gradations of meiotic crossing over are not distributed in a simple stochastic fashion. If the centromere effect were simply the result of the distribution of chromosomes in the nucleus and/or the spreading of a protein from centromeres there should be a steady gradation of frequency of crossing over along the chromosome arm. This is not the case in *Drosophila* (Ashburner, 1989). Unfortunately if there is a sequence requirement to the centromere effect, no other likely candidate sequence that has been found.

5.1.3 Implications for Recombination Nodules

Chromosome 4 sequences associated with euchromatin have recombination nodules otherwise found only on sequences associated with centric heterochromatin (Carpenter, 1975). The results here suggest if "euchromatic nodules" are required for crossing over, their formation is dependent on position relative the centromere rather than on the sequence they form next to. If they were dependent on sequence rather than position, then "heterochromatic nodules" would form and no crossing over would have taken place on chromosome 4 sequences of $T(1;4)w^{m5}$. A requirement for euchromatic nodules in crossing over can not be ascertained from the results of this experiment. The types of recombination nodules that are presumed to form on $T(1;4)w^{m5}$ translocated chromosome 4 sequences were not examined.

5.1.4 Implications for Copolymer Repeat Distribution

Given the result of crossing over on distal chromosome 4, how can one explain the absence of $(dC\text{-}dA)_n$-$(dG\text{-}dT)_n$ copolymer repeats on chromosome

4? It is clear now that the absence of these sequences does not mean *ceteris paribus* (all other things being equal) that a chromosome can not undergo crossing over. A more likely explanation to account for their presence in regions of crossing over and absence in regions of no crossing over is given by Lowenhaupt et al (1989). They suggested that if the absence of these sequences is not responsible for the lack of crossing over, then their distribution may be the consequence of the lack of crossing over, rather than the cause of it. Thus these sequences would be only be transferred to regions that undergo crossing over via crossing over. They would therefore not be represented in regions that do not undergo crossing over, which is consistent with the results obtained here.

5.2 The Frequency of Chromosome 4 Crossing Over on *T(1;4)w^{m5}*

5.2.1 Map Distance Comparison with the Data of Sturtevant (1951)

The map distance between the *shaven* and *eyeless* genes on the *T(1;4)w^{m5}* translocation chromosome is approximately 0.1cM. This number is only one-tenth the size of the map distance that Sturtevant (1951) calculated when he used triploid females that were diploid for chromosome 4 to map these two genes. The very different genetic composition of the heterozygous females used in Sturtevant's experiment versus the translocation experiment described here makes it unclear what this difference means. Given that Sturtevant mapped these genes in triploids, whereas the distance obtained here was found in diploid translocation homozygotes it seems to me that a measure of a "centromere effect free" distance between these two genes is more useful, although both are under abnormal conditions. This is because meiotic crossing over is measured is being measured in a female with a diploid karyotype, not in a triploid which is disomic for chromosome 4.

5.2.2 Can both Sequence and Position be Responsible for Lack of Crossing Over on Chromosome 4?

The small map distance between *ey* and *sv* in this translocation could be interpreted such that the centromere effect may not be totally responsible for the lack of crossing over on chromosome 4. That is to say that the complete absence of crossing over is a combination of the centromere effect and chromosome 4 sequences act to repress recombination on chromosome 4. While this can not be ruled out entirely, it is not likely because a distance of 0.1 map units is not atypical for two genes located at as far apart as *ey* and *sv* near the telomere.

The *eyeless* gene has been cloned and its cytological location determined to be 102D by in-situ hybridization (Quiring et al, 1994). The *shaven* locus has also been cloned (Fu et al, 1998) and its cytological location is between 102E2 and 102F10 (Lindsey and Zimm, 1992). The map distance calculated on the *T(1;4)w^{m5}* translocation were very low, it could be argued that the centromeric heterochromatin regions translocated with chromosome 4 were still exerting some suppression effect on crossing over suppression effect. Unfortunately it is

not easy to tell whether this is the case. A comparison of the cytological distance between *eyeless* and *shaven* with similar distances near the telomeres of other chromosomes reveals a wide variation in genetic map distance (see Table 5.1). This varies from 0 map units on the left tip of chromosome 3 (3L) to 5.9 map units for the right tip of the same chromosome. With such wide variation in map distances (which overlap with the translocation value), it is impossible to determine whether the map distance calculated is would be what one would expect for 2 genes on a "normal" chromosome tip in *D. melanogaster*. However given that the distance calculated does fall within this wide range, there is no need to suppose that chromosome 4 sequence is necessarily acting to prevent crossing over.

Table 5.1. Comparative Estimates of the Genetic Distance between *ey* and *sv* for other Chromosome Tips

Tip	Range	Genetic Distance
4	*ey-sv*	0
T(1;4)w^{m5}	*ey-sv*	0.1
3R	*M(3)100CF-l(3)SG71*	5.9
3L	*Klp61F-Lspl-g*	0
2R	*E(br)-tipdD*	2.5
2L	*M(2)21C-l(2)ey*	0.65
X	*cc-tw*	0

*data compiled from Lindsey and Zimm (1992)

5.3 Chromosome Evolution

5.3.1 History of Chromosome 4

Because chromosome 4 sequences are capable of undergoing crossing over when moved some distance from the centromere, this supports a model in which chromosome 4 did not lose its ability to undergo crossing over until it became the dot chromosome. If chromosome 4 first lost the ability to undergo crossing over, and then was subsequently moved adjacent to the centromere one would expect no recombination on *T(1;4)w^{m5}*. Thus the results here suggest that in order for a sequence to become permanently non-recombining, translocation to a centromere can only be a possible first step.

Although chromosome 4 has repeated sequences in abundance (Miklos, 1988) relative to other chromosomes, it has not yet been shown to contain the $(dC\text{-}dA)_n\text{-}(dG\text{-}dT)_n$ copolymer repeat found on chromosomes that do undergo crossing over. This inability of chromosome 4 sequence type to fit neatly into a euchromatin/heterochromatin dichotomy suggests that this is a chromosome in transition (or has reached an evolutionarily stable state) between those two categories and their genetically distinct regulatory apparatus (Carpenter and Baker, 1982) for crossing over. The type of sequences chromosome 4 contains may provide useful information of the evolution of crossing over. Given that chromosome 6 of *D. virilis* (the chromosome 4 homologue) has these repeated

sequences AND has crossing over, it suggests that the presence of this sequence comes after the chromosome gains the ability to undergo crossing over. Alternatively, these sequences may have been lost from *D. melanogaster* chromosome 4 after it lost the ability to cross over.

5.3.2. Model for the Development of NON-recombining Sequences

How permanently non-recombining centric heterochromatic sequences are produced is still not clear. However, one possible first step in the production of these sequences is their translocation to a chromosome adjacent to a non-recombining area of the genome, such as a centromere or a non-exchanging sex chromosome. This would be followed by a subsequent gradual accumulation of repetitive or deleterious sequences (like mobile elements) on the now non-recombining chromosome through a mechanism like Muller's Ratchet (Muller, 1964). In chromosome 4 the mobile element would be Dr.D., in *D. miranda* this would be TRAM and other new translocations would likely have their own mobile elements, depending on which sort are active in the genome at the time of the translocation. At some point, the accumulation becomes so severe that these translocated sequences lose their ability to be regulated by meiotic mutants and cross over as they are converted into centric heterochromatin. It is this final step, which is only a theoretical possibility, which lacks evidence.

So this model implies direct production of highly repetitive sequences from any sequence source, not a gradual transition from middle repetitive sequences and diminishing crossing over to highly repetitive sequence unable to undergo crossing over. Smith (1976) has argued that highly repeated sequences are the natural product of any sequence that is no longer subject to selection and are produced by unequal crossing over which supports the model put forward here.

5.4 Future Research and Prospects

Although the results presented here strongly suggest that the centromere effect is responsible for the lack of crossing over on chromosome 4, and that the copolymer repeat $(dC\text{-}dA)_n\text{-}(dG\text{-}dT)_n$ is not a requirement for crossing over, very little is yet known about the regulation of crossing over. The exact mechanism by which the "centromere effect" functions is unclear. The most promising approach to determine the mechanism at work here is characterize the meiotic mutants that regulate crossing over on euchromatic sequences in Drosophila. One such meiotic mutant gene has already been cloned, *mei-218* (McKim, Dahmus and Hawley, 1996). The predicted protein sequence had no similarity to any other proteins in the database at that time. Studying the binding of proteins from the wild type alleles of *mei-218* and other meiotic mutant genes should tell us which sequences (if any) these proteins bind to, and hopefully lead to an understanding of how the centromere effect works.

Furthermore, the recent identification in both *C. elegans* and *S. cerevisiae* of meiotic mutants and genes encoding structural components of the synaptonemal complex should facilitate the discovery of *Drosophila* homologues. A molecular and genetic analysis of these homologues will provide a better understanding of cross over control in this organism whose centromeres

probably closely resemble our own (Albertson and Thomson, 1982, Fitzgerald-Hayes et al, 1982).

Chapter 6.

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