AN INVESTIGATION INTO A MEIOTIC CHECKPOINT THAT MONITORS SEX CHROMOSOME PAIRING

Thesis submitted for the Degree of Doctor of Philosophy
Tristan Argéo Rodríguez
National Institute of Medical Research
University College London
University of London
1997
This thesis is dedicated to my parents, Caroline Farningham and Argéo Rodríguez de León.
ACKNOWLEDGEMENTS

First of all I would like to thank Paul Burgoyne for all his help and guidance during these three and a half years. I would also like to express my gratitude to past and present members of the lab for all their patience and help, specially Shanthi, Àine, Vladimir and Teresa, and to Robin and his group for all that advice and group meetings. Thanks of course to Muz and all the coffee group for keeping me sane.

I am indebted to Dr. Rosa Beddington, without whose computer this would have taken much longer, and to Dr. T. Evans, Mr. Andy Barlow and Professor M. Hultén for their collaboration with some of these projects.

Finally, I would specially like to thank my parents and Sara, not only for their patient correcting of my thesis, but also for all their support during all this time.

This work was supported by an MRC studentship.
ABSTRACT

There is extensive evidence for the existence of a meiotic "quality control" that acts to eliminate those germ cells which fail to achieve full sex chromosome synapsis at the pachytene stage of the first meiotic prophase. A study was undertaken of the role this meiotic checkpoint in a number of cases of sex chromosomally induced spermatogenic arrest in mice. XYY mice are sterile, and sex chromosome pairing anomalies and the double Y gene dosage, have both been implicated as the cause of sterility. Meiosis in mice with three sex chromosomes but only one dose of Y specific DNA (XYY\textsuperscript{x}) was compared to that of XYY mice. It was determined that failure of sex chromosome synapsis is the major factor in the sterility of XYY mice, but double Y gene dosage couldn't be excluded from playing a role. Mice with four sex chromosomes were also produced (XYYY\textsuperscript{x} and XYY\textsuperscript{x}XY\textsuperscript{x}) and it was determined that the presence of extra sex chromosomal material (whether or not it increased Y-specific gene dosage) interferes with spermatogenesis, possibly by causing problems with the inactivation of the sex chromosomes at pachytene. Two other examples of mice with partial spermatogenic impairment, XYSx\textsuperscript{a} and XY\textsuperscript{*}, were also studied and found to have a reduction in sperm counts proportional to the efficiency of sex chromosome pairing. Finally an inherited genetic effect was characterised that could partially overcome the requirement for synapsis of the meiotic "quality control". This effect allowed the usually sterile XYO mice to become fertile. Two factors, one major and another minor were found to be responsible for this inherited genetic effect.
CONTENTS

ABSTRACT ................................................................................................................... 4
PREFACE ....................................................................................................................... 14

CHAPTER 1. GENERAL INTRODUCTION
1.1 SPERMATOGENESIS ............................................................................................ 16
1.2 MEIOSIS ................................................................................................................... 18
   1.2.1 The synaptonemal complex .................................................................... 18
   1.2.2 Important events during the first meiotic prophase. ......................... 23
       1.2.2.1 Homology searching, presynaptic alignment and synapsis ............. 23
       1.2.2.2 Recombination .......................................................................... 24
   1.2.3 After pachytene ........................................................................................ 27
1.3 REGULATION OF MEIOSIS .............................................................................. 28
1.4 THE MOUSE Y CHROMOSOME ......................................................................... 30
   1.4.1 Y Chromosome rearrangements and deletions ................................... 33
1.5 UNPAIRED SEX CHROMOSOMES AND GAMETOGENIC FAILURE ...... 38
1.6 OUTLINE OF PROJECTS ...................................................................................... 44

CHAPTER 2. SYNAPSIS, RECOMBINATION AND FERTILITY OF XY* MALES
2.1 INTRODUCTION ................................................................................................... 47
2.2 MATERIALS AND METHODS ............................................................................ 51
   2.2.1 Mice ........................................................................................................... 51
   2.2.2 Karyotyping ............................................................................................. 51
   2.2.3 Sperm Counts ........................................................................................... 51
   2.2.4 Synaptonemal Complex Analysis. ......................................................... 51
   2.2.5 Air-dried preparations ............................................................................. 51
CHAPTER 2. A COMPARISON OF THE FERTILITY AND SYNAPSIS OF THE SEX CHROMOSOMES OF XY*, XYLT, XYSxr and XYRIII Males

2.2 Antibodies and Immunodetection .................................................................................................................. 52
2.3 RESULTS ......................................................................................................................................................... 55

2.3.1 Testis weights and sperm counts of XY*, XYLT, XYSxr and XYRIII males .............................................. 55
2.3.2 Pachytene in XYSxr and XYRIII males ...................................................................................................... 55
2.3.3 Pachytene in XY* and XYLT males ............................................................................................................... 55
2.3.4 Recombination in XY* males ....................................................................................................................... 56
2.3.5 Evidence for the conversion into the staggered configuration of the X-Y* parasynapsed bivalents .......... 57
2.3.6 Diakinesis and Metaphase I in XY*, XYLT, XYSxr and XYRIII males ......................................................... 57

2.4 DISCUSSION .................................................................................................................................................... 79

CHAPTER 3. A COMPARISON OF THE FERTILITY AND SYNAPSIS OF THE SEX CHROMOSOMES OF XYY*X AND XYY MICE

3.1 INTRODUCTION .............................................................................................................................................. 85
3.2 MATERIALS AND METHODS ........................................................................................................................ 86

3.2.1 Production of XYY*, XYY- and XYy- mice ................................................................................................. 86
3.2.2 Karyotyping ................................................................................................................................................... 86
3.2.3 Sperm Counts ............................................................................................................................................... 87
3.2.4 Synaptonemal Complex Analysis ................................................................................................................ 87
3.2.5 Air-dried preparations ................................................................................................................................. 87

3.3 RESULTS ......................................................................................................................................................... 89

3.3.1 Fertility, testis weights and sperm counts of XYY*X and XYY- males .......................................................... 89
3.3.2 Pachytene in XYY*X, XYY- and XY males ................................................................................................. 89
3.3.3 Diakinesis/MI in XYY*X, XYY- and XY males ............................................................................................... 91
3.3.4 Analysis of older XYY*X and XY- males ..................................................................................................... 91

3.4 DISCUSSION .................................................................................................................................................... 112
CHAPTER 4. ANALYSIS OF THE FERTILITY AND SYNAPSIS OF
THE SEX CHROMOSOMES OF X^{Y*Y*}, XYY-Y^*X AND XYY*XY*X MICE

4.1 INTRODUCTION...................................................................................................118

4.2 MATERIALS AND METHODS.............................................................................119
  4.2.1 Production of X^{Y*Y*} males .................................................................119
  4.2.2 Production of XYY-Y^*X, XYY*XY*X and XYy-Y^*X males ...............119
  4.2.3 Karyotyping .............................................................................................119
  4.2.4 Sperm Counts ..........................................................................................120
  4.2.5 Synaptonemal Complex Analysis..........................................................120

4.3 RESULTS..................................................................................................................122
  4.3.1 Fertility, testis weights, sperm counts and pairing of the sex
      chromosomes at pachytene in X^{Y*Y*} males ........................................122
  4.3.2 Fertility, testis weights and sperm counts of XYY-Y^*X and
      XYY*XY*X males ......................................................................................122
  4.3.3 Sex chromosome synapsis at pachytene in XYY-Y^*X, and
      XYY*XY*X males ......................................................................................123
  4.3.4 Fertility, testis weights, sperm counts and sex chromosome
      pairing at pachytene in XYy-Y^*X males ...................................................124

4.4 DISCUSSION ..........................................................................................................140

CHAPTER 5. IDENTIFICATION OF TWO GENETIC FACTORS THAT
BY-PASS THE ACTION OF THE MEIOTIC CHECKPOINT THAT
MONITORS SYNAPSIS/RECOMBINATION IN X^{Y*O} MICE.

5.1 INTRODUCTION...................................................................................................146

5.2 MATERIALS AND METHODS.............................................................................147
  5.2.1 Production of X^{Y*O} males .................................................................147
  5.2.4 Sperm Counts ..........................................................................................147
  5.2.5 Synaptonemal Complex Analysis..........................................................147
MANUSCRIPT: The "meiotic quality control" which acts to remove spermatocytes with incompletely synapsed chromosomes utilises a p53-independent apoptotic pathway.
# LIST OF FIGURES

## CHAPTER 1.
- Figure 1.1. Diagrammatic representation of the mouse Y chromosome ..........31
- Figure 1.2. Diagramatic representations of the Y* rearrangement and of the products of it's recombination with the X chromosome .................36
- Figure 1.3. Diagrammatic representation of the sex chromosomes of XSxr³O and XSxr³Y*× mice .................................................................40

## CHAPTER 2.
- Figure 2.1. Orientations in which the X and Y* chromosomes can synapse ....49
- Figure 2.2. Examples of pachytene cells at early, mid and late pachytene ......53
- Figure 2.3. Examples of pachytene cells from XYLT and XYRIII males showing autosome asynapsis or in the process of being eliminated ..........67
- Figure 2.4. Examples of the positions of the centromeres in pachytene cells of XY males ........................................................................69
- Figure 2.5. Examples of Mlh1 foci on pachytene cells of XY* and XY males, stained with Mlh1 and A1 antibodies ............................................71
- Figure 2.6. Examples of sex chromosome configurations in XYSxr³ and XYRIII males ..............................................................................73
- Figure 2.7. Examples of sex chromosome configurations in XY* and XYLT males ...............................................................................75

## CHAPTER 3
- Figure 3.1. Crosses set up to generate XYY*× and XYY− males ..................88
- Figure 3.2. Plot of the testis weights vs the sperm counts of the XY males from the cross XXY− x XYY*× ..............................................................104
- Figure 3.3. Examples of sex chromosome configurations in pachytene spreads from XYY*× males ................................................................105
Figure 3.4. Examples of sex chromosome configurations in pachytene cells of XYY- and XYy- males.......................................................... 107

Figure 3.5. Plot of the relationship between the proportion of cells with all the sex chromosomes synapsed and the sperm counts of XYY**, XYY- and XY males...................................................................................... 110

Figure 3.6. Plot of the relationship between the proportion of cells with all the sex chromosomes synapsed and the sperm counts of XYY**, XYY- and XY males ...................................................................................... 111

Figure 3.7. Plot of the mean ratio of XYY/euploid germ cells present at different stages in 5 XY-y/XY-y/Xy germ line mosaic males......................... 116

CHAPTER 4.

Figure 4.1. Cross set up to generate XY*Y- males.............................................. 121

Figure 4.2. Examples of sex chromosome configurations in an XY*Y- male ...... 131

Figure 4.3. Plot of the proportion of cells with all the sex chromosomes synapsed vs. the sperm counts of XYY-Y**X and XYY*XY*X males compared to the relationship between synapsis and sperm counts of XYY**X, XYY- and XY males.......................................................... 133

Figure 4.4. Examples of sex chromosome associations in pachytene spreads from XYY-Y**X and XYy-Y**X males ........................................ 134

Figure 4.5. Sex chromosome configurations of an XYY*XY*X male ............ 137

CHAPTER 5.

Figure 5.1 Crosses set up to generate XTaY*O males........................................... 148

Figure 5.2. Synaptonemal Complex of the XTaY* chromosome from the original fertile XTaY*O male showing no evidence of self pairing................ 154

Figure 5.3. Distributions of the sperm counts of the XTaY*O males analysed.... 157
Figure 5.4. Distributions of the sperm counts of the $X^{TaY^*O}$ offspring of $In(X)/X$ females and $X^{TaY^*O}$ males with either (a) 100,000-800,000 sperm/caput, or (b) over 800,000 sperm/caput. .......................... 160

Figure 5.5. The $X^{TaY^*O}$ males were divided into pedigrees according to the identity of the $X^{TaY^*}$ studs used to produce them. Presented here are the sperm counts of the $X^{TaY^*O}$ males from Pedigree 7 ..................... 162

Figure 5.6. Sperm counts of the $X^{TaY^*O}$ males from Pedigree 5 .......................... 164

LIST OF TABLES

CHAPTER 2.

Table 2.1. Mean testis weights and sperm counts of $XY^*$, $XYLT$, $XYSxr^a$ and $XYRIII$ males......................................................................................... 59

Table 2.2. Levels of sex chromosome asynapsis in pachytene cells of $XYSxr^a$ and $XYRIII$ males....................................................................................... 61

Table 2.3. Levels of sex chromosome asynapsis in pachytene cells of $XY^*$ and $XYLT$ males........................................................................................... 62

Table 2.4. Sex chromosomes configurations in early, mid and late pachytene cells of $XY^*$ and $XYLT$ males ................................................................. 63

Table 2.5. Sex chromosome configurations in pachytene cells of $XYSxr^a$ and $XYRIII$ males....................................................................................... 64

Table 2.6. Sex chromosome configurations at Diakinesis and MI, and MI/Diakinesis ratios in $XY^*$ and $XYLT$ males ....................................................... 65
Table 2.7. Sex chromosome configurations at Diakinesis and MI, and MI/Diakinesis ratios in XYSxr^a and XYRIII males ........................................... 66

Table 2.8. Observed frequency of each of the configurations observed in XY* males at pachytene, and predicted fate of these configurations by each of the models for the meiosis of XY* males ............................................................................................................ 83

CHAPTER 3.

Table 3.1. Record of the relative numbers of males produced of each expected genotype from the cross XXY^- x XYY*^ ................................. 93

Table 3.2. Average testes weights and sperm counts for males of each genotype produced by the cross XYY**x x XXY^- ........................................... 94

Table 3.3. Testis weights and sperm counts of XYY**x and XYY^- males used to analyse sex chromosome synapsis during the first meiotic prophase. ................................................................. 95

Table 3.4. Testis weights and sperm counts of the XY males selected for SC analysis ........................................................................................................... 96

Table 3.5. Synapsis of the sex chromosomes of XYY**x males during pachytene and diakinesis/MI ........................................................................ 97

Table 3.6. Synapsis of the sex chromosomes of XYY^- males during pachytene and diakinesis/MI ................................................................. 98

Table 3.7. X-Y Pairing during the first meiotic prophase of the low sperm count XY males .............................................................................. 99

Table 3.8. Record of the relative numbers of mice produced of each possible genotype from the cross XXy^- x XYY**x ......................................... 100
Table 3.9. Average testis weights for males of each genotype produced by the cross XYY* x XXy- ................................................................. 100

Table 3.10. Testis weights and sperm counts of XYY* x XXy- males tested for fertility and used for MI and SC analysis .................. 101

Table 3.11. Synapsis at pachytene of the sex chromosomes in the cells analysed from XYY* x XXy- mice.................................................. 102

Table 3.12. Diakinesis/MI synapsis configurations for the three sex chromosomes of the XYY* x XXy- mice analysed ................. 103

CHAPTER 4.

Table 4.1. Testis weights and sperm counts of X^Y*Y- males................................................. 125

Table 4.2. Pairing of the sex chromosomes at pachytene in X^Y*Y- males.............. 125

Table 4.3. Testis weights and sperm counts of XYY-Y* and XYY*XY*X males analysed for sex chromosome synapsis at pachytene.......... 126

Table 4.4. Synapsis of the sex chromosomes at pachytene in XYY-Y*X males................................................................. 127

Table 4.5. Synapsis of the sex chromosomes at pachytene in XYY*XY*X males.................................................................................. 128

Table 4.6. Testis weights and sperm counts of XYy-Y*X males ....................... 129

Table 4.7. Configurations at pachytene of the sex chromosomes in the cells analysed by synaptonemal complex preparation of XYy-Y*X animals ................................................................ 130

CHAPTER 5.

Table 5.1. Numbers of fertile and sterile mice produced from each of the crosses set up to test for a heritable genetic effect being the cause for the fertility of the XTaY*O males ............................................. 156
PREFACE

Three aspects of this work were carried out with other members of the lab or in collaboration with members of other labs:

1. In Chapter 2, the immunofluorescent work was carried out in Dr. Hultein's laboratory (Regional Genetics Laboratory, Birmingham Heartlands Hospital) with the help of Andy Barlow.

2. Dr. Ted Evans (MRC Radiobiology Unit, Hartwell) carried out the scoring of diakinesis/metaphase I ratios and the levels of univalency at these stages for those males analysed in Chapter 2.

3. The identification of the genetic factors that by-pass the action of the meiotic checkpoint that monitors synapsis/recombination in \(X^YO\) mice was done with Dr. Paul Burgoyne who carried out the breeding of the mice used in this study.
CHAPTER 1.
GENERAL INTRODUCTION
Mammalian testes form the focal point of the male reproductive system. Their development and differentiation occur during foetal life. During the postnatal period, the testes mature to perform two functions in the adult, one hormonal (e.g. production of testosterone and other sex steroids) and the other the production of spermatozoa (spermatogenesis). These processes are localised in two distinct morphological compartments, the vascularized Leydig cells of the interstitium and the avascular seminiferous tubules (Guraya, 1980).

The seminiferous tubules generally constitute 75-90% of the total testicular mass in the adult because of the intense and continuous cell proliferation occurring during spermatogenesis. Their lining (seminiferous epithelium) consists of a proliferating, interdependent, population of germ cells and a non proliferating population of supporting somatic cells (the Sertoli cells). The Sertoli cells comprise 25% of the normal seminiferous epithelium (Cavicchia and Dym, 1977). The seminiferous epithelium has a limiting membrane, the basal lamina, which consist of flat, myoid cells, fibroblasts and collagen fibres (Guraya, 1980; Guraya, 1987).

1.1 SPERMATOGENESIS

Spermatogenesis is a general term used to describe a whole collection of processes involved in the development of a haploid spermatozoon from a diploid spermatogonial stem cell. In adult mammals, the generation of mature sperm cells is a continuous process and takes several weeks. The process proceeds in clearly defined stages, these can be broadly grouped into three main phases: spermatogoniogenesis (production and differentiation of the spermatogonia -Hilscher, 1981), meiosis and spermiogenesis. These three phases are each of approximately equal duration, with the whole process requiring 35 days in mice (Oakberg, 1956).
Spermatogonial stem cells arise from the primordial germ cells. The latter cell type can first be distinguished at 8 days post coitum (d.p.c.) at the base of the allantois. The primordial germ cells migrate through the hindgut and into the genital ridges, populating them by the time the first signs of sexual differentiation are evident - 11-12 d.p.c. (Chiquoine, 1954; Tam and Snow, 1981; Wylie and Heasman, 1993). After a period of substantial cell growth and proliferation, taking about 3 days (Beaumont and Mandl, 1963), the primordial germ cells in the testis enter mitotic arrest in the G1 phase of the cell cycle, and do not divide again until shortly after birth (Franchi and Mandl, 1964; Baille, 1964; Bellvé et al., 1977a; Bellvé et al., 1977b; Hilscher, 1981). By this time they have differentiated into type A spermatogonia and can undergo a species specific number of mitotic divisions that mark the beginning of spermatogenesis. These divisions serve both to replenish the stem cell population and to generate the type B spermatogonia. The type B spermatogonia, in their turn, undergo a final mitotic division to produce the preleptotene spermatocytes (Clermont and Perey, 1957; Bellvé et al., 1977a; Bellvé et al., 1977b).

It is during the spermatocyte stage that meiosis occurs. Meiosis comprises a round of DNA replication, occurring at the preleptotene stage, followed by two reduction divisions which produce four haploid spermatids from each primary spermatocyte. The remainder of male germ cell development is called spermiogenesis and is the period during which the spermatids undergo a complex morphogenesis to produce terminally differentiated spermatozoa (Oakberg, 1956; Bellvé, 1979; Guraya, 1987).

The pre-meiotic stages of development occur in the basal compartment of the seminiferous tubules. With the onset of the meiotic prophase, the spermatocytes migrate to the adluminal compartment where subsequent differentiation occurs.
During this period they are in intimate association with Sertoli cells, a relationship that is essential for spermatogenic development (Guraya, 1987). Another factor that determines the spatial distribution of the germ cells in the seminiferous epithelium is the fact that the initiation of spermatogenesis occurs in segments round the circumference of the tubule, so that along the length of an entire tubule there are particular combinations of cell types at different stages of the spermatogenic pathway - this is the spermatogenic wave (Oakberg, 1956; Bellvé, 1979).

1.2 MEIOSIS

As mentioned above, meiosis comprises a single round of DNA replication followed by two successive nuclear divisions, namely meiosis I and II. After the pre-meiotic S phase, during the prophase of meiosis I, homologous chromosomes condense and pair, the non-sister chromatids of homologous chromosomes recombine with each other and then disjoin. As a result, at meiosis I, nuclei end up with novel assortments of genes. Subsequently, at meiosis II, the chromatids of each chromosome segregate, as in a mitotic division. The chromatin rearrangements of meiotic prophase are accompanied by the assembly and disassembly of synaptonemal complexes (SCs).

1.2.1 The synaptonemal complex

SCs were first described 40 years ago (Moses, 1956; Fawcett, 1956) and have since been found in almost all sexually reproducing eukaryotes analysed so far (Von Wettstein et al., 1984; Loidl, 1994). They are proteinaceous, zipper like structures which are assembled between homologous chromosomes during the prophase of the first meiotic division. Their assembly and disassembly are intimately related to the chromatin rearrangements of meiotic prophase, namely the condensation, pairing, recombination and disjunction of homologous chromosomes (Heyting, 1996).
The synaptonemal complex is of a tripartite nature. It's basic structure consists of two lateral elements (LE) that lie parallel to each other, about 100nm apart, and a central element (CE) that lies between them. The LEs are made of a proteinaceous axis (axial element or axial core) that develops early in the pathway of SC assembly, between the two sister chromatids. The axial cores of homologous chromosomes are connected along their length by numerous transverse filaments (TF). The central element (CE) lies on the TFs. Although in most species this structure of the SC has been conserved, there is much variation in the detailed structure (Von Wettstein, 1984; Schmekel et al., 1993; Heyting, 1996). At least two species, namely Schizosaccharomyces pombe (Kohli and Baehler, 1994) and Aspergillus nidulans (Egel-Mitani et al., 1982), display otherwise normal meiosis but do not have a detectable SC. Meiotic prophase cells of S. pombe contain linear elements, which possibly correspond to the LEs of the SC (Kohli and Baehler, 1994; Baehler et al., 1993).

The chromatin loops are anchored to the axial elements, and most of the DNA is located outside the SC. In situ hybridization with unique sequence probes suggests that homologous DNA sequences interact with each other outside the confines of the SC (Moens, 1994). The average size of the loops attached to the SCs is species specific (Izaurralde et al., 1988). Attempts have been made to study the SC-associated DNA sequences that should serve as attachment sites for these chromatin loops. This study was carried out on DNase treated, isolated SCs from rat or golden hamster spermatocytes (Pearlman et al., 1992; Karpova et al., 1995). The residual DNA in such preparations was found to be enriched in long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs) and (GT/CA)n repeats. However, in situ hybridization of a probe containing part of a LINE sequence to surface spread spermatocytes did not result in preferential labelling of SCs (Moens and Pearlman, 1990).
Usually the SC forms between chromosomes and chromosome regions that are preselected by presynaptic alignment. In these cases the SC forms strictly between homologous regions. But if the chromosomes do not come in two homologous sets (e.g. haploids -Loidl et al., 1991) or if the mechanisms for recognising or moving homologous chromosomes fail (e.g. Jenkins and Okumus, 1992) extensive SC may nevertheless be formed (Loidl, 1990). In these cases it is not clear whether the initiation of the SC is based on at least some short homology stretch or on homology of a low degree, or if it is totally independent of homology. The formation of so-called SC polycomplexes (stacks of SC material without the participation of chromosomes- Goldstein, 1987) suggests the capability of the SC to organise autonomously and hence in the absence of homology (Loidl, 1994; Moens, 1994). The anomalous SCs of nonhomologously synapsed chromosomes in inversions and duplications is another example of the neglect of homology by the SC (Moses and Poorman, 1981). But it is possible that dispersed repetitive DNA sequences that are very frequent in higher eukaryotes could serve as secondary sources for homology (Shaw and Wilkinson, 1978) for many types of nonhomologous pairing and even allow for crossing-over (Neijzing, 1982; Metzenberg et al., 1991).

A number of structural components of the SC have been identified in recent years in preparations of isolated SCs from rodents (Heyting et al., 1989) and Lilium (Anderson et al., 1994) or by the analysis of meiotic mutants in yeast (Roeder, 1995) and visualised by immunofluorescence or immunogold labelling. These include components of the central region such as the rodent SCP1 (SYN1) (Dobson et al., 1994; Meuwissen et al., 1992), an unnamed rat 48kDa protein (Smith and Benavente, 1992a and 1992b), and the yeast ZIP1 protein (Sym et al., 1993). Components of the LEs such as the rodent SCP2 (Offenberg, 1993) and SCP3 (COR1) (Lammers et al., 1994; Dobson et al., 1994), the yeast HOP1
(Thompson and Roeder, 1989) and RED1 (Hollingsworth et al., 1990), and an unnamed 55-70kDa Lilium protein (Anderson et al., 1994), have also been identified.

A number of meiosis specific proteins have been found to be associated to the SC such as DMC1 (yeast) (Bishop et al., 1992) and LIM1 (lily) (Terasawa et al., 1995), the homologues of the E. coli RECA protein, that are found in foci associated with ZIP1 in meiotic prophase nuclei. Similarly a number of non-meiosis specific proteins have been found associated with the SCs. Examples include RAD50 in yeast (Alani et al., 1990), RAD51 in yeast (Shinohara et al., 1992) and mouse (Haaf et al., 1995; Ashley et al., 1995), RAP1 in yeast (Klein et al., 1992) and topoisomerase II (Klein et al., 1992; Moens and Earnshaw 1989).

I will not enter into a description of all these proteins but will instead focus on one that has revealed fundamental insights into the function of the SC. Without doubt one of these molecules is the yeast ZIP1 protein (Sym et al., 1993). ZIP1 is localised continuously along the lengths of the mature SC, but is not present in unsynapsed axial elements, indicating that ZIP1 is a component of the central region. In zip1 null mutants, axial elements are full length and homologously paired, but the distance between them is greater and more variable than the distance between lateral elements in mature SC. It has been proposed that ZIP1 could be an element of the TFs (Roeder, 1995). Historically the SC had been viewed as a structure responsible for bringing homologous chromosomes together into apposition for correct genetic exchange, but analysis of zip1 mutants has revealed that full length aligned axial cores can be assembled in the absence of the tripartite SC structure. Also in some yeast strains, zip1 mutants produce viable spores with a near normal cross-over frequency. However cross-over interference is abolished in these mutants (Sym and Roeder, 1994). Cross-over interference can be considered as a manifestation of a regulatory mechanism
that also ensures at least one cross-over per bivalent, even if the average number of cross-over in each bivalent is close to one. This information, combined with the fact that *S. pombe* and *Aspergillus nidulans*, that fail to make SCs, do not exhibit crossover interference either (Egel-Mitani *et al.*, 1982; Olson *et al.*, 1978), indicates that the tripartite SC structure is not required for meiotic recombination, but instead has a regulatory role on the frequency and distribution of recombination.

The rodent protein SCPl (Dobson *et al.*, 1994; Meuwissen *et al.*, 1992) has a similar secondary structure to ZIP1: both proteins possess a long coiled-coil domain and randomly coiled amino and carboxy termini. The amino and carboxyl termini of ZIP1 and the carboxyl terminus of SCPl are enriched in Ser/Thr-Pro motifs, which are common in DNA binding proteins (Suzuki, 1989). Despite their similar structure and position within SCs, SCPl and ZIP1 do not show amino acid sequence homology apart from that expected for two coiled-coil proteins.

Two further yeast proteins have added to the concept that the SC has a regulatory role in recombination. In yeast *red1* mutants (which fail to make axial elements), recombination occurs at about 25% of the wild type (Nicolas *et al.*, 1989). These crossovers do not ensure the proper disjunction of homologues and it has been proposed that RED1 (that is associated with the SC) is important for the incorporation of cross-overs into stable chiasmata (Roeder, 1995). HOP1 gene product is also associated with the SC, and mutants for this gene have a defect in interchromosomal but not intrachromosomal recombination. The recombination (10% of the wild type recombination that is initiated by double strand breaks) occurs preferentially between sister chromatids rather than between homologues (Schwach and Kleckner, 1994), indicating that the HOP1 gene product plays a role in directing recombination towards the homologue rather than the sister chromatid. All this led to the conclusion that an important function of the SC is
to steer recombination in the right direction, and to link the recombination events to the mechanism of chromosome segregation (Heyting, 1996).

1.2.2 Important events during the first meiotic prophase.

1.2.2.1 Homology searching, presynaptic alignment and synapsis

Chromosome synapsis and the assembly of the SC is preceded by a homology search that results in the side by side alignment of homologues at a distance that exceeds the width of the SC (>300nm) (Von Wettstein et al., 1984). In yeast, homologous chromosomes are already paired to some extent in pre-meiotic cells (Weiner and Kleckner, 1994; Loidl et al., 1994); in mammals on the other hand, this doesn't seem to be the case (O’Keeffe et al., 1996; Schertan et al., 1996). This pre-meiotic pairing in yeast is disrupted during S phase (Weiner and Kleckner, 1994), so that at the start of the meiotic prophase homologues are not paired. It is the end of the pre-meiotic S phase that marks the initiation of chromosome pairing (Roeder, 1995; Schertan et al., 1996).

In mouse and man (O’Keeffe et al., 1996; Schertan et al., 1996) the meiotic pairing process initiates with the movement of the centromeres to the nuclear envelope (NE). This is followed by the movement of the telomeres to the NE (late pre-leptotene in the mouse). The chromosomal domains then elongate, and the telomeres cluster at one side of the nuclear envelope, so that chromosomes form a bouquet. This bouquet is a common feature of the meiotic prophase (Von Wettstein et al., 1984). The clustering of telomeres allows for numerous pairing encounters between the now elongated chromosomes which contribute to homology testing at the exposed pairing sites. It has been proposed that the convergence of chromosome ends (telomeres) increases the efficacy of homology search and leads to pre-alignment of the now bent chromosomes (Schertan et al., 1996). This pre-alignment allows for initial pairing interactions between homologues. In yeast it has been demonstrated that these interactions occur at
multiple sites along each chromosome pair (Weiner and Kleckner, 1994). Similar interactions seem to occur in other organisms (Loidl, 1990). It has been proposed that initially unstable and reversible paranemic interactions between homologues occur and these interactions will be progressively stabilised, by bringing adjacent sequences into close apposition and by recombinational interactions. They will become permanent when a cross-over occurs (Kleckner and Weiner, 1993). It has been suggested that in mammals, the RAD51 protein may have a role in the stabilisation of these paranemic interactions (Plug et al., 1996).

At one or several regions where chromosomes have approached each other within the critical distance of 300nm (the maximum distance that the TFs can bridge), they initiate the typical tripartite SC structure (synapsis) which then extends by zipper-like growth (Loidl, 1994).

1.2.2.2 Recombination

*S. cerevisiae* is the only organism in which SC assembly (and therefore synapsis) has been analysed systematically in relation to successive steps in recombination, namely by the determination of the order of events at the DNA and chromosomal levels, and by the analysis of SC assembly and recombination in meiotic mutants of yeast. This implies that most of what is known about these events is due to the analysis of this system.

Observations that the total number of initial pairing interactions per nucleus (about 190) is similar to the number of recombination events led to the hypothesis that recombination initiates at the sites of early pairing (Kleckner and Weiner, 1993; Weiner and Kleckner, 1994). In support of this hypothesis is the fact that the mouse homologue of the yeast recombination enzyme RAD51 is localised at the sites of synaptic initiation (Plug et al., 1996). The notion that pairing connections precede recombination (and not vice versa) is supported by
the following observations: (i) Pairing occurs in premeiotic cells in yeast which presumably contain no strand interruptions (Weiner and Kleckner, 1994); (ii) some meiotically induced pairing is observed in mutants that fail to initiate recombination (Nag and Petes, 1993; Loidl et al., 1994; Weiner and Kleckner, 1994; Rockmill et al., 1995); (iii) In one particular mutant the number of pairing connections exceeds the number of exchanges initiated (Weiner and Kleckner, 1994).

Biochemical stages of the yeast recombination reaction have been revealed by physical analysis of meiotic DNA (Roeder, 1995; Schwacha and Kleckner, 1995; Goldman and Lichten, 1996; Kleckner, 1996). There is very strong evidence that double strand breaks (DSB) initiate recombination. This mechanism of meiotic recombination initiation seems to be evolutionarily conserved (at least in budding yeast, fission yeast, nematodes and archaeabacteria -Keeney et al., 1997). 5' termini are rapidly resected, leaving 3' single-stranded tails suitable for strand invasion and polymerase extension. DSBs are then converted into double Holliday junctions, which are thought to be the first stable interactions between homologues at the DNA level (Kleckner, 1996). Finally, cross-over and non-crossover products appear. In a non cross-over, also known as gene conversion, a local DNA interaction is resolved without concomitant exchange of flanking chromosome arms. Cross-overs and non cross-overs seem to arise via double Holliday junctions (Schwacha and Kleckner, 1994).

So initial pairing interactions and recombination interactions occur in succession at the same positions along each pair of interacting homologues. DSBs therefore occur at the sites of a weak initial interaction and have a role in stabilising the weak initial bonds. It is important to note that DSBs and recombination can occur outside the context of homologous interactions, as shown by the presence of DSBs in haploid yeast (Gilbertson and Stahl, 1994; de
Massy et al., 1994) and by examples of ectopic recombination (Jinks-Robertson and Petes, 1985; Lichten et al., 1987). This indicates that these events (initial pairing interactions and DSBs) are probably independent of each other, but both occur in the same vicinity where DNA is accessible due to chromatin conformation (Kleckner, 1996). One protein that could act at the interface between pairing, recombination and the development of axial chromosome structure is RAD50. A rad50 mutant is defective in all three processes (Weiner and Kleckner, 1994; Alani et al., 1990).

Further support for the hypothesis that initial weak pairing interactions are stabilised by recombinational interactions comes from the observation that successive steps in recombination determine successive steps in SC assembly (therefore of synapsis). Analysis of a number of meiotic mutants in yeast that have blocks in different steps of recombination have blocks at different steps of SC assembly (Roeder, 1995; Heiting, 1996). The dependence of SC formation/maintenance on different steps of recombination is also observable in mouse. Mice without the mismatch repair gene PMS2 (hypothesised to have a role in recombination), exhibit extensive asynapsis (Baker et al., 1995). In a similar way, mice lacking the mismatch repair gene MLH1, that does have a role in recombination, lose the initial synapsis of homologous chromosomes (Baker et al., 1996).

Another important aspect of recombination is the regulation of crossover frequency. The distribution of crossovers is non random in two respects (Carpenter, 1988; Roeder, 1995). (i) Crossovers tend not to occur close together. (ii) Every pair of chromosomes (no matter how small) undergoes at least one reciprocal change. These two phenomena, referred to as crossover-interference and the obligate chiasma, have been thought to be mechanistically related (Roeder, 1995). Crossover interference has been postulated to involve the
transmission of an inhibitory signal from one crossover site to nearby potential sites for crossover (Egel, 1978; Maguire, 1988; King and Mortimer, 1990). As described above the SC is necessary for crossover interference and is therefore an obvious conduit for this signal. It has been put forward that crossover interference serves to choose one amongst the many initial sites where DSBs occur (Kleckner, 1996).

I would like to finish this section on recombination by commenting on recombination nodules (RN)s (Carpenter, 1994). These are electron-dense structures that accompany the assembly of SCs. Two types can be distinguished: (i) Late recombination nodules are found on top of the tripartite structure of SCs. Their distribution and frequency along bivalents correlate with the frequency and distribution of crossovers and chiasmata. Late RNs are therefore presumed to mark the sites of reciprocal crossover (Carpenter, 1994). An RN-independent pathway for recombination at telomeres and recombination hot-spots has been proposed (Ashley, 1994). (ii) Early RNs are more numerous than late RNs. They occur along the unpaired axial elements during zygotene, on (part of) the fibrous connections between axial elements of aligned chromosomes, and on top of the tripartite structure of the SC during zygotene and early pachytene. It has been suggested that early RNs are involved in homology search and leave behind gene conversions as footprints of their activity (Carpenter, 1994). The relationship between early and late RN is still to be elucidated (Heyting, 1996).

1.2.3 After pachytene

After pachytene the SC disassembles. At the same time, or shortly after, axial elements are lost and the homologue axes reorganise (Solari, 1974; Heyting and Dietrich, 1992). Then higher order axial compaction occurs in progressive stages. After reorganisation of homologue axes, individualised chromatid axes appear in close parallel array. At this stage only chiasmata hold homologues together.
Then, the microtubules of the spindle become attached to the centromeres and appear to pull the centromeres of a bivalent to opposite poles. Metaphase ends as the separating chromosomes move simultaneously and rapidly to the poles of the cell. The first meiotic anaphase is the period when the mixture of two genomes is sorted out into two diploid genomes. The random orientation of bivalents at metaphase I and recombination will have ensured that no two spermatocytes carry the same genetic information. From here the cells can enter a second meiotic metaphase. Metaphase II is followed by a second meiotic anaphase which reduces the diploid genomes of duplicated chromosomes to haploid genomes with single DNA content (Moens, 1987).

1.3 REGULATION OF MEIOSIS

It is clear that a number of meiotic checkpoints exist that serve to control the entry and progression through meiosis (Honigberg et al., 1993). I will concentrate on the work that has studied those checkpoints monitoring progression through the meiotic prophase. In yeast (again due to the wide availability of meiotic mutants, yeast is one of the best studied systems) the RAD9 gene has been found to be an important component of at least one of these checkpoints. RAD9 causes G2 arrest in the progression of the mitotic cell cycle in response to DNA damaging elements (Weinert and Hartwell, 1988). During meiosis, RAD9 has a similar checkpoint function. In the *cdc13* mutant, meiosis is arrested after pre-meiotic DNA replication but prior to synapsis or recombination. The *rad9* mutation allows *cdc13* cells to progress through meiosis, although spore viability is lowered, consistent with its checkpoint function (Weber and Biers, 1992). The presence of a second checkpoint is evident from the analysis of mutants with defects in chromosome metabolism - *zip1* (Nag et al., 1995), *dmc1* (Bishop et al., 1992), *top2* (Rose and Holm, 1993), *rad50S* (Alani et al., 1990), *rad51* (Shinohara et al., 1992) and *sep1* (Tishkoff et al., 1995). Some or all of these mutants probably arrest in response to the accumulation of intermediates in the recombination
and/or synapsis pathway (Roeder, 1995). In support of this hypothesis, the prophase arrest of the dmc1 (Bishop et al., 1992), top2 (Rose et al., 1990) and zip1 (Sym et al., 1993) mutants is alleviated by mutations that block the initiation of recombination and synapsis. The fact that the complete absence of recombination does not trigger a checkpoint, as evidenced by the fact that mutants defective in DSB formation sporulate efficiently (Roeder, 1995) also supports this view. RAD9 doesn’t seem to be involved in this checkpoint, as a rad9 mutant does not bypass the meiotic arrest of dmc1, sepl and zip1 mutants; however RAD17, RAD24 and MEC1 are required for this meiotic arrest (Lydall et al., 1996). Similarly RED1 and MEK/MRE4 are required for the rad50S, rad51, dmc1 and zip1 arrest (Xu et al., 1997). Differences between yeast strains suggests that, at least in the case of zip1 and dmc1, the meiotic arrest may be triggered by different signals or effected by different mechanisms (Roeder, 1995). This suggests at least two checkpoints acting at this stage. Therefore it seems clear that mitotic checkpoints do operate during yeast meiosis, but meiosis specific signals may modify the signals generated and the response machinery (Roeder, 1995).

In vertebrates there is also strong evidence for checkpoints operating to control progression through the first meiotic prophase. Mice with asynapsed sex chromosomes suffer from a meiotic block during the first meiotic prophase, but when synapsis is restored, the arrest is overcome (Burgoyne et al., 1992). It has been proposed that the checkpoint monitors synapsis (Burgoyne and Mahadeviah, 1993) or both recombination and synapsis (Hale, 1994). This specific block due to asynapsed chromosomes will be dealt with further on, but it is interesting to note that the mouse ATR protein (a homologue of the yeast meiotic checkpoint protein MEC1) has been found to be associated with asynapsed but not synapsed axial elements (Keegan et al., 1996). It has been suggested that this protein could be monitoring asynapsis of chromosomes (Keegan et al., 1996). There is also evidence for a mechanism that monitors the proper chromosome
segregation both during insect meiosis (Li and Nicklas, 1995) and vertebrate mitosis (Rieder et al., 1994) cells. This checkpoint seems to monitor kinetochore attachment to the spindle by detecting tension at the kinetochore. It has been proposed that this checkpoint could also act, with some modifications, during meiosis in mammals (Rieder et al., 1994).

1.4 THE MOUSE Y CHROMOSOME.

In this section I will describe the mouse Y chromosome and a number of Y rearrangements and deletions that have been used as tools to gain a greater understanding of the meiotic checkpoint that monitors synapsis and/or recombination.

The mouse Y chromosome encodes for a very limited number of functions, being involved almost exclusively in controlling primary sex determination, spermatogenesis and fertility (Figure 1.1). Structurally, the Y chromosome is divided into a short arm, and a long arm that includes the pseudoautosomal region. The Y pseudoautosomal region is homologous to the X chromosome pseudoautosomal region and mediates pairing between these two chromosomes. At the molecular level the majority of DNA on the Y chromosome has been shown to comprise highly repeated sequences of no obvious function. These include Murvy (murine repeated virus Y linked) (Eicher and Washburn, 1986, Eicher et al., 1989), the simple repeat GATA (Jones and Singh, 1981; Singh et al., 1994) and a large number of anonymous sequences detected by probes isolated by various laboratories (Lamar and Palmer, 1984; Bishop et al., 1985; Baron et al., 1986; Nallaseth and Dewey, 1986; Nishioka and Lamothé, 1986; Harbers et al., 1986, Bishop and Hatat, 1987; Avner et al., 1987; Garchon et al., 1989; Nishioka, 1989; Tucker et al., 1989; Harbers et al., 1990; Prado et al., 1992). Very few of these repeats cross hybridize with DNA from other mammalian species, including close relatives such as rat, indicating that Y linked repetitive sequences change
The mouse Y chromosome

- H-Y antigen (Hya)
- Spermatogonial proliferation (Spy=Ube1y?)
- Testis determination (Sry)

- Normal sperm head development (Smy=Sstv family?)

Figure 1.1 Diagrammatic representation of the mouse Y chromosome
rapidly in evolution as might be expected for DNA which may only function as "bulk" for the chromosome.

The study of genes present on the Y chromosome has been hampered by the fact that recombination between the sex chromosomes is restricted to the tiny pseudoautosomal or pairing/exchange region located at the long arm (Yq) telomere. This makes normal meiotic mapping nearly impossible outside this region (Laval et al., 1995). The localisation of genes/functions/DNA probes has depended on a combination of direct in situ hybridization to metaphase spreads and/or Y "deletion mapping" approaches. Sxr which is derived from the minute short arm of the mouse Y chromosome (McLaren et al., 1988; Roberts et al., 1988), has proved particularly valuable, allowing the assignment of a number of genes/gene functions to this region of the short arm. Sxr includes the testis determining gene Sry (Gubbay et al., 1990), a gene, Hya, needed for the expression of the male specific H-Y antigen (McLaren et al., 1984) and a gene (Spy) needed for the normal proliferation of differentiating A spermatogonia (Burgoyne et al., 1986; Sutcliffe and Burgoyne, 1989). XO males carrying a copy of Sxr attached to the X chromosome are sterile owing to an almost complete arrest during the meiotic metaphase stages. This block, as will be explained later, can be overcome by providing the XSxr chromosome with a meiotic partner carrying no Y specific DNA, but the sperm produced have abnormal heads. From this experiment it can be concluded that the Y specific region of the long arm contains a gene (Smy) involved in the development of the sperm head, but, it would seem, little else vital for the spermatogenic process.

The pseudoautosomal or X-Y homologous region of the mouse Y chromosome is situated at the tip of the long arm of the chromosome. The only gene localised to this region so far is the steroid sulphatase gene - Sts (Keitges et al., 1985, Salido et al., 1996). The highly repeated sequence Mov15 (Harbers et al., 1986), the
polymorphic hypervariable locus DXYH1 (Fennelly et al., 1996) and intermale aggression (Roubertoux et al., 1994), have also been assigned to the pseudoautosomal region. An important function of the pseudoautosomal region is that it mediates pairing between the X and Y chromosomes, which is necessary for normal segregation of the X and Y chromosomes and as will be explained later, to avoid elimination of the cell through a meiotic quality control mechanism.

1.4.1 Y Chromosome rearrangements and deletions.

A number of Y chromosome variants involving either rearrangements or deletions, have been of critical importance for the assignment of functions to the Y chromosome or allocating genes to these functions. Some of these have been introduced previously but a more detailed explanation of these rearrangements is essential for the better understanding of the work carried out.

Sxr

The most useful rearrangement for the purpose of mapping genes has been the sex reversed mouse mutant Sxr, first described by Cattanach and colleagues in 1971. Sxr or [Tp(Y)1Ct] as it is more properly termed, is a small chromosomal fragment comprising most of the Y chromosome short arm, transposed distal to the pseudoautosomal region of the Y in XYSxr carriers (Singh and Jones, 1982, Roberts et al., 1988, McLaren et al., 1988) that is transferred to the X by the obligate X-Y crossover during male meiosis. The Sxr region carries the primary testis determining gene Sry so that XXSxr mice develop as males. It also has been shown to carry the Y chromosome spermatogenesis gene termed Spy (Levy and Burgoyne, 1986a and b) and genes controlling H-Y antigen (Hya) expression.

In 1984 McLaren and co-workers described a deletion variant of Sxr termed Sxr' (now referred to as Sxr^ and the original Sxr as Sxr^a). Sex reversed mice carrying Sxr^b were male but were found to be H-Y negative (McLaren et al., 1984, Simpson et al., 1986; Simpson et al., 1987). Spy was also found to be localised in
the DNA deleted in Sxr\textsuperscript{b} with respect to Sxr\textsuperscript{a} (Burgoyne et al., 1986). Molecular genetic analysis of the deletion has identified a gene, \textit{Ube1y}, encoding ubiquitin activating enzyme E1, which is a strong candidate for \textit{Spy} (Mitchel et al., 1991, Kay et al., 1991, Odorisio et al., 1996). Two other genes, \textit{Smcy} and \textit{Uty}, have been identified which encode epitopes of the H-Y antigen (Scott et al., 1995, Greenfield et al., 1996).

\textbf{Y*, Y*X and X\textsuperscript{Y*}.}

\textit{Y*} is a complex rearranged mouse Y chromosome first reported by Eicher and Washburn (1986). In essence it is a Y chromosome that has been hijacked by a non-Y centromere attached distal to the pseudoautosomal region (PAR), the original Y centromere being 'inactivated'. In conjunction with the addition of this foreign centromere there has been the addition of a segment of inverted PAR and a segment of X specific DNA (Figure 1.2- Eicher et al., 1991; Hale et al., 1991, Burgoyne et al., unpublished observations).

Recombination between the X and the \textit{Y*} chromosomes generates two new sex chromosomes (Figure 1.2):

(1) a large chromosome (X\textsuperscript{Y*}) comprised of the X chromosome attached at its distal end to all of the Y* chromosome via a shared pseudoautosomal region and
(2) a small chromosome (Y*X) containing the centromeric portion of the Y* chromosome attached to the G-band negative material of the X chromosome. The mode of generation of the Y*X chromosome precludes it from carrying any Y specific DNA and it is indeed negative for all the Y specific probes that have been tested (Eicher et al., 1991). It should, however, have an intact PAR (Figure 1.2).

\textbf{Sry deletion}

This is a 11kb deletion in the short arm of the Y chromosome which has
removed the testis determining gene, Sry (Lovell Badge and Robertson, 1990; Gubbay et al., 1990).

**Yd deletions**

These are a series of deletions encompassing a region of the short arm of the Y chromosome, proximal to the centromere but not including Sry (Capel et al., 1993; Laval et al., 1995).

**Large visible interstitial deletions of Yq**

Three have been described, one derived from the B10.BR Y (Styrna al 1991a; Styrna al 1991b), another from the RIII Y (Conway et al., 1994) and a final one from the Sry-negative 129Y chromosome (Mahadevaiah et al., 1993). The B10 and RIII Y deletions have been shown to be associated with an increased frequency of abnormal sperm head morphology, supporting the view that genes necessary for proper development of the sperm head are located on the long arm of the Y chromosome. A complex family of transcribed Y sequences (Ssty), related to the probe Y353B, exclusively expressed in spermatids and mapping to the Y long arm, have been proposed as candidate genes (Conway et al., 1994).
Figure 1.2. Diagramatic representations of the $Y^*$ rearrangement and of the products of its recombination with the $X$ chromosome.

(a) The $Y^*$ chromosome compared with the normal $Y$. In conjunction with the addition of a foreign centromere there has been the addition of a segment of inverted PAR (ABA instead of ABC), and of a segment of $X$ specific DNA ($X$).

(b) Synapsis and recombination of the $X$ and $Y^*$ chromosomes.

(c) During meiosis in $XY^*$ males, synapsis and crossing over within the regions that are homologous between these two chromosomes generates two recombinant chromosomes: $X^Y^*$, comprising the $X$ with an attached $Y^*$, and $Y^*X$, comprising the non-$Y$ centromere of the $Y^*$, the $Y^*$'s segment of $X$ specific DNA and a PAR.
Y

'Inactive' Y Centromere

Active non-Y Centromere

XY* Meiosis
1.5 UNPAIRED SEX CHROMOSOMES AND GAMETOGENIC FAILURE

It has long been noted that there is a correlation between the presence of an unpaired sex chromosome and spermatogenic failure. In 1974 Miklos proposed that the presence of an unpaired sex chromosome during the meiotic prophase would lead to the elimination of the cell with this type of error. In 1984 Burgoyne and Baker extended the Miklos hypothesis to include female meiosis. The most solid evidence in support of the existence of this type of checkpoint will be summarised here. Further evidence is reviewed in later chapters.

Mice with a single sex chromosome.

XSxr^O mice are sterile due to an almost total spermatogenic block at the meiotic metaphase I stage (Kot and Handel, 1991; Sutcliffe et al., 1991), any sperm that are produced being grossly abnormal and often diploid (Levy and Burgoyne, 1986a). If a pairing partner is provided for the XSxr^ chromosome, by using the Y^X chromosome, the meiotic block is overcome (Figure 1.3- Burgoyne et al., 1992). This provided clear evidence of the existence of a meiotic checkpoint that monitors synapsis and/or recombination.

XY^*O mice, which carry the large recombinant product of the Y^* rearrangement described above, also have a single sex chromosome and suffer from a very similar meiotic block to the one of XSxr^O males (Eicher and Washburn, 1986). Unfortunately attempts to provide this chromosome with a pairing partner have proved unsuccessful. For example, in XY^*Y^*X mice, the Y^*X chromosome fails to pair with the XY^* chromosome in all the pachytene spermatocytes analysed (Burgoyne et al., 1992). This is probably due to the fact that the XY^* chromosome has an interstitial PAR. In mouse, the movement of telomeres to the nuclear envelope seems to precede the initial stages of chromosome pairing (Scherthan et al., 1996). This movement would leave the PARs of both the sex chromosomes
very far apart at the time the initial pairing interactions are occurring, and would therefore explain why these chromosomes fail to synapse. Nevertheless, in $X^{Y*}O$ mice, it seems clear that the meiotic arrest present is due to the absence of synapsis and/or recombination of the single sex chromosome.

$XO$ female mice also suffer from a partial meiotic arrest. It has been demonstrated that these mice have less than half the number of oocytes and that the oocyte loss occurs in the late pachytene stage (Burgoyne and Baker, 1985). Self synapsis of the X chromosome has been shown to occur in XO mice (Speed, 1986) and it has been argued that the oocytes remaining survive due to this self pairing (Burgoyne and Mahadevaiah, 1993). This provides evidence that the checkpoint discussed above operates during female meiosis.
Figure 1.3 Diagrammatic representation of the sex chromosomes of $X Sxr^a O$ and $X Sxr^a Y^{*X}$ mice.
Mice with two sex chromosomes

Strong evidence for a checkpoint that monitors synapsis and/or recombination can be found when spermatogenic failure is analysed in mice in which the pairing between the two sex chromosomes is interfered with for some reason. In such a way XYSxr^ (Chandley and Fletcher, 1980; Evans et al., 1980; Mahadevaiah et al., 1988; Tease and Cattanach 1989), XSxr^Y (Cattanach et al., 1990) and XY* males (Eicher et al., 1991; Hale et al., 1991) present a reduction in the amount of pairing between the sex chromosomes due to the presence of the rearranged X or Y chromosomes, and this is accompanied by a partial meiotic arrest (Burgoyne and Mahadevaiah 1993). In a similar way, XY interspecific hybrids have a nearly complete failure of sex chromosome pairing at MI (due to the genetic divergence between the two pseudoautosomal regions), that is accompanied by an almost total meiotic arrest (Matsuda et al., 1991; Burgoyne and Mahadevaiah, 1993).

In females, mice with two sex chromosomes have a reduction in oocyte numbers (that is reflected in a reduction of reproductive lifespan), if the amount of pairing between the two sex chromosomes is reduced for any reason, as is the case of XY^Sry^ females (Mahadevaiah et al., 1993), In(X)/X females (with an inversion in one of the X chromosomes) (Burgoyne and Baker, 1985; Tease and Fisher, 1988) and XY*X females (Hunt, 1991).

Mice with three sex chromosomes

There is also clear evidence for the operation of the meiotic checkpoint in mice with three sex chromosomes, but in this type of mice additional factors, such as the possible deleterious effect of the excess of genetic material from the extra chromosome, need to be taken into account when analysing gametogenic failure. XYY mice have a partial meiotic block at MI and are almost always sterile. The
majority of spermatocytes in these males have a univalent sex chromosome at MI. It has been argued therefore, that the presence of an unpaired sex chromosome is the main or only cause for the meiotic block observed (Miklos, 1974, Burgoyne and Biddle, 1980, Burgoyne and Mahadevaiah, 1993). The analysis of XYY*^ males has challenged this view. These mice have only one dose of Y specific DNA, are fertile and have the same level of sex chromosome pairing failure at MI as XYY males (Hunt and Eicher, 1991). This fertility, in the face of low sex chromosome pairing levels at MI in XYY*^ mice, gave rise to the suggestion that the sterility of XYY males is due to the deleterious effects of excess Y gene dosage (Hunt and Eicher, 1991). However, as pointed out by Burgoyne and Mahadevaiah (1993), this doesn't necessarily have to be the case, as it is possible that XYY*^ males have a much higher percentage of cells with all three sex chromosomes paired (forming a trivalent) at pachytene than at MI. A higher level of pairing at pachytene than at MI could occur if only one chiasma per trivalent is formed. If this was the case, the trivalents would resolve into a bivalent plus univalent configuration by MI. The argument that this is occurring in XYY*^ but not XYY males is based upon the fact that the Y*^ chromosome is a more efficient pairing partner than the Y (Hunt and Eicher, 1991) and could therefore have a higher propensity to form trivalents during pachytene (Burgoyne and Mahadevaiah, 1993). It is therefore essential to assess the rate of trivalent formation at pachytene in XYY*^ males before deciding whether excess Y gene dosage, sex chromosome failure, or both, are responsible for the sterility of XYY mice.

In females with three sex chromosomes the evidence in favour of a meiotic checkpoint that monitors synapsis and/or recombination is clearer. Both XXY and XYY female mice are fertile but have reduced oocyte counts. This reduction in oocyte levels is directly correlated with the presence of a univalent sex chromosome during pachytene, but in both types of females, as well as in XY.
females, there does also seem to be a negative effect of Y genes on the quality of
the oocytes (Mahadevaiah et al., 1993, Burgoyne and Mahadevaiah, 1993).

All these observations point strongly to the conclusion that the presence of
unpaired sex chromosomes is causally related to gametogenic failure. This
implies the existence of a meiotic checkpoint that acts during the first prophase
to ensure correct chromosome pairing and/or recombination. The question as to
whether this checkpoint monitors pairing alone or pairing and recombination is a
complex issue and will be dealt with in detail further on.

Regarding the mechanism for the action of the meiotic quality control, Miklos
(1974) suggested that meiotic 'pairing sites' distributed along the chromosome (in
the case of the sex chromosomes along the pseudoautosomal region), must be
saturated by pairing during the meiotic prophase. If chromosome regions remain
unpaired at pachytene, Miklos proposed that these pairing sites become
activated and set in motion a process that leads to the elimination of the cell with
the meiotic pairing error. Evidence at the molecular level in support of this
hypothesis remains elusive. Miklos based his initial hypothesis on observations
made in the achiasmate Drosophila male. In this organism, it is clear that rDNA
sequences serve as X-Y pairing sites (Mckee and Karpen, 1990; Merrill et al.,
1992), but no evidence exists that suggests that these could act as the pairing sites
described by Miklos, or that they have a role in other chiasmate species. It is
possible that the use of these sequences evolved only in the context of achiasmate
meiosis, as an alternative mechanism to ensure proper chromosome segregation.
This argument is supported by the fact that centric heterochromatin in female
Drosophila seems to have a similar backup role, by ensuring proper segregation
of achiasmate chromosomes (Karpen et al., 1996; Dernburg et al., 1996). In lily,
1000 repeats, and in mouse 400 repeats, of a specific pachytene small DNA
fraction, PsnDNA, seem to be dispersed throughout the genome at 160kb
intervals. Their nicking is regulated by PsnRNA and a non-histone protein, and is dependent on homologous pairing (Stern and Hotta, 1987). Again the importance of these sequences and molecules in chromosome synapsis, recombination and the checkpoint that regulates them remains unknown. Of course a number of other models that could explain the mechanism for a checkpoint can be envisaged. For example, as seems to occur in yeast, the accumulation of synapsis or recombination intermediates could be recognised, and trigger the elimination of the cell; but until solid experimental support appears, they will remain hypotheses.

1.6 OUTLINE OF PROJECTS

A number of questions still remain to be resolved concerning the mode of action of the meiotic checkpoint that monitors sex chromosome synapsis and/or recombination. The aim of the experiments described in this thesis was to resolve some of these questions and thus gain a clearer understanding of the circumstances in which this checkpoint acts.

The first question I sought to address was whether this meiotic checkpoint monitors sex chromosome synapsis, recombination or both. The most cogent arguments in favour of a recombination checkpoint are based on data from XY* males (Hale et al., 1991; Hale, 1994; but see counter arguments in Burgoyne and Mahadevaiah, 1993). In Chapter 2, the meiotic pairing and sperm output of XY* males is compared with XYSxr^ males in order to provide further evidence for or against the existence of a recombination checkpoint.

The next two sets of experiments were designed to investigate further the reasons for the spermatogenic failure of XYY mice. As mentioned earlier, XYY mice are sterile, and excess Y gene dosage, sex chromosome pairing failure, or a combination of both, have been put forward as the reasons for this sterility.
The most compelling data supporting a Y dosage effect comes from the study of XYY*^ mice (Hunt and Eicher, 1991). These mice have three sex chromosomes, but only a double dose of Y-specific genes; since they are fertile, Hunt and Eicher reasoned that excess Y gene dosage was the major factor in the infertility of XYY mice. However, as pointed out by Burgoyne and Mahadevaiah (1993), this conclusion is jeopardised by the lack of information on the level of trivalent formation at pachytene in XYY*^ mice - it is formally possible that these mice have much higher levels of trivalent formation than XYY mice. Accordingly, in Chapter 3, the degree of sex chromosome synapsis in XYY*^ mice is compared with that of XYY mice from pachytene through to MI, and the level of trivalent formation is correlated with testis weight and sperm output.

As an alternative approach to addressing the question as to whether excess Y gene dosage contributes to the infertility of XYY mice, Chapter 4 describes an analysis of sex chromosome synapsis, testis weights and sperm output in XYYY*^ and X^*Y mice. Both these types of mice have the potential to satisfy pairing requirements in the presence of excess Y gene dosage, the former by forming two sex bivalents and the latter by forming a single sex bivalent.

During the course of a collaborative project looking at the apoptotic elimination of spermatocytes in X^*O males, in which the presence of a single sex chromosome triggers the synapsis/recombination checkpoint, an exceptional X^*O male was identified that had sufficient sperm to allow fertility. Chapter 5 describes experiments designed to determine the genetic basis of this circumvention of the meiotic checkpoint.
CHAPTER 2
SYNAPSIS, RECOMBINATION AND FERTILITY OF XY* MALES.
2.1 INTRODUCTION
As discussed in Chapter 1, a great deal of evidence exists in support of the view that in mammals, a failure of the sex chromosomes to pair activates a meiotic checkpoint. The original hypothesis put forward by Miklos in 1974, and then extended by Burgoyne and Baker in 1984, proposed that this meiotic checkpoint monitors the synopsis of homologous chromosomes. Modifications of this hypothesis have been put forward, the most notable being Hale's "obligate recombination" hypothesis (Hale et al., 1991; Hale, 1994). Hale suggested that X-Y synopsis alone is not sufficient for spermatogenic success, and that an additional meiotic checkpoint exists that monitors recombination. He also proposed that as XO females produce viable gametes, this additional control doesn't act during female meiosis.

The fact that there is a mechanism to ensure one chiasma per bivalent which seems to drive chiasma formation in the PAR of the X-Y bivalent (Burgoyne et al., 1982), means that it is very difficult to separate a requirement for synopsis from a requirement for synopsis and recombination. It has been suggested that this linkage is broken in two situations:

(a) Recombination without PAR synopsis. The observation that in XSxr<sup>a</sup>Y males a crossover can occur between Sxr<sup>a</sup> and Yp (Y short arm) without it occurring between the PARs, led Cattanach et al. (1990) to suggest that Sxr<sup>a</sup>-Yp pairing and crossing over, without PAR synopsis, avoids elimination. However, Burgoyne and Mahadevaiah (1993) have pointed out that in this example there could have been PAR-PAR and Sxr<sup>a</sup>-Yp synopsis, with the Sxr<sup>a</sup>-Yp recombination satisfying the obligate chiasma requirement.

(b) PAR synopsis without recombination. In XY* males, due to the inverted
nature of the pseudoautosomal region, the sex chromosomes can synapse in one of two orientations (Figure 2.1): (i) staggered (which allows for extensive homologous synapsis and has previously been reported to be present in 2% of pachytene cells - Hale et al., 1991), and (ii) parasynapsed (which in the majority of the cases will provide nonhomologous synapsis and has been observed in 42% of pachytene cells - Hale et al., 1991). Since, in the XY* males, the vast majority of products observed at MII are those predicted from the staggered orientation, Hale et al. suggested that in these mice the majority of parasynapsed bivalents are failing to recombine and are then eliminated at MI. However, Burgoyne and Mahadevaiah (1993), suggested that the majority of parasynapsed bivalents may in fact, not have PAR-PAR synapsis, but centromere-PAR synapsis, leaving one PAR unsynapsed. These cells would then be eliminated because of a failure to achieve full PAR synapsis.

The problem with Hale's, and with Burgoyne and Mahadevaiah's explanations, is that the fertility and testis weights of the XY* males are not compatible with the level of spermatocyte elimination predicted by either model. Furthermore, one of the main planks of Hale's argument - the increase in univalence between pachytene and diakinesis/MI taken to indicate a separation of parasynapsed bivalents lacking a chiasma - is undermined by our recent findings that spermatocytes with univalent chromosomes arrest and accumulate at MI prior to being eliminated by apoptosis (see attached manuscript). Therefore it was decided to reinvestigate sex chromosome synapsis throughout the first meiotic prophase in XY* males and relate this to their testis weights and sperm counts as parameters of spermatogenic proficiency. XYSxr^ males were also analysed as "controls" in which a more straightforward relationship between sex chromosome pairing failure and spermatocyte loss is expected (Chandley and Speed, 1987; Mahadevaiah et al., 1988; Tease and Cattanach, 1989).
Figure 2.1. Orientations in which the X and Y* chromosomes can synapse:

(a) In the parasympapsed configuration there is only a short region of homology in the correct direction (A) between the X and Y* chromosomes.

(b) In the staggered configuration this homology is much greater (XAB).
2.2 MATERIALS AND METHODS

2.2.1 Mice

XY* and XYSxr^ males.

XY* (Eicher et al., 1991) and XYSxr^ males (Evans et al., 1982) were obtained from stocks maintained on an MF1 background. The Y* is of a LT strain origin (Eicher et al., 1991) and the Y and Sxr^ of the XYSxr^ males is of a RIII origin (Cattanach et al., 1971). XYLT and XYRIII males were therefore used as controls; the XYLT had been backcrossed to MF1 for 5 generations, and the XYRIII mice (originally from the Sxr^ stock) was fully backcrossed to MF1. The XY* and XYLT males were processed at the age of 3.5 to 4 months. XYSxr^ and XYRIII males were processed at the age of 4 months.

2.2.2 Karyotyping

Air-dried metaphase spreads were obtained from bone marrow cells post mortem using standard procedures (See Appendix 1).

2.2.3 Sperm Counts.

As a measure of sperm production, sperm counts were estimated for each caput epididymis by a procedure modified from that described by Searle and Beechey (1974) (See Appendix 1).

2.2.4 Synaptonemal Complex Analysis.

The procedure used to prepare pachytene spreads for synaptonemal complex analysis was a modification of that described by Mahadevaiah and Mittwoch (1986) -See Appendix 1. The spreads were stained with silver nitrate (Howell and Black, 1980). Cells were scored as being at the early, mid or late pachytene stage as described by Moses and Poorman (1984), Figure 2.2. See Appendix 1.

2.2.5 Air-dried preparations.
Two air-dried slides of fixed testicular suspensions (Evans et al., 1964) were prepared from each of the XY*, XYSxr³, and XY control males and were stained with giemsa (See Appendix 1). Diakinesis and metaphase I spermatocytes, classified as to stage on the basis of the degree of condensation and "opening out" of the bivalents, were scored using a low power objective. Subsequently the X-Y pairing status of these cells was scored using a high power objective.

2.2.6 Antibodies and Immunodetection.

It has been suggested that the Mlh1 antibody detects late recombination nodules in pachytene cells (Baker et al., 1996), and was therefore used to detect recombination foci in XY* and XYLT males. This antibody was obtained from PharMingen. The GS antibody detects centromeres (Earnshaw and Rothfield, 1985) and was a gift from W. Earnshaw (University of Edinburgh). The A1 antibody (that reacts with the lateral element protein SCP3 -Lammers et al., 1994) was used in combination with the Mlh1 or the GS antibody as a way of positioning the chromosomes in the nucleus. This antibody was a gift from C. Heyting (Wageningen Agricultural University). The immunochemical detection procedure used for all antibodies was a modification of the protocol by Moens et al. (1987) (See Appendix 1).
Figure 2.2. Examples of pachytene cells at early, mid and late pachytene:

(A) At early pachytene the sex chromosome axial elements are thin and uniform, and the nucleoli (indicated with an arrow) are observed as individual units.

(B) At mid pachytene the sex chromosome axial elements are branched and the nucleoli are clustered (indicated with an arrow).

(C) At late pachytene the sex chromosome axial elements have thick ends and the nucleoli are clumped, but more condensed than at mid pachytene (indicated with an arrow).
2.3 RESULTS

2.3.1 Testis weights and sperm counts of XY*, XYLT, XYSxr\(^a\) and XYRIII males.

The mean testis weights and mean sperm counts of the XY* males (82.6±6.9 mg and 846,600±105,808 sperm/caput) and XYSxr\(^a\) males (86.3±10.2 mg and 735,000±144,498 sperm/caput) are very similar, and clearly reduced as compared to the XY controls (Table 2.1). It should also be noted that the mean testis weights and mean sperm counts of the XYLT males (127±7.0 mg and 2,011,500±188,500 sperm/caput) are slightly higher than the mean testis weights and sperm counts of the XYRIII males (99.3±22.3 mg and 1,500,167±424,311 sperm/caput). This could be due to an effect associated with the YLT chromosome, or to a genetic effect associated with the MFl background, as the XYLT mice, unlike the XY*, XYSxr\(^a\) and XYRIII males, are not fully backcrossed to MFl (they have only been backcrossed for 5 generations). The finding that the sperm counts of two XYLT males that had been backcrossed to MFl for 9 generations, were lower than those of the first set of XYLT males (1,781,500±158,500 sperm/caput), indicates that it is possibly the MFl background that is causing these higher testis weights and sperm counts of the XYLT males.

2.3.2 Pachytene in XYSxr\(^a\) and XYRIII males.

XYSxr\(^a\) males show a clearly increased level of sex chromosome asynapsis at pachytene (34% -range 28-45%) when compared to the XYIII controls (2% - range 0-10%) - Table 2.2, Figure 2.6. In both these types of male a proportion of cells had autosome asynapsis; these were all eliminated by the mid-pachytene stage and were not included in the levels of synapsis presented above (Figure 2.3).

2.3.3 Pachytene in XY* and XYLT males.

XY* males have somewhat lower levels of sex chromosome asynapsis (25% -range 13-44% - Table 2.3) than the XYSxr\(^a\) males. However, this underestimates the level of PAR asynapsis since a proportion of the parasynapsed bivalents have
PAR asynapsis (Hale et al., 1991). To estimate this proportion, the GS antibody (that detects centromeres -Earnshaw and Rothfield, 1985) was used on pachytene cells of XY* males (Figure 2.4). 3 out of the 26 (12%) cells that had their sex chromosomes parasyapsed had PAR asynapsis. Using this estimate to adjust the overall level of PAR asynapsis in XY* males, increases the proportion of XY* cells with PAR asynapsis (33%- range 22-52% -Table 2.3) to a level that is very similar to that seen in XYSxr^ mice. Thus, XYSxra males and XY* males are very similar with respect to their levels of PAR asynapsis and with respect to their reduced sperm counts. This implies that sperm production in XY* males is supported by most, if not all, the cells with PAR synapsis, including the majority of parasyapsed X-Y* bivalents. The possibility that the parasyapsed bivalents are not being eliminated contradicts the MII and offspring data which have been taken to imply that only cells with their sex chromosomes staggered are progressing through meiosis (Hale et al., 1991). This contradiction can only be resolved if these bivalents are (i) crossing over and then resolving as if staggered, or (ii) converting into the staggered configuration so as to achieve recombination.

A proportion of cells of the XY* and XYLTA males had autosome asynapsis, these were all eliminated by the mid-pachytene stage (Figure 2.3). These cells were not included in the levels of synapsis presented above.

2.3.4 Recombination in XY* males.

To test if the parasyapsed bivalents of XY* males are regularly crossing over and then resolving as if staggered, the Mlh1 antibody (that recognises recombination sites) was used on pachytene cells of XY* and XYLTA males (Figure 2.5). 5 out of 10 XYLTA cells presented clear foci, but only 2 out of 22 (9%) XY* cells that had their sex chromosomes parasyapsed had foci. The 2 XY* cells studied that had their sex chromosomes staggered also had Mlh1 foci. Given this very low frequency of parasyapsed bivalents with Mlh1 foci, the possibility that
the sex chromosomes of the XY* males recombine whilst parasynapsed and resolve at MI as if they had been staggered, is untenable.

2.3.5 Evidence for the conversion into the staggered configuration of the X-Y* parasynapsed bivalents.

Evidence for the conversion of a proportion of parasynapsed X-Y* bivalents into the staggered configuration so as to achieve recombination, comes from comparing the synaptic configurations of the X-Y* bivalent at early, mid and late pachytene. Between early and mid pachytene the proportion of cells with the X-Y* bivalent staggered remains more or less constant (9% and 13% respectively), but by late pachytene this proportion has increased dramatically (35%). This increase is paralleled by a fall from 60% to 25% of the proportion of the cells with the parasynapsed orientation between mid and late pachytene (Table 2.4). This situation is completely different to that seen in XYSxr^ males, where there is no increase in any synaptic configuration between these stages (Table 2.5, Figure 2.4).

It is unlikely that the decrease in the parasynapsed configuration between mid and late pachytene is due to the elimination of cells that have chromosomes that have failed to recombine, as there doesn't seem to be selection against any other configuration that lacks recombination (such as univalents) between these stages (Table 2.4). Further evidence that these observations are due to a switch between the parasynapsed and staggered configurations, is the presence of an intermediate orientation that seems to be halfway between parasynapsed and staggered (Table 2.4, Figure 2.7).

2.3.6 Diakinesis and Metaphase I in XY*, XYLT, XYSxr^ and XYRIII males.

At Diakinesis the level of X-Y separation is very similar in XY* (27%- Table 2.6) and XYSxr^ males (25%-Table 2.7), and in both these types of mice it is clearly
higher than the XY controls (XYRIII- 1%; XYLT- 2%). It is to be expected that by diakinesis those configurations lacking a chiasma will have separated, so this equivalence in levels of X-Y separation is in agreement with the equivalent levels of PAR asynapsis at early/mid pachytene in XYSxr^a and XY* males. It also supports the view that the majority of parasynapsed bivalents seen at early/mid pachytene must eventually be forming chiasma, although this process is probably deferred until they have converted to the staggered orientation.

Between diakinesis and MI there is a very clear pile up of those cells with a univalent sex chromosome. This pile up is evident from the drastic increase in the levels of X-Y separation between these stages in all types of male analysed (Tables 2.6 and 2.7). This piling up is reflected in the MI/Diakinesis ratio of cells with and without X-Y separation. This ratio ranges between 2.22 and 13 for cells with X-Y separation, but is only between 0.2 and 1.1 for those cells without (Tables 2.6 and 2.7).
<table>
<thead>
<tr>
<th>XY*</th>
<th>Testis Weight</th>
<th>Sperm Counts/Caput</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY* 1</td>
<td>74mg</td>
<td>801,000</td>
</tr>
<tr>
<td>XY* 2</td>
<td>76mg</td>
<td>816,000</td>
</tr>
<tr>
<td>XY* 3</td>
<td>88mg</td>
<td>688,000</td>
</tr>
<tr>
<td>XY* 4</td>
<td>83mg</td>
<td>975,000</td>
</tr>
<tr>
<td>XY* 5</td>
<td>92mg</td>
<td>953,000</td>
</tr>
<tr>
<td>XY* Means ± SD</td>
<td>82.6±6.9 mg</td>
<td>846,600±105,808</td>
</tr>
</tbody>
</table>

| XYLT 1 | 120mg         | 1,823,000          |
| XYLT 2 | 134mg         | 2,200,000          |
| XYLT Means ± SD | 127±7.0 mg   | 2,011,500±188,500 |

| XYSxr^ 1 | 72mg          | 980,000            |
| XYSxr^ 2 | 88mg          | 725,000            |
| XYSxr^ 3 | 82mg          | 543,000            |
| XYSxr^ 4 | 101mg         | 852,000            |
| XYSxr^ 5 | 97mg          | 685,000            |
| XYSxr^ 6 | 78mg          | 625,000            |
| XYSxr^ Means ± SD | 86.3±10.2 mg | 735,000±144,498    |

| XYRIII 1 | 103mg         | 1,320,000          |
| XYRIII 2 | 73mg          | 823,000            |
| XYRIII 3 | 84mg          | 1,807,000          |
| XYRIII 4 | 84mg          | 1,173,000          |
| XYRIII 5 | 140mg         | 1,901,000          |
| XYRIII 6 | 112mg         | 1,977,000          |
| XYRIII Means ± SD | 99.3±22.3 mg | 1,500,167±424,311  |

Table 2.1. Mean testis weights and sperm counts of XY*, XYLT, XYSxr^ and XYRIII males. The difference between the mean testis weights and sperm counts
of the XY* and the XYLT males is highly significant as revealed by a Student's t-test (For mean testis weights: t=6.5, P<0.005; and for the mean sperm counts: t=8.7, P<0.001). The difference between the mean sperm counts of the XYSxr^d and the XYRIII males are also highly significant as revealed by a Student's t-test (t=3.8, P<0.01), although the difference between the mean testis weights was not (t=1.2, P>0.2), probably due to the large variation of these values for the XYRIII mice. A Student's t-test also reveals that the difference between the mean testis weights and mean sperm counts of the XY* and XYSxr^d males is not significant (For the mean testis weights: t=0.6 P>0.5; and for the mean sperm counts: t=1.3, P>0.2).
<table>
<thead>
<tr>
<th></th>
<th>XYSr</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>19</td>
<td>27</td>
<td>26</td>
<td>30</td>
<td>28</td>
<td>32</td>
<td>162</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>AS</td>
<td>10</td>
<td>14</td>
<td>21</td>
<td>14</td>
<td>11</td>
<td>14</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>(33%)</td>
<td>(33%)</td>
<td>(45%)</td>
<td>(32%)</td>
<td>(28%)</td>
<td>(30%)</td>
<td>(34%)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>42</td>
<td>47</td>
<td>44</td>
<td>39</td>
<td>47</td>
<td>249</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>XYRIII</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>36</td>
<td>45</td>
<td>36</td>
<td>40</td>
<td>44</td>
<td>38</td>
<td>239</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AS</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>(10%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(2%)</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>45</td>
<td>36</td>
<td>40</td>
<td>44</td>
<td>39</td>
<td>244</td>
</tr>
</tbody>
</table>

Table 2.2. Levels of sex chromosome asynapsis in pachytene cells (both early and mid pachytene) of XYSr and XYRIII males (P= Parasyngapsed, S=Staggered, AS= Asynapsed).
### Table 2.3. Levels of sex chromosome asynapsis in pachytene cells (both early and mid pachytene) of XY* and XYLT males (P-S= Predicted number of cells that will have their sex chromosomes parasynapsed and both the PAR's synapsed, P-AS= Predicted number of cells that will have their sex chromosomes parasynapsed and have at least one PAR asynapsed, S=Staggered, NS= Not Synapsed).

#### XY*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-S</td>
<td>11</td>
<td>14</td>
<td>27</td>
<td>26</td>
<td>14</td>
<td>92 (55%)</td>
</tr>
<tr>
<td>P-AS</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>14 (8%)</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>20 (12%)</td>
</tr>
<tr>
<td>AS</td>
<td>11</td>
<td>9</td>
<td>12</td>
<td>8</td>
<td>3</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>(44%)</td>
<td>(32%)</td>
<td>(24%)</td>
<td>(19%)</td>
<td>(13%)</td>
<td>(25%)</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>28</td>
<td>50</td>
<td>43</td>
<td>23</td>
<td>169</td>
</tr>
</tbody>
</table>

#### XYLT

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>30</td>
<td>29</td>
<td>59</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AS</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(14%)</td>
<td>(9%)</td>
<td>(12%)</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>32</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>XY*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>NP</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>XY LT</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>9</td>
<td>14</td>
<td>22</td>
<td>25</td>
<td>11</td>
<td>81 (60%)</td>
<td></td>
<td>22</td>
<td>20</td>
<td>42 (85%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>8 (6%)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>2 (4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>17 (13%)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>28 (21%)</td>
<td></td>
<td>4</td>
<td>1</td>
<td>5 (10%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>24</td>
<td>36</td>
<td>37</td>
<td>17</td>
<td>134</td>
<td></td>
<td>27</td>
<td>22</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>12 (25%)</td>
<td></td>
<td>3</td>
<td>6</td>
<td>9 (90%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (5%)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>17 (35%)</td>
<td></td>
<td>0</td>
<td>1</td>
<td>1 (10%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>17 (35%)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>15</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>48</td>
<td></td>
<td>3</td>
<td>7</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.4. Sex chromosomes configurations in early, mid and late pachytene cells of XY* and XYLT males (EP= Early Pachytene, MP= Mid Pachytene, LP= Late Pachytene, P= Parasynapsed, I= Intermediate, S= Staggered, NP= Not Paired).
<table>
<thead>
<tr>
<th></th>
<th>XYSxr^a</th>
<th></th>
<th>XYRIII</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>8</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NP</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>T</td>
<td>8</td>
<td>13</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>13</td>
<td>19</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NP</td>
<td>8</td>
<td>9</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>T</td>
<td>22</td>
<td>29</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NP</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T</td>
<td>9</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.5. Sex chromosome configurations in pachytene cells of XYSxr^a and XYRIII males (EP= Early Pachytene, MP= Mid Pachytene, LP= Late Pachytene, P= Parasympased, S= Staggered, NP= Not Paired, T= Totals).
<table>
<thead>
<tr>
<th></th>
<th>Diakinesis</th>
<th>Metaphase I</th>
<th>MI/Dia.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>NP</td>
<td>Total</td>
</tr>
<tr>
<td>XY* 1</td>
<td>11</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>XY* 2</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>XY* 3</td>
<td>16</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>XY* 4</td>
<td>20</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>XY* 5</td>
<td>16</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>74</td>
<td>27</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>(73%)</td>
<td>(27%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>XYLT 1</th>
<th>XYLT 2</th>
<th>Total</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32</td>
<td>0</td>
<td>32</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>1</td>
<td>24</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>55</td>
<td>1</td>
<td>56</td>
<td>38</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(98%)</td>
<td>(2%)</td>
<td>(98%)</td>
<td>(86%)</td>
<td>(14%)</td>
</tr>
</tbody>
</table>

Table 2.6. Sex chromosome configurations at Diakinesis and Metaphase I, and metaphase I/Diakinesis ratios in XY* and XYLT males (P= Cells with sex chromosomes paired, NP= Cells with sex chromosomes not paired, MI/Dia= Metaphase I/Diakinesis ratios).
<table>
<thead>
<tr>
<th></th>
<th>Diakinesis</th>
<th>Metaphase I</th>
<th>MI/Dia.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>NP</td>
<td>Total</td>
</tr>
<tr>
<td>XYSr^a 1</td>
<td>10</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>XYSr^a 2</td>
<td>15</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>XYSr^a 3</td>
<td>23</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>XYSr^a 4</td>
<td>17</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>XYSr^a 5</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>XYSr^a 6</td>
<td>16</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>(75%)</td>
<td>(25%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>XYRII 1</th>
<th>XYRII 2</th>
<th>XYRII 3</th>
<th>XYRII 4</th>
<th>XYRII 5</th>
<th>XYRII 6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31</td>
<td>34</td>
<td>35</td>
<td>32</td>
<td>35</td>
<td>35</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>35</td>
<td>35</td>
<td>33</td>
<td>35</td>
<td>36</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>12</td>
<td>8</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>15</td>
<td>15</td>
<td>17</td>
<td>15</td>
<td>13</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>0.45</td>
<td>0.26</td>
<td>0.37</td>
<td>0.34</td>
<td>0.34</td>
<td>0.23</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>(99%)</td>
<td>(1%)</td>
<td>(71%)</td>
<td>(29%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.7. Sex chromosome configurations at Diakinesis and Metaphase I, and metaphase I/Diakinesis ratios in XYSr^a and XYRII males (P= Cells with sex chromosomes paired, NP= Cells with sex chromosomes not paired, MI/Dia= Metaphase I/Diakinesis ratios).
Figure 2.3. Examples of pachytene cells from XYLT and XYRIII males showing autosome asynapsis or in the process of being eliminated:

(A-B) XYLT (A) and XYRIII (B) pachytene cells showing autosome asynapsis.

(C) Pachytene cell from an XY* male degenerating.
Figure 2.4. Examples of the positions of the centromeres in pachytene cells of XY (A) and XY* males (B-D) stained with the GS (that detects the centromeres) and A1 (that detects the lateral elements) antibodies (the arrow indicates the sex bivalent):

(A) The X and Y chromosomes are synapsed, and the centromeres of both these chromosomes are located distal to the paired region, therefore there is PAR-PAR synapsis.

(B-C) The X and Y* chromosomes are parasynapsed, and only the centromere of the Y* chromosome is located by the paired segment. As the PAR of the Y* chromosome is proximal to the active centromere there is PAR-PAR synapsis.

(D) The X and Y* chromosomes are staggered, and only the centromere of the Y* chromosome is located in the paired segment, therefore there is PAR-PAR synapsis.
Figure 2.5. Examples of Mlh1 foci on pachytene cells of XY* (A-C) and XY males (D-E), stained with Mlh1 (that detects recombination foci) and A1 (that detects lateral elements) antibodies (the arrow indicates the position of the sex bivalent):

(A) The X and Y* chromosomes are parasynapsed, and have no Mlh1 foci.
(B) The X and Y* chromosomes are staggered, and have a Mlh1 focus.
(C) The X and Y* chromosomes are parasynapsed, and have a Mlh1 focus.
(D) The X and Y chromosomes are synapsed, and have a Mlh1 focus.
(E) The X and Y chromosomes are synapsed, and have no Mlh1 focus.
Figure 2.6. Examples of sex chromosome configurations in XYSxr\(^a\) (A-D) and XYRIII males (E-F).

(A-B) The X and YSxr\(^a\) chromosomes are synapsed.

(C) Both the X and the YSxr\(^a\) chromosome are self synapsed.

(D) Both the X and YSxr\(^a\) chromosomes are present as univalents.

(E) The X and Y chromosomes are synapsed in a XYRIII male.

(F) Both the X and Y chromosomes are present as univalents. The Y chromosome is self synapsed.
Figure 2.7. Examples of sex chromosome configurations in XY* (A-M) and XYLT males (N-O) (The arrow indicates the position of the telomeres that are proximal to the PARs):

(A-B) The X and Y* chromosomes are parasynapsed.

(C-F) The X and Y* chromosomes are in the intermediate configuration. The end of the Y* chromosome proximal to the PAR is bridging between this chromosome and the X chromosome.

(G-L) The X and Y* chromosomes are staggered. In some cases (G-I) the Y* chromosome seems to have a region of (or close to) the PAR selfsynapsed. This region probably corresponds to the duplicated segment of the Y* PAR (see Figure 2.1). It is possible that the intermediate configurations allow for contact, and subsequent synapsis, to occur between these duplicated segments, and this would explain why this selfsynapsis is observable in some of the staggered configurations, but not in any of the parasynapsed ones.

(M) The X and Y* chromosomes are present as univalents.

(N) The X and Y chromosomes are synapsed.

(O) The X and Y chromosomes are present as univalents.
2.4 DISCUSSION

XY\* males have previously been considered to provide the strongest evidence in favour of a requirement for recombination during meiosis. The observation that the vast majority of cells present at MII in these mice are products from the staggered configuration, led Hale et al., (1991) to argue that those parasynapsed bivalents that do not recombine are eliminated at MI. However, Burgoyne and Mahadevaiah (1993) pointed out that it was possible that the majority of parasynapsed bivalents lack PAR-PAR synapsis, and are therefore eliminated for this reason. Both these models assume that the majority of bivalents must be eliminated (Table 2.8), but this doesn't fit with the testis weights and sperm counts of the XY\* males (Hale et al., 1991; present data).

To resolve this contradiction Hale et al. in 1991, argued that artefactual disruption caused by the physical stress of the surface spreading procedure could preferentially affect the staggered configuration, and therefore be a reason for the very low levels of this type of synapsis observed. This seems unlikely, as the staggered configuration should be in theory, the more stable one, as a mature chiasma should be holding together the bivalents, as indicated by the presence of Mlh1 foci on these bivalents. By contrast, the majority of parasynapsed bivalents do not have Mlh1 foci and would therefore be predicted to be less stable.

Burgoyne and Mahadevaiah's model is clearly rejected by the current GS antibody data. Instead of the majority of parasynapsed bivalents having incomplete PAR synapsis, the majority (88%) had PAR-PAR synapsis.

An insight into the reasons for this discrepancy between the level of staggered synapsis and XY\* fertility, was provided by the comparison of the sperm counts and overall levels of PAR synapsis in XY\* and XYSxr\(^a\) males. This comparison
led to the conclusion that the majority of spermatocytes with the X-Y* bivalent parasynapsed must be progressing through to sperm. Two possibilities were considered that could account for the lack of nonrecombinant products and the rarity of predicted parasynapsed products at MII (Hale et al., 1991): (i) the parasynapsed bivalents could be recombining and resolving as if they had been in the staggered configuration, or (ii) these bivalents could be switching into the staggered configuration to achieve recombination.

The first possibility is rejected by the very low frequency of parasynapsed bivalents that have Mlh1 foci. It has been suggested that the Mlh1 antibody detects late recombination nodules (Baker et al., 1996), therefore these foci can be taken as sites of recombination. This indicates that it is probable that very few of the parasynapsed bivalents have recombined.

On the other hand, the present data provides strong evidence for the conversion of parasynapsed bivalents to the staggered configuration. Both the dramatic increase in the proportion of cells with staggered X-Y* bivalents between mid and late pachytene in XY* males (from 13% to 35%), that is accompanied by a parallel decrease in parasynapsed bivalents between these stages (from 60% to 25%), and the presence of an intermediate configuration that seems to be halfway between parasynapsed and staggered, support the view that this configuration change is occurring between mid and late pachytene.

It seems that for some reason the initial congression of telomeres on the nuclear envelope (Schertan et al., 1996) favours the X and Y* chromosomes pairing in the parasynapsed configuration. As in the majority of cases this will not provide for homologous interactions, these chromosomes will have to convert to the staggered configuration to recombine. The view that the sex chromosomes of XY* males in the parasynapsed configuration can be switching to the staggered
configurations to achieve recombination, fits with the view that recombinational interactions act to stabilise the initial weak interactions between homologues (Kleckner and Weiner, 1994). This view states that initial pairing interactions should involve very unstable contacts. These interactions are progressively stabilised by recombinational interactions, and the only truly permanent connection is a crossover. Pairing via unstable interactions has the advantage of providing a mechanism with flexibility to eliminate nonhomologous interactions.

In the case of the X and Y* chromosomes that are parasynapsed, the possibility of replacing the unstable nonhomologous interactions for more stable recombinational interactions could provide the driving force behind the switch to staggered. In the parasynapsed configuration, the proximity of regions with chromatin loops that could provide homologous and therefore recombinational interactions (specially in the region close to the inversion point of the pseudoautosomal region- Figure 2.1), will provide for the initial contacts. As these contacts will be homologous, they will allow for the initial recombinational interactions. The stability of these contacts will provide for additional interactions in the neighbouring regions and will extend from there, as longer stretches of homology are found. Eventually, the extent of this pairing will lead to a switch to the staggered configuration.

It is puzzling why this switch in orientation only becomes evident by late pachytene and not earlier. It is possible that the final force that allows the interruption of the SC, is the recruitment to the site of recombination of the bulk of the machinery needed for the final steps of recombination. The second possibility is that by late pachytene nonhomologous interactions are weakened or lost, and therefore at this stage those interactions that are stabilised by the initial recombination interactions take over. In support of this view is the fact that mice that lack Mlh1, and therefore are deficient in the final stages of
recombination, exhibit sex chromosome desynapsis by late pachytene (Baker et al., 1996). The same occurs in other organisms. Mutants that can't initiate recombination lose the initial association of homologues (Weiner et al., 1994, Loidl et al., 1994, Nag et al., 1995). These observations therefore provide a plausible basis for the switch from the parasympapsed to the staggered configuration.

In conclusion, the observations that in the XY* males all (or at least the majority) of the parasympapsed bivalents do finally recombine, a few whilst parasympapsed (that will give rise to the rare dicentric products observed at MII by Hale et al. in 1991), as shown by the Mlh1 antibody, and the rest after resolving into the staggered configuration, is in agreement with the fertility and sperm counts of these mice. But this finding also re-opens the question whether there is a requirement for recombination during meiosis for two reasons:

(a) The fact that all (or at least the majority) the parasympapsed bivalents that synapse also recombine makes it impossible to separate these two events in XY* mice.

(b) The MI pile up of cells with univalent sex chromosomes removes the need to propose that there are univalents at MI which had previously been synapsed (as seen here for both XY* and XYSxr^ mice).

Therefore, to determine if there really is a requirement for recombination during meiosis, other systems that do allow PAR recombination and synapsis to be separated, will have to be found.
Table 2.8. Observed frequency of each of the configurations observed in XY* males at pachytene, and predicted fate (x = eliminated, ✓ = survive, ? = no prediction made) of these configurations by each of the models (P = Parasynapsed, S = Staggered, AS = Asynapsed, Obs. = Observed frequencies of each configuration, Hale = Predicted fate by the Hale model (1994), B&M = Predicted fate by the Burgoyne and Mahadevaiah model (1993), R&B = Predicted fate by the model presented here. 1 = The Hale (1994) model would predict that the majority of bivalents will be eliminated, but a few will achieve recombination and avoid elimination, 2 = The Burgoyne and Mahadevaiah model (1993) predicted that the majority of parasynapsed bivalents will have PAR synapsis and will therefore be eliminated, but a few will have PAR-PAR synapsis and avoid elimination, 3 = The model presented here suggests that the majority of parasynapsed bivalents will have PAR-PAR synapsis and not be eliminated, but a few (12%) will have PAR asynapsis and therefore be eliminated).
CHAPTER 3.
A COMPARISON OF THE FERTILITY AND SYNAPSIS OF THE
SEX CHROMOSOMES OF XYY**X and XYY MICE.
3.1 INTRODUCTION.

XYY mice are almost always sterile (Cattanach and Pollard, 1969; Ratthenberg and Muller, 1973; Evans et al., 1978; Das and Kar, 1981; Tease, 1990), and have clear signs of spermatogenic impairment. There has been a long running debate as to whether this sterility is due to an excess Y chromosomal gene dosage due to the presence of two Y chromosomes (Hunt and Eicher, 1991), or whether it is a consequence of an unpaired sex chromosome, as originally postulated by Miklos (1974; reviewed by Burgoyne and Mahadevaiah, 1993). Although there may be considerable losses of XYY spermatocytes during pachytene (Palmer et al., 1990), the more obvious arrest is at the first meiotic metaphase (MI). At this stage the majority have a bivalent plus a univalent configuration, but in some cells the three sex chromosomes associate to form a trivalent which could satisfy pairing requirements. Burgoyne and Biddle (1980) have suggested that it is this trivalent formation that allows some cells to complete spermatogenesis, and that this could in some cases generate sufficient sperm for fertility and transmission of aneuploid XY and YY gametes.

More recently, Hunt and Eicher (1991) described mice with a normal X and Y chromosome plus the tiny Y*^X chromosome containing only the Y chromosome pseudoautosomal region and no other Y specific DNA. These mice, in marked contrast to XYY males, proved to be regularly fertile, although the trivalent frequencies at MI were similar. Since these mice have three copies of the pseudoautosomal region but only one dose of Y specific DNA, Hunt and Eicher concluded that the infertility of XYY mice must, at least in part, be due to an excess dosage of Y linked genes. However their MI analysis indicated that the Y*^X is a very efficient pairing partner; this led Burgoyne and Mahadevaiah (1993) to suggest the possibility of an increase in trivalent formation at pachytene that would allow survival of sufficient spermatocytes to account for the fertility of XYY*^X males.
To establish if there is any difference in the level of trivalent formation at pachytene between XYY*X and XYY males, which might contribute to their differing fertility, synaptonemal complex (SC) analysis was carried out for both these types of males. Trivalent frequencies were then related to their spermatogenic proficiency as measured by testis weights, sperm counts and fertility.

3.2 MATERIALS AND METHODS

3.2.1 Production of XYY**, XYY^ and XYy~ mice

The breeding crosses are summarised in Figure 3.1. The initial cross was used to generate XYY** males (Eicher et al., 1991) for use in the two subsequent crosses. For this purpose MF1 XY** females (Eicher et al., 1991) were crossed to 129 males, so that the XYY** offspring carried a 129 Y chromosome, an MF1 X chromosome and a Y** chromosome that is a product of a recombination between an LT Y* chromosome and an MF1 X chromosome. These offspring were initially crossed to MF1 XXY~ females where the Y~ denotes a Y129, Sry - negative chromosome (Koopman et al., 1989; Gubbay et al., 1992). This cross generated the two genotypes desired, XYY** (with an X of MF1 origin, the Y of 129 origin and the Y** of MF1/LT origin) and XYY~ males (with an MF1 X and the two 129 Y chromosomes). All mice were processed at between 2 and 2.5 months old. To produce the XYy~ males, MF1 XXy~ females (with the Sry deletion and a large interstitial deletion of ~2/3 of the long arm - Mahadevaiah et al., 1993) were used instead of the XXY~ females. These males were processed at 9.5 to 10 months of age.

3.2.2 Karyotyping

See Chapter 2 and Appendix 1.
3.2.3 **Sperm Counts.**
See Chapter 2 and Appendix 1.

3.2.4 **Synaptonemal Complex Analysis.**
See Chapter 2 and Appendix 1.

3.2.5 **Air-dried preparations.**
Air-dried slides of fixed testicular suspensions were prepared as described by Evans *et al.* (1964) and were stained with 2% giemsa (See Appendix 1). X-Y pairing in diakinesis/metaphase I spermatocytes, was scored using a high power objective.
Figure 3.1. Crosses set up to generate XYY*^ and XYY males. Note that the strain origin of the pairing region of the XYY* and XYY males is not indicated as their pairing region is recombinant and there are a number of possibilities for their pseudoautosomal region strain origin.
3.3 RESULTS.

3.3.1 Fertility, testis weights and sperm counts of XYY** and XYY* males

Tables 3.1 and 3.2 provide a summary of the male genotypes generated by the XXY* x XYY** cross and their mean testis weights and sperm counts. In this chapter the focus is on the comparison between XYY** and XYY* males.

There is a clear difference in the fertility of XYY** and XYY* mice. All except one of the XYY** males, but none of the XYY* males tested, were fertile. This difference in fertility is clearly reflected in the higher testis weights and sperm counts of the XYY** males (Table 3.2). Table 3.3 highlights the relationship between testis weights, sperm counts and fertility by showing the individual values for the males used for SC analysis. The only XYY** male (#5) that wasn't fertile by the time it was processed, was the male that had the lowest testis weight and sperm count. The fact that the XYY** males have lower testis weights and sperm counts than the XY controls (Table 3.2), indicates that they also have a certain degree of spermatogenic impairment, but this impairment is obviously much smaller than that of XYY* males. Some of the control XY males generated by the cross had unusually low sperm counts for this type of male. Plotting the testis weight and sperm count data (Figure 3.2) indicated that the males fell into two groups, one group with a mean sperm count of 1,243,083±180,199 sperm/caput and a mean testis weight of 107.7±14.6 mg, equivalent to that of XYRIII males in chapter 2 (1,500,167±424,311 sperm/caput and 99.3±22.3 mg), and a second group with a mean of only 549,600±49,277 sperm/caput and testes of 90.2±5.7 mg. Three males from each group were selected for subsequent SC analysis (Table 3.4).

3.3.2 Pachytene in XYY**X, XYY* and XY males.

The analysis of the degree of synopsis of the sex chromosomes of XYY**X (Table 3.5) and XYY* males (Table 3.6) at pachytene reveals that the amount of trivalent formation is very different between these types of males. XYY**X males have a
trivalent formation rate of 58% (range 45-75%) at pachytene (Figure 3.3). This is very clearly higher than the amount of trivalents in XYY− males, which is 29% (19-37%) at pachytene (Figure 3.4). Chi square tests on the totals confirmed that the differences are highly significant ($X^2 = 58.6, P<0.005$).

The XY males analysed had clearly higher levels of XY pairing (93% at pachytene -Table 3.7) than the levels of trivalent formation in XYY*X males, providing evidence that the partial spermatogenic impairment in XYY*X mice is due to pairing failure. The lack of differences in the level of sex chromosome synapsis between the two groups of XY males excludes univalent sex chromosomes from being responsible for the variability in sperm counts within the XY controls (Table 3.7).

In both XYY*X and XYY− males, those that had the higher sperm counts and testis weights tended to have the higher trivalent formation rate (Figure 3.5 and 3.6). In the case of the XYY*X mice, males 1 (with 688,000 sperm per caput) and 6 (423,000 sperm per caput) had clearly higher sperm counts and trivalent formation rates at pachytene (75%) than the rest of XYY*X males. This is also true for the XYY− males, as observed from the fact that male 7 (50,000 sperm/caput) and 8 (98,000 sperm/caput) had the highest sperm counts of all XYY− males analysed, as well as the highest trivalent rates (33% and 37% respectively). This evidence establishes a direct correlation between fertility (expressed in the sperm counts) and the level of trivalent formation, not only between the two genotypes but also within each genotype.

In all genotypes of males analysed, a proportion of cells had autosome asynapsis. This autosome asynapsis was predominantly observed at early pachytene, being greatly reduced by the mid pachytene stage, suggesting a progressive elimination of this type of cell.
3.3.3 Diakinesis/MI in XYY*^, XYY* and XY males.
It is clear that both XYY*^ and XYY* males present a drastic decrease in the frequency of trivalents by diakinesis/MI. By this stage both types of males have a similar level of trivalent formation (10% in XYY*^ mice- Table 3.5, and 8% in XYY* males- Table 3.6). In the case of the XY males, there is also a decrease in the level of pairing by diakinesis/MI. This is very probably due to the piling up of cells with univalent sex chromosomes at MI (see Chapter 2).

3.3.4 Analysis of older XYY*^ and XYy~ males.
As part of a preliminary experiment, XYY*^ males were mated to XYy~ females in order to produce XYY*^ and XYy~ males. Table 3.8 summarises the progeny frequencies for these crosses, and Table 3.9 gives the testis weight data for the males. Four XYY*^ and four XYy~ mice were produced, and were processed between the ages of 6 and 10 months (Table 3.10). All the XYY*^ males were immediately fertile, while the XYy~ mice were all initially sterile. Three of these XYy~ males became fertile after the age of 8 months, this fertility being due to breeding from euploid cell lines, as revealed by the absence of aneuploid offspring and the meiotic data presented below. This mosaicism in the germ line of the XYy~ mice is one reason for the higher testis weights (69.5±10.6mg) and sperm counts (367,050±283,623 sperm/caput) of these males when compared to those of the younger XYY~ males from the main experiment (40.6±4.0mg and 11,647±24,430 sperm/caput). This mosaicism rendered invalid any further attempt to compare the fertility of these two genotypes.

Although due to technical problems (see Appendix 1) insufficient cells could be analysed at pachytene for either type of male in this initial experiment, it is clear that the levels of trivalents in XYY*^ males are higher than the levels of trivalents of XYy~ mice (Table 3.11, Figure 3.4). By diakinesis/MI the level of
trivalent formation in XYY*\textsuperscript{x} mice has fallen to a level similar to that in XYy\textsuperscript{-} mice, as occurred in the main experiment (Table 3.12). Diakinesis/MI analysis reveals that in the XYy\textsuperscript{-} mice, between 18\% and 49\% of the cells had an euploid constitution, providing evidence for mosaicism in these males. Those mice that had higher levels of mosaicism had higher sperm counts and testis weights, providing further evidence that the fertility of older XYy\textsuperscript{-} males is due to breeding from euploid cell lines. This mosaicism of older XYy\textsuperscript{-} mice led to the study being repeated, as described above, by analysing only males of 2 to 2.5 months of age. Previous experience in our laboratory had shown that at this age mosaicism wouldn't be a problem.
Table 3.1. Record of the relative numbers of males produced of each expected genotype from the cross XXY\(^{-}\) x XYY\(^{**}\) for those litters where all males were karyotyped. An XYY\(^{**}\)Y\(^{**}\) male was also found when analysing the offspring of this cross. This male must have arisen due to a meiotic (paternal) or mitotic (early embryonic) non-disjunction event.

<table>
<thead>
<tr>
<th>Paternal Gametes</th>
<th>Y</th>
<th>XY</th>
<th>Y(^{**})X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Gametes</td>
<td>X</td>
<td>XY</td>
<td>XXY</td>
</tr>
<tr>
<td>Gametes</td>
<td>13</td>
<td>32</td>
<td>5</td>
</tr>
<tr>
<td>X(^{-})</td>
<td>XYY(^{-})</td>
<td>XYYY(^{-})</td>
<td>XYY(^{**})Y(^{-})</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Genotype</td>
<td>Number of mice</td>
<td>Testis Weight (mg.)</td>
<td>Sperm Count/Caput</td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
<td>---------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>XY</td>
<td>17</td>
<td>102.5 ± 15.0</td>
<td>1,039,118±351,397</td>
</tr>
<tr>
<td>XYY*^x</td>
<td>6</td>
<td>61.0 ± 13.8</td>
<td>340,000±171,072</td>
</tr>
<tr>
<td>XYY^-</td>
<td>17</td>
<td>40.6 ± 4.0</td>
<td>11,647±24,430</td>
</tr>
<tr>
<td>XYY^-Y^x</td>
<td>9</td>
<td>39.8 ± 4.2</td>
<td>2,000±2,828</td>
</tr>
<tr>
<td>XXY</td>
<td>32</td>
<td>27.4 ± 3.7</td>
<td>-</td>
</tr>
<tr>
<td>XXYY^-</td>
<td>28</td>
<td>27.1 ± 4.6</td>
<td>-</td>
</tr>
<tr>
<td>XYY*XYY^-</td>
<td>1</td>
<td>39</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3.2. Average testes weights and sperm counts for males of each genotype produced by the cross XYY*^x x XYY^- . The difference between the mean testis weights and mean sperm counts of the XYY*^x and XYY^- mice is highly significant as revealed by a Student's t-test (For the testis weights t= 5.22, P<0.001; for the sperm counts t= 7.36, P<0.001). [Note: XXY and XXYY^- males have very small testes and produce no sperm, because two X chromosomes in the germline are incompatible with spermatogenesis]
<table>
<thead>
<tr>
<th></th>
<th>Testis Weight (mg)</th>
<th>Sperm Count/ Caput</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYY*</td>
<td>84</td>
<td>688,000</td>
</tr>
<tr>
<td>XYY*</td>
<td>50</td>
<td>222,000</td>
</tr>
<tr>
<td>XYY*</td>
<td>50</td>
<td>257,000</td>
</tr>
<tr>
<td>XYY*</td>
<td>63</td>
<td>238,000</td>
</tr>
<tr>
<td>XYY*</td>
<td>46</td>
<td>212,000</td>
</tr>
<tr>
<td>XYY*</td>
<td>73</td>
<td>423,000</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>61.0 ± 13.8</td>
<td>340,000±171,072</td>
</tr>
<tr>
<td>XYY^-</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>XYY^-</td>
<td>42</td>
<td>3,000</td>
</tr>
<tr>
<td>XYY^-</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>XYY^-</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>XYY^-</td>
<td>38</td>
<td>4,000</td>
</tr>
<tr>
<td>XYY^-</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>XYY^-</td>
<td>45</td>
<td>50,000</td>
</tr>
<tr>
<td>XYY^-</td>
<td>51</td>
<td>98,000</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>41.4±4.1</td>
<td>17,000±32,265</td>
</tr>
</tbody>
</table>

Table 3.3. Testis weights and sperm counts of XYY* and XYY^- males used to analyse sex chromosome synapsis during the first meiotic prophase.
<table>
<thead>
<tr>
<th></th>
<th>Testis Weight (mg)</th>
<th>Sperm Count/Caput</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY 1</td>
<td>84</td>
<td>482,000</td>
</tr>
<tr>
<td>XY 2</td>
<td>85</td>
<td>513,000</td>
</tr>
<tr>
<td>XY 3</td>
<td>92</td>
<td>610,000</td>
</tr>
<tr>
<td>XY 4</td>
<td>86</td>
<td>1,107,000</td>
</tr>
<tr>
<td>XY 5</td>
<td>96</td>
<td>1,122,000</td>
</tr>
<tr>
<td>XY 6</td>
<td>106</td>
<td>1,360,000</td>
</tr>
</tbody>
</table>

Table 3.4. Testis weights and sperm counts of the XY males selected for SC analysis. Males 1-3 are from the low sperm count group and males 4-6 are the males with normal control male sperm counts.
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Y</td>
<td>4</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>9</td>
<td>3</td>
<td>60 (22%)</td>
</tr>
<tr>
<td>Y**x</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>21 (8%)</td>
</tr>
<tr>
<td>Univ.</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>Chain</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>12</td>
<td>26 (9%)</td>
</tr>
<tr>
<td>Triv.</td>
<td>30</td>
<td>28</td>
<td>18</td>
<td>24</td>
<td>24</td>
<td>37</td>
<td>161 (58%)</td>
</tr>
<tr>
<td></td>
<td>(75%)</td>
<td>(55%)</td>
<td>(45%)</td>
<td>(51%)</td>
<td>(60%)</td>
<td>(65%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>51</td>
<td>40</td>
<td>47</td>
<td>40</td>
<td>57</td>
<td>275</td>
</tr>
</tbody>
</table>

|      | 5  | 6  | 3  | 2  | 1  | -  | 17 (7%) |
| Y    | 12 | 33 | 33 | 26 | 27 | -  | 131 (53%) |
| Y**x | 19 | 6  | 6  | 10 | 9  | -  | 50 (20%) |
| Univ.| 4  | 2  | 3  | 5  | 4  | -  | 18 (7%)  |
| Chain| 2  | 1  | 1  | 1  | 3  | -  | 8 (3%)   |
| Triv.| 8  | 2  | 4  | 6  | 6  | -  | 26 (10%) |
|      | (16%) | (4%) | (8%) | (12%) | (12%) |       |       |
| Total| 50 | 50 | 50 | 50 | 50 | -  | 250   |

Table 3.5. Synapsis of the sex chromosomes of XYY**x** males during pachytene and diakinesis/MI. P= Pachytene, D/MI= Diakinesis/Metaphase I, X= X chromosome univalent and YY**x** paired, Y= Y chromosome univalent and XY**x** paired, Y**x** = Y**x** chromosome univalent and XY paired, Univ.= All sex chromosomes present as univalents, Chain= Sex chromosomes forming a chain, Triv= Sex chromosomes forming a trivalent.
Table 3.6. Synapsis of the sex chromosomes of XYY⁻ males during pachytene and diakinesis/MI. P= Pachytene, D/MI= Diakinesis/Metaphase I, X= X chromosome univalent and YY paired, Y= Y chromosome univalent and XY paired, Univ.= All sex chromosomes present as univalents, Chain= Sex chromosomes forming a chain, Triv= Sex chromosomes forming a trivalent.
Table 3.7. X-Y Pairing during the first meiotic prophase of the low sperm count (1-3) XY males and the more normal XY controls (4-6). P= pachytene, D/MI= Diakinesis/Metaphase I. Paired= Sex chromosomes synapsed. NP= Sex chromosomes unsynapsed.
Table 3.8. Record of the relative numbers of mice produced of each possible genotype from the cross XXy" x XYY*^ for those litters where all mice were karyotyped.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of mice</th>
<th>Testis Weight (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY</td>
<td>10</td>
<td>119.9 ± 3.5</td>
</tr>
<tr>
<td>XYY*^x</td>
<td>8</td>
<td>77.4 ± 4.2</td>
</tr>
<tr>
<td>XYy&quot;</td>
<td>9</td>
<td>60.0 ± 4.2</td>
</tr>
<tr>
<td>XXY*^xYy&quot;</td>
<td>11</td>
<td>62.0 ± 3.0</td>
</tr>
<tr>
<td>XXY</td>
<td>31</td>
<td>25.2 ± 1.1</td>
</tr>
<tr>
<td>XXYy&quot;</td>
<td>38</td>
<td>23.8 ± 1.1</td>
</tr>
</tbody>
</table>

Table 3.9. Average testis weights for males of each genotype produced by the cross XYY*^x Xxy".
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age processed</th>
<th>Testis Weight (mg)</th>
<th>Sperm per caput (x10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYY^x</td>
<td>6 months</td>
<td>71.9</td>
<td>660.0</td>
</tr>
<tr>
<td>XYY^x</td>
<td>9.5 months</td>
<td>51.6</td>
<td>194.8</td>
</tr>
<tr>
<td>XYY^x</td>
<td>8.6 months</td>
<td>88.2</td>
<td>1005.0</td>
</tr>
<tr>
<td>XYY^x</td>
<td>9 months</td>
<td>77.5</td>
<td>578.0</td>
</tr>
<tr>
<td>XYy^-</td>
<td>10 months</td>
<td>68.4</td>
<td>144.4</td>
</tr>
<tr>
<td>XYy^-</td>
<td>10 months</td>
<td>87.05</td>
<td>381.3</td>
</tr>
<tr>
<td>XYy^-</td>
<td>9.5 months</td>
<td>59.3</td>
<td>117.5</td>
</tr>
<tr>
<td>XYy^-</td>
<td>9 months</td>
<td>63.2</td>
<td>825.0</td>
</tr>
</tbody>
</table>

Table 3.10. Testis weights and sperm counts of XYY^x and XYy^- males tested for fertility and used for MI and SC analysis.
### Table 3.11. Synapsis at pachytene of the sex chromosomes in the cells analysed from XYY*^x and XYy^- mice. (percentages are only calculated for the total number of cells where three sex chromosomes were visible). * 3 sex chromosomes only.

<table>
<thead>
<tr>
<th>Configurations</th>
<th>XYY*^x 3</th>
<th>XYY*^x 4</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYY*^x</td>
<td>7 (70%)</td>
<td>10 (58.8%)</td>
<td>17 (63%)</td>
</tr>
<tr>
<td>XY*^x + Y</td>
<td>3 (30%)</td>
<td>2 (11.8%)</td>
<td>5 (18.5%)</td>
</tr>
<tr>
<td>XY + Y*^x</td>
<td>0 (0%)</td>
<td>1 (5.9%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>X + YY*^x</td>
<td>0 (0%)</td>
<td>1 (5.9%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>X + Y + Y*^x</td>
<td>0 (0%)</td>
<td>3 (17.6%)</td>
<td>3 (17.7%)</td>
</tr>
<tr>
<td>XY</td>
<td>2</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>X + Y</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>XY*^x</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total Cells</td>
<td>14</td>
<td>24</td>
<td>27*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Configurations</th>
<th>XYy^- 2</th>
<th>XYy^- 3</th>
<th>XYy^- 4</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYy^-</td>
<td>0 (0%)</td>
<td>2 (7.7%)</td>
<td>2 (13.3%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>XY + y^-</td>
<td>2 (22.2%)</td>
<td>8 (30.8%)</td>
<td>1 (6.7%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Xy^- + Y</td>
<td>3 (33.4%)</td>
<td>2 (7.7%)</td>
<td>1 (6.7%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>X + Y + y^-</td>
<td>2 (22.2%)</td>
<td>3 (11.5%)</td>
<td>6 (40%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>X + Yy^-</td>
<td>2 (22.2%)</td>
<td>11 (42.3%)</td>
<td>5 (33.3%)</td>
<td>18 (36%)</td>
</tr>
<tr>
<td>X + y^-</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Xy^-</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>X + Y</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>XY</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Total Cells</td>
<td>14</td>
<td>31</td>
<td>24</td>
<td>50*</td>
</tr>
<tr>
<td>Configurations</td>
<td>XYY*^ 2</td>
<td>XYY*^ 3</td>
<td>XYY*^ 4</td>
<td>Totals</td>
</tr>
<tr>
<td>----------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>XYY*^</td>
<td>13 (11.8%)</td>
<td>5 (7.6%)</td>
<td>11 (11%)</td>
<td>29 (10.5%)</td>
</tr>
<tr>
<td>XY*^ + Y</td>
<td>69 (62.3%)</td>
<td>39 (59%)</td>
<td>54 (54%)</td>
<td>162 (58.9%)</td>
</tr>
<tr>
<td>X + YY*^</td>
<td>14 (12.7%)</td>
<td>7 (10.6%)</td>
<td>10 (10%)</td>
<td>31 (11.3%)</td>
</tr>
<tr>
<td>XY + Y*^</td>
<td>8 (7.3%)</td>
<td>11 (16.7%)</td>
<td>15 (15%)</td>
<td>34 (12.4%)</td>
</tr>
<tr>
<td>X + Y + Y*^</td>
<td>6 (5.4%)</td>
<td>4 (6.1%)</td>
<td>9 (9%)</td>
<td>19 (6.9%)</td>
</tr>
</tbody>
</table>

| XY            | 3 | - | 8 | - |
| X + Y         | 3 | 2 | 5 | - |

Total cells | 116 | 68 | 112 | 275 |

<table>
<thead>
<tr>
<th>Config.</th>
<th>XYy^- 1</th>
<th>XYy^- 2</th>
<th>XYy^- 3</th>
<th>XYy^- 4</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYy^-</td>
<td>3 (6.4%)</td>
<td>4 (8.2%)</td>
<td>6 (11.5%)</td>
<td>0 (0%)</td>
<td>13 (8.1%)</td>
</tr>
<tr>
<td>Xy^- + Y</td>
<td>11 (23.4%)</td>
<td>16 (32.7%)</td>
<td>10 (19.3%)</td>
<td>5 (38.5%)</td>
<td>42 (26.1%)</td>
</tr>
<tr>
<td>XY + y^-</td>
<td>11 (23.4%)</td>
<td>8 (16.3%)</td>
<td>6 (11.5%)</td>
<td>2 (15.4%)</td>
<td>27 (16.8%)</td>
</tr>
<tr>
<td>X + Yy^-</td>
<td>11 (23.4%)</td>
<td>13 (26.5%)</td>
<td>22 (42.3%)</td>
<td>2 (15.4%)</td>
<td>48 (29.8%)</td>
</tr>
<tr>
<td>X + Y + y^-</td>
<td>11 (23.4%)</td>
<td>8 (16.3%)</td>
<td>8 (15.4%)</td>
<td>4 (30.7%)</td>
<td>31 (19.2%)</td>
</tr>
<tr>
<td>XY</td>
<td>1</td>
<td>25</td>
<td>5</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>Xy^-</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>X + y^-</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>X + Y</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Total | 57 | 89 | 64 | 41 | 161 |

Table 3.12. Diakinesis/MI synapsis configurations for the three sex chromosomes of the XYY*^ and XYy^- mice analysed. (Percentages are only calculated for the total number of cells where three sex chromosomes were visible). Config. = Configurations.
Figure 3.2. Plot of the testis weights vs. the sperm counts of the XY males from the cross XXY* x XYY*x, showing how these mice fall into two groups. A student t-test reveals that the mean sperm counts and the mean testis weights of these two groups are significantly different (For the sperm counts: t=3.24, P< 0.001; and for the testis weights: t=2.43, P<0.05).
Figure 3.3. Examples of sex chromosome configurations in pachytene spreads from XYY**X males:

(A-C) The X, Y and Y**X chromosomes are forming a trivalent.
(D) The X and Y**X chromosomes are synapsed, and the Y chromosome is present as a univalent.
(E-F) The X and Y chromosomes are synapsed, and the Y**X chromosome is present as a univalent.
Figure 3.4. Examples of sex chromosome configurations in pachytene cells of XYY− and XYy− males:

(A-C) The X, Y and Y− chromosomes are forming a trivalent.

(D) The X, Y and Y− chromosomes are forming a chain.

(E) Both Y chromosomes are synapsed, and the X is forming a circle.

(F-G) The X and Y chromosomes are synapsed, and the other Y chromosome is present as a univalent.

(H) Both Y chromosomes are synapsed, and the X chromosome is present as a univalent.

(I) The X and both Y chromosomes are present as univalent. One of the Y chromosomes is selfsynapsed (indicated with an arrow).

(J) The X, Y and y− chromosomes of an XYy− male are forming a trivalent.
Figure 3.5. Plot showing the relationship between the proportion of cells with all the sex chromosomes synapsed and the sperm counts of XYY*, XYY- and XY males.
Figure 3.6. Plot showing the relationship between the proportion of cells with all the sex chromosomes synapsed and the testis weights of XYY*¥, XYY- and XY males.
3.4 DISCUSSION

The present study was designed to determine the basis of the difference in fertility between XYY*^ and XYY" males. Both these types of mice were produced on an identical genetic background, allowing a direct comparison between the two genotypes. The mean testis weights and sperm counts of XYY*^ males (61.0±13.8 mg and 340,000±171,072 sperm/caput) were higher than those of XYY" males (40.6±4.0 mg and 11,647±24,430 sperm/caput), but lower than those of XY mice (101.6±15.0 mg and 1,039,118±351,397 sperm/caput). This indicates that, as previously reported (Hunt and Eicher, 1991), XYY*^ mice do have some degree of spermatogenic impairment, but significantly less than that of XYY" males.

Having established the relative spermatogenic proficiency of the two genotypes compared to the controls, the percentage of cells for each genotype that would satisfy the requirements of a meiotic checkpoint monitoring synapsis and/or recombination was determined by synaptonemal complex (SC) analysis. This analysis revealed that at pachytene large differences exist between genotypes in the amount of cells with all sex chromosome synapsed. At pachytene XY males have clearly the highest levels of synapsis (93% of cells), XYY*^ males have intermediate levels of cells with all sex chromosomes synapsed (58%), and in XYY" mice these levels are clearly even lower (29%). The large difference in trivalent formation between XYY*^ and XYY" mice (nearly 30%), provides direct evidence for the suggestion, made by Burgoyne and Mahadevaiah (1993), that the difference in fertility between these two types of mice might be a reflection of different levels of sex chromosome synapsis at pachytene. The fact that within each genotype, those males with the highest sperm counts have the highest trivalent rates, further supports this view that the level of sex chromosome synapsis is the major factor affecting fertility. It is also in agreement with Burgoyne and Biddle’s (1980) original suggestion that it is only the cells with trivalents which escape elimination at MI.
It should be noted that the relationship between sperm count and percent full synapsis (Figure 3.5) is non-linear and that sperm counts of zero are reached when there still is 24% synapsis. We take this to imply that apoptotic elimination of spermatocytes triggered by asynapsed sex chromosomes (see Chapter 5) can lead to the death of adjacent spermatocytes (with which they are joined by intercellular bridges) - indeed, it has been observed that cell death in the testis tends to affect groups of spermatogenic cells (Huckins, 1978; Henriksen et al., 1996).

One striking feature of the present data is the drop in frequency of trivalents between pachytene and Diakinesis/MI: from 58% to 10% in XYY* males and from 29% to 8% in XYY- males. The similarity in frequencies at Diakinesis/MI between these two genotypes is as previously noted by Hunt and Eicher (1991). Three possible causes could account for this drop in trivalent frequencies: either (i) a selection against trivalents between these stages, (ii) a separation of those trivalents with only one chiasma into a bivalent and a univalent, or (iii) a pile-up of cells with univalent sex chromosomes at MI (as observed in Chapter 2).

The strong correlation in XYY* and XYY- males between trivalent frequencies and amount of sperm produced (Figure 3.5) rules out the possibility of a selection occurring against trivalents between pachytene and diakinesis/MI in XYY mice. On the other hand, previous studies carried out on five XY^-y/XY^-/Xy germ line mosaic males by Mahadevaiah, Evans and Burgoyne (Unpublished observations - Figure 3.7) indicate that a separation of trivalents with only one chiasma and a pile-up at MI of univalent sex chromosomes does occur in XYY males. The analysis of XY^-y/XY^-/Xy males reveals that despite the selection against XY^-y cells between pachytene and diakinesis, the frequency of XY^-y cells with bivalent plus univalent configurations goes up markedly. As there seems to
be no selection against trivalents in XYY mice, this increase in cells with bivalent plus univalent configurations is very likely due to the separation of trivalents with only one chiasma. The analysis of these XY'y/XY'/Xy mosaics also shows that there is a pile up of XY'y cells between diakinesis and MI, and as observed in Chapter 2, this pile up preferentially affects the cells with univalents.

It is intriguing that the drop in trivalent frequency is greater in XYY** males than in XYY* mice. It is possible, that in the case of XYY** trivalents, in which the region of homology is restricted to the short segment of the pseudoautosomal region, crossover interference could inhibit in the majority of the cases the occurrence of a second chiasma. In the case of the XYY* males, it is possible that a second crossover could occur outside the PAR, but within the region of homology between the two Y chromosomes, thereby avoiding this constraint.

The potential for fertility of XYY men seems much greater than in mouse. The fertility of XYY humans ranges from near normal to severe spermatogenic impairment (Skakkebaek et al., 1973; Baghdassarian et al., 1975). As in the mouse, the spermatogenic impairment in XYY men is thought to be due, at least in part, to disruption of chromosome pairing (Chandley, 1984). The conclusion, drawn from the comparison of XYY** and XYY* mice, that the level of sex chromosome pairing is a major factor in the sterility if XYY males, supports this view. However, only two cases of transmission of a YY gamete giving rise to XYY sons have been reported (Tzoneva-Maneva et al., 1966; Sundequist and Hellstrom, 1969). This is explained by the fact that there is frequently a loss of one Y chromosome in some spermatogonic cells of XYY men (Thompson et al., 1967; Melynk et al., 1969; Evans et al., 1970; Chandley et al., 1976; Faed et al., 1976; Benet and Martin, 1988). This is equivalent to the situation found in the older XYY* mice analysed in the present study, which became fertile but produced only euploid offspring. It is therefore likely that in XYY humans a selection over time
occurs in favour of those germ cells that have lost one Y chromosome due to a mitotic error. The XYY mice that become mosaic when older, should provide a good model to investigate the basis of this selection, and allow parallels to be drawn with human.

In conclusion, it is clear that the difference in sex chromosome synapsis at pachytene between XYY* and XYY- mice is sufficient to account for the difference in fertility. However this does not rule out a deleterious role of double Y gene dosage. Attempts to test for a Y dosage effect are described in the next chapter.
Figure 3.7. Plot of the mean ratio of XYY/euploid germ cells present at different stages in 5 XY−y/XY−/Xy germ line mosaic males. The total number of cells analysed at each stage are indicated above the correspondent bar. G= Spermatogonia, EP= Early Pachytene, MP= Mid Pachytene, LP= Late Pachytene, DIA= Diakinesis, MI= Metaphase I, MII= Metaphase II.
CHAPTER 4.
ANALYSIS OF THE FERTILITY AND SYNAPSIS OF THE SEX
CHROMOSOMES OF $X^Y Y^*$, $XY Y^* X$ AND $XY Y^* Y^* X$ MICE.
4.1 INTRODUCTION.

The deleterious role of excess Y gene dosage on spermatogenesis (Hunt and Eicher, 1991) and the presence of an unpaired sex chromosome during meiosis (Burgoyne and Mahadevaiah, 1993) have both been suggested as possible reasons for the sterility of XYY males, but are not necessarily mutually exclusive. The comparison of the fertility and sex chromosome synapsis at pachytene of XYY*^ and XYY° mice in Chapter 3, led to the conclusion that disruption of sex chromosome synapsis is the major factor leading to the impairment of XYY spermatogenesis. However, this does not exclude the possibility that double Y gene dosage could also have a deleterious effect.

Aside from the study of XYY*^ mice by Hunt and Eicher (1991), some other lines of evidence have been used to support the idea that the presence of two intact Y chromosomes affects germ cell survival. Evans et al., (1978) examined histological sections from the testes of an XYY male mouse and observed a progressive decline in spermatogenesis that appeared to begin at an early stage. From this observation, they suggested that XYY males have a reduction in germ cell numbers prior to the onset of homologous pairing, with degeneration of germ cells as early as the type-A spermatogonia stage. Similarly, Skakkebaek et al., (1973) reported a range of histological abnormalities in human XYY males, from tubules undergoing apparently normal spermatogenesis to those containing Sertoli cells but completely lacking germ cells, suggesting very early disruption of germ cell development in human XYY males. Together, these data have been taken to suggest that there are factors, other than the disruption of synapsis, but still associated with the presence of two Y chromosomes, that contribute to the sterility of XYY males. However, as Burgoyne and Mahadevaiah (1993) have pointed out, these pre-pachytene deficiencies may simply be secondary consequences of continuing spermatocyte death - just as has been documented in XSex°O mice (Sutcliffe et al., 1991).
Nevertheless, given the accumulating evidence for the presence of genes involved in spermatogenesis on the mouse and human Y chromosomes (Burgoyne, 1996), it remains entirely possible that an increase in Y gene dosage may impair sperm production or function. In order to investigate this possibility, two types of mice were produced in which there was the potential for full sex chromosome synapsis, despite the presence of two Y chromosomes. First, $X^Y^*Y$ males, that have the potential to form one bivalent in the presence of two doses of Y specific genes were generated. Second, $XYYY^*X$ males, that have the potential to form two bivalents in the presence of two doses of Y specific genes, were compared to $XYY^*XY^*X$ mice, that have the potential to form two bivalents in the presence of only one dose of Y specific genes. The analysis of spermatogenesis in these animals, when put in the context of their relative pairing failure levels should address this question.

4.2 MATERIALS AND METHODS

4.2.1 Production of $X^Y^*Y^-$ males

The breeding crosses used to generate $X^Y^*Y^-$ males are summarised in Figure 4.1. For this purpose $XY^*$ males (Eicher et al., 1991) were mated to $XY^-$ females (Mahadevaiah et al., 1993).

4.2.2 Production of $XYY^*Y^X$, $XYY^*XY^*X$ and $XYy^*Y^*X$ mice.

The cross set up to produce $XYY^*Y^X$ and $XYy^*Y^*X$ mice has already been described in Chapter 2. One $XYY^*XY^*X$ male was produced by this cross, presumably as the result of a meiotic or mitotic non-disjunction event.

4.2.3 Karyotyping

See Chapter 2 and Appendix 1.
4.2.4 Sperm Counts.

See Chapter 2 and Appendix 1.

4.2.5 Synaptonemal Complex Analysis.

See Chapter 3 and Appendix 1.
Figure 4.1. Cross set up to generate $X^Y*Y^-$ males.
4.3 RESULTS

4.3.1 Fertility, testis weights, sperm counts and pairing of the sex chromosomes at pachytene in \(X^Y^*Y^-\) males.

\(X^Y^*Y^-\) males have two doses of Y specific DNA and have the potential to satisfy the pairing requirements by forming one bivalent. The two males of this genotype analysed were found to be sterile, with mean testis weights of 48±2 mg, and no sperm in the caput (Table 4.1). From the analysis of the pairing of the sex chromosomes at pachytene in these mice, it is clear that in only a very small proportion of cells (6% -Table 4.2, Figure 4.2) both the pseudoautosomal regions are paired. This fact alone could be responsible for the spermatogenic arrest observed, and it is therefore impossible to determine in these males if the presence of two Y chromosomes has a deleterious effect or not.

4.3.2 Fertility, testis weights and sperm counts of \(XYY^-Y^{*X}\) and \(XYY*-Y^{*X}\) males.

\(XYY^-Y^{*X}\) mice have the potential to satisfy the pairing requirements (by forming two bivalents) in the presence of double Y gene dosage. All the males of this genotype analysed were sterile, had low testis weights (39.8 ± 4.2 mg ) and low sperm counts (2,000±2,828 sperm/caput). These testis weights and sperm counts of \(XYY^-Y^{*X}\) mice are clearly very reduced when compared to those of the XY males produced in the same cross (101.6 ± 15.0 mg and 1,039,118±351,397 sperm/caput). This level of spermatogenic impairment accounts for the sterility observed in these animals.

Amongst the progeny of the \(XYY^- x XYY^{*X}\) cross was an \(XYY^*Y^{*X}\) male. This type of mouse has the potential to form two bivalents in the presence of only one Y chromosome, and therefore can be used as a control when analysing the effects of Y gene dosage and sex chromosome pairing failure in \(XYY^-Y^{*X}\) males. When test mated it was found to be sterile. Once again this sterility is clearly due to
severe spermatogenic impairment (testis weight = 39mg, sperm count = 0 sperm/caput). This testis weight and sperm count is not significantly different from those of XYY−Y*X males, indicating that both types of mice have very similar levels of spermatogenic impairment.

This XYY*Y*X and five XYY−Y*X mice were selected for SC analysis. The individual testis weight and sperm count data are given in Table 4.3.

4.3.3 Sex chromosome synapsis at pachytene in XYY−Y*X, and XYY*XY*X males.

To test if the requirements of a checkpoint monitoring sex chromosome synapsis are being fulfilled in XYY−Y*X and XYY*XY*X males, synaptonemal complex (SC) analysis of pachytene cells was carried out for these mice. In the XYY−Y*X males analysed, a significant proportion of cells had all their sex chromosomes synapsed. Overall, 51% of cells at pachytene (Table 4.4), had the sex chromosomes forming two bivalents or a quadrivalent (Figure 4.4). Both these configurations should satisfy the requirements for sex chromosome synapsis. These levels of cells with all the sex chromosomes synapsed (51% at pachytene) are equivalent to the levels observed in some of the XYY*X males (Chapter 3), that exhibited fertility and had much higher mean testis weights and sperm counts. This is highlighted in Figure 4.3, which plots the levels of synapsis vs sperm counts for XYY−Y*X males alongside those for the XYY*X and XYY− males generated from the same cross. This indicates that in addition to the spermatogenic impairment resulting from incomplete sex chromosome synapsis, there must be an additional factor related to the presence of the extra Y chromosome.

What is the nature of this additional factor? Analysis of the pairing of the sex chromosomes at pachytene in the XYY*XY*X male suggests that the spermatogenic failure of the XYY−Y*X mice is due to the presence of an extra sex
chromosome *per se*, rather than an increase in Y gene dosage. In the XYY*XY* male, 40% of the cells at pachytene, had all their sex chromosomes synapsed (Table 4.5, Figure 4.5). From the relationship between sperm count and level of synapsis established in Chapter 3 (Figure 3.2), this level of cells satisfying the requirements for synapsis would be predicted to give a sperm count of 136,000 sperm/caput, instead of the observed sperm count of 0 (see Figure 4.3). Thus, the presence of extra sex chromosome material appears to have a deleterious effect on spermatogenesis, whether or not it increases Y gene dosage.

4.3.4 Fertility, testis weights, sperm counts and sex chromosome pairing at pachytene in XYY-Y* males.

As part of a preliminary experiment, a number of XYY-Y* males were produced that had the Y chromosome instead of the Y chromosome. Eight of these males were tested for fertility and all were found to be sterile. The average testis weights (64.03 mg) and sperm counts (67,800±11,800 sperm/caput) of these males (Table 4.6) indicates that their sterility is due to severe spermatogenic impairment.

Although due to technical problems (see Appendix 1) insufficient XYY-Y* pachytene cells were analysed (Table 4.7), it is clear that a proportion of cells are mosaic, making any relationship between pairing levels and spermatogenic failure invalid (Figure 4.4).
### Table 4.1. Testis weights and sperm counts of $X^{Y^*}Y^-$ males.

<table>
<thead>
<tr>
<th>$X^{Y^*}Y^-$</th>
<th>Testis Weight (mg)</th>
<th>Sperm Count/Caput</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>48±2</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 4.2. Pairing of the sex chromosomes at pachytene in $X^{Y^*}Y^-$ males. Paired PAR-PAR = Pseudoautosomal region of the $X^{Y^*}$ chromosome paired with the pseudoautosomal region of the $Y^-$ chromosome. Paired SA-SA = Short arm of the $Y^*$ chromosome paired with the short arm of the $Y^-$ chromosome.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paired PAR-PAR</td>
<td>2</td>
<td>2</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Paired SA-SA</td>
<td>4</td>
<td>7</td>
<td>11 (16%)</td>
</tr>
<tr>
<td>Not Paired</td>
<td>19</td>
<td>34</td>
<td>53 (78%)</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>43</td>
<td>68</td>
</tr>
</tbody>
</table>
Table 4.3. Testis weights and sperm counts of XYY−Y*Y and XYY*XY*X males analysed for sex chromosome synapsis at pachytene.

<table>
<thead>
<tr>
<th></th>
<th>Testis Weight (mg)</th>
<th>Sperm Count/Caput</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYY−Y*Y 1</td>
<td>36</td>
<td>1,000</td>
</tr>
<tr>
<td>XYY−Y*Y 2</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>XYY−Y*Y 3</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>XYY−Y*Y 4</td>
<td>46</td>
<td>9,000</td>
</tr>
<tr>
<td>XYY−Y*Y 5</td>
<td>40</td>
<td>3,000</td>
</tr>
<tr>
<td>XYY−Y*Y Average</td>
<td>40.2±3.2</td>
<td>2,600±3,382</td>
</tr>
<tr>
<td>XYY<em>XY</em>X 1</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>XYY**X</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(2%)</td>
<td>(2%)</td>
</tr>
<tr>
<td>X Y*X+YY</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>(29%)</td>
<td>(40%)</td>
</tr>
<tr>
<td>X Y+YY**X</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(4%)</td>
<td>(2%)</td>
</tr>
<tr>
<td>XY+Y+Y*</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>X Y*X+Y+Y</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>XYY*X+Y</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>XYY+Y*X</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>X+Y+YY**X</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>X+YYY**X</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>X+YY+Y**X</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Chain</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>X+Y+Y+Y**X</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>42</td>
</tr>
</tbody>
</table>

Table 4.4. Synapsis of the sex chromosomes at pachytene in XYY-Y**X males. In bold are sex chromosomes that are synapsed. P= Pachytene.
Table 4.5. Synapsis of the sex chromosomes at pachytene (P) in an \textit{XYY*XY**X} male. In bold are sex chromosomes that are synapsed. P = Pachytene.

<table>
<thead>
<tr>
<th>P</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>XYY*XY**X</td>
<td></td>
<td>4 (10%)</td>
</tr>
<tr>
<td>XY*XY+YY**X</td>
<td></td>
<td>7 (17%)</td>
</tr>
<tr>
<td>XY+Y*XY**X</td>
<td></td>
<td>5 (13%)</td>
</tr>
<tr>
<td>XY+Y*XY+Y**X</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>XY*XY+Y**X+Y</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>XYY*XY+Y**X</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>XY*XY**X+Y</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>X+YY*XY**X</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>X+Y*XY+YY**X</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>X+Y+Y*XY**X</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Chain</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>X+Y+Y<strong>X+Y</strong>X</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>
### Table 4.6. Testis weights and sperm counts of XYy^{-}Y^{x} males.

<table>
<thead>
<tr>
<th>XYy^{-}Y^{x} mice</th>
<th>Age Processed</th>
<th>Testis Weight (mg.)</th>
<th>Sperm per Caput (x10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.5 months</td>
<td>56.4</td>
<td>19.7</td>
</tr>
<tr>
<td>2</td>
<td>7 months</td>
<td>69.2</td>
<td>106.7 *</td>
</tr>
<tr>
<td>3</td>
<td>12.3 months</td>
<td>67.6 *</td>
<td>82.5</td>
</tr>
<tr>
<td>4</td>
<td>10.7 months</td>
<td>53.3</td>
<td>36.2</td>
</tr>
<tr>
<td>5</td>
<td>13 months</td>
<td>84.5</td>
<td>80.0</td>
</tr>
<tr>
<td>6</td>
<td>9.7 months</td>
<td>50.8 *</td>
<td>110.0 *</td>
</tr>
<tr>
<td>7</td>
<td>12.5 months</td>
<td>68.0</td>
<td>45.0</td>
</tr>
<tr>
<td>8</td>
<td>12.5 months</td>
<td>62.5</td>
<td>54.1</td>
</tr>
<tr>
<td>Average</td>
<td>-</td>
<td>64.03</td>
<td>67.8 ± 11.8</td>
</tr>
</tbody>
</table>

* Data from only one testis.
### Table 4.7. Configurations at pachytene of the sex chromosomes in the cells analysed by synaptonemal complex preparation of XYy^*-Y*^ animals. Due to low amount of cells analysed the pairing configurations were only classified into: II = sex chromosomes in bivalent configuration; III = sex chromosomes forming trivalent association; IV = sex chromosomes as quadrivalent; I = sex chromosomes as univalent.

<table>
<thead>
<tr>
<th>Configurations</th>
<th>XYy^<em>-Y</em>^ male 1</th>
<th>XYy^<em>-Y</em>^ male 2</th>
<th>XYy^<em>-Y</em>^ male 4</th>
<th>Total number cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>II + II</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>12 (20%)</td>
</tr>
<tr>
<td>II + I + I</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>18 (30%)</td>
</tr>
<tr>
<td>III + I</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7 (11.7%)</td>
</tr>
<tr>
<td>I + I + I + I</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>8 (13.3%)</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>5 (8.3%)</td>
</tr>
<tr>
<td>II + I</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>7 (11.7%)</td>
</tr>
<tr>
<td>I + I + I</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>II</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>Total n° cells</td>
<td>19</td>
<td>29</td>
<td>12</td>
<td>60</td>
</tr>
</tbody>
</table>
Figure 4.2. Examples of sex chromosome configurations in an $X^Y*$ $Y^-$ male.

(A) The $X^Y*$ and $Y^-$ chromosomes are showing possible synapsis between their PAR's.

(B-C) The $X^Y*$ and $Y^-$ chromosomes are showing possible synapsis between their short arms.

(D-F) The $X^Y*$ and $Y^-$ chromosomes are both present as univalents.
Figure 4.3. Plot of the proportion of cells with all the sex chromosomes synapsed vs. the sperm counts of XYY-Y*X and XYY*Y*X males compared to the relationship between synapsis and sperm counts of XYY*X, XYY- and XY males observed in Chapter 3.
Figure 4.4. Examples of sex chromosome associations in pachytene spreads from XYY-Y^{*X} (A-J) and XYy-Y^{*X} males (K-L) (when not indicated otherwise, the arrow shows the position of the Y^{*X} chromosome).

(A) Two bivalents are forming. The X and Y chromosomes are forming one bivalent, and the Y and Y^{*X} chromosomes the other one.

(B-C) Two bivalents are forming. The X and Y^{*X} chromosomes are forming one bivalent, and both the Y chromosomes the other one.

(D) All sex chromosomes are synapsed forming a quadrivalent.

(E) The X and both the Y chromosomes are synapsed forming a trivalent, and the Y^{*X} chromosome is associated with the centromeric end of the Y chromosomes.

(F-G) The X, the Y and the Y^{*X} chromosomes are synapsed forming a trivalent. The other Y chromosome is present as a univalent.

(H) The Y and Y^{*X} chromosomes are synapsed, and the other Y chromosome and the X chromosome are present as univalents.

(I-J) The X and Y chromosomes are synapsed, and the other Y and the Y^{*X} chromosome are present as univalents.

(K) Two bivalents are forming in a XYy-Y^{*X} male. The X and the Y^{*X} are forming one bivalent and the Y and y^- chromosomes the other one.

(L) The Y and y^- chromosomes are synapsed, and the X and Y^{*X} chromosomes are present as univalents.
Figure 4.5. Examples of sex chromosome configurations from an XYY*XY* male (unless stated otherwise the arrow indicates the position of the Y* chromosome).

(A-C) Two bivalents are formed by the synapsis of the X and Y* chromosome and by the synapsis of the Y and Y* chromosome.

(D-E) All the sex chromosomes are synapsed forming a quadrivalent.

(F) The X and both the Y* chromosomes are synapsed forming a trivalent, and the Y chromosome is present as a univalent.

(G-H) Both the Y* chromosomes are synapsed, and the X and Y chromosomes are present as univalents.
4.4 DISCUSSION
The objective of this study was to determine whether there was any deleterious effect on spermatogenesis of a double Y gene dosage. The first approach taken was to generate mice with the potential to form one bivalent in the presence of two doses of Y specific DNA. For this purpose $X^Y*Y^-$ males were generated. Previously, $X^Y*Y^{xx}$ mice (with only one dose of Y specific DNA) had been produced but found to be sterile, due to the failure to pair of the $Y^{xx}$ and $X^Y*$ chromosomes in all the pachytene spermatocytes analysed (Burgoyne et al., 1992). This failure to pair of the sex chromosomes is probably due to the fact that the $X^Y*$ chromosome has an interstitial PAR. In the mouse, the movement of telomeres to the nuclear envelope seems to precede the initial stages of chromosome pairing (Scherthan et al., 1996). This movement would leave the PARs of both the sex chromosomes very far apart at the time the initial pairing interactions are occurring, and would therefore explain why these chromosomes fail to synapse. By using a $Y^-$ chromosome instead of the $Y^{xx}$ chromosome it was hoped that the initial alignment process would bring the two telomeres proximal to the Y short arm together, and this should align both Y chromosomes and allow for synapsis to occur between the PARs. This PAR to PAR synapsis would satisfy the pairing requirements. Unfortunately this wasn't the case. Both $X^Y*Y^-$ males analysed were sterile and had not only very low levels of PAR to PAR pairing, but very low overall levels of pairing. This very low level of pairing could either be due to a low incidence of the initial pairing interactions that occur between homologues, or a failure to confirm and extend these interactions due to lack of homology. A lack of homology between a $Y^*$ chromosome (of an LT origin) and the $Y^-$ chromosome (of 129 origin) is possible due to the rapid divergence in the absence of recombination, of the 'junk' repetitive sequences that constitute the majority of the Y chromosome (Bishop, 1992). Since the $X^Y*$ and $Y^-$ chromosomes failed to synapse in the majority of the cases at pachytene, and this alone could explain the sterility of the $X^Y*Y^-$ males, it was impossible to determine if double
Y gene dosage also had a deleterious effect on spermatogenesis.

As a second approach, XYY-Y*X mice were generated. These males have the potential to satisfy the requirements for sex chromosome synapsis by forming two bivalents, in the presence of two Y chromosomes. All the males of this genotype analysed were found to be sterile and had much lower testis weights and sperm counts than the XY controls. SC analysis revealed that a significant proportion of cells (51% at pachytene), had all the sex chromosomes synapsed. This level of synapsis is similar to the levels of cells with all sex chromosomes synapsed in some XYY*X mice (Chapter 3). The plot of sperm counts against level of synapsis (Figure 4.3) clearly indicates that in XYY-Y*X mice the presence of an extra Y chromosome has a deleterious effect on spermatogenesis in addition to the deleterious effects of incomplete synapsis.

To analyse if this deleterious effect of an extra Y chromosome on spermatogenesis is due to the double dosage of Y genes or just to the negative effect that an extra sex chromosome could have on meiosis, an XYY*XY*X mouse was analysed. This male has the potential to form two bivalents in the presence of only one dose of Y specific DNA. The male of this genotype analysed was found to be sterile, with a spermatogenic impairment very similar to that of the XYY-Y*X males analysed. Once again analysis of sex chromosome pairing at pachytene revealed that a significant proportion of cells (40% at pachytene) had all their sex chromosomes synapsed and that the sperm count was lower than predicted from the level of synapsis. This provides evidence that the presence of an extra sex chromosome per se, independent of it increasing Y gene dosage, has a deleterious effect on spermatogenesis.

What is the basis for this deleterious effect of an extra sex chromosome? One possibility is that it is a deleterious effect of excess dosage of PAR genes.
However, this is not a convincing explanation since the presence of three PAR’s in a trivalent is compatible with spermatogenesis (Chapter 3). During the pachytene stage of meiosis, the X and Y chromosomes assume a chromatin configuration strikingly different from that of the autosomes, namely, the sex body, also termed the sex vesicle or XY body (Solari, 1989; Handel and Hunt, 1992). The sex body mainly consists of the sex chromosome axis and the surrounding chromatin fibrils (Solari, 1974), and due to its failure to incorporate radiolabelled uridine, it is thought to be transcriptionally inactive (Monesi, 1965; Kierszenbaum and Tres, 1974; Handel et al., 1994). It is possible that with two sex bivalents only one sex body is formed. Indeed, this would be predicted by those who view sex body formation to be a consequence of the localised production of Xist (the gene that is essential for X inactivation in females) RNA by the X chromosome. A failure of sex chromosomes to form a sex body has been proposed as the cause of spermatogenic failure in mice with a 11:19 translocation (Richler et al., 1989).

The predicted consequences of having sex chromosomes which fail to become inactivated at pachytene, by forming a sex body, vary according to the presumed function of this inactivation. One suggestion is that the gene products of the X chromosome may be harmful to spermatocytes (Lifschytz and Lindsley, 1972; Forejt, 1982). As a number of Y chromosome genes have a role during spermatogenesis, it could also be argued that their misexpression during pachytene could also have deleterious effects. There is no direct evidence for a negative effect of X chromosome gene products on spermatocytes, but Hotta and Chandley (1982) have reported that there are higher levels of certain X linked genes in some sterile male mouse translocation heterozygotes.

Alternatively, since sex chromosome inactivation is a meiotic phenomenon contemporaneous with chromosome pairing and recombination, inactivation has
been proposed as a mechanism to prevent promiscuous onset of recombination in the nonhomologous portions of the X and Y chromosomes (McKee and Handel, 1993). Evidence against this view is the recombination of the short arm of the Y chromosome and Sxr\(^a\) in XSxr\(^a\)Y males (Laval et al., 1995), even though these segments are presumably inactivated. A modification of this view is that inactivation of the sex chromosomes serves to stop the nonhomologous, and therefore unpaired, segments of the X and Y chromosomes from triggering the checkpoint that monitors sex chromosome pairing and/or recombination. The fact that the PARs of both these chromosomes are still transcriptionally active, supports this view (Raman and Das, 1991). Similarly, in their analysis of a number of X-autosome translocations, Handel et al., (1994) found that the only translocation that was fertile (even if only transiently) was the one that had the potentially unpaired segment of the autosome inserted into the X chromosome, where it is likely to be condensed and inactivated, and therefore unable to trigger the meiotic checkpoint that monitors synapsis.

Essentially nothing is known about the chromosomal requirements and genetic control of the condensation of the sex chromosomes and the formation of the sex body in mammalian spermatocytes. Sex body formation seems to be regulated by extracellular factors and the testicular environment appears to be a prerequisite (Whitten et al., 1979; Hogg and McLaren, 1985; Handel et al., 1994; Hendriksen et al., 1995). As to the recognition of this inactivating signal by the sex chromosomes and the process of spreading of the inactivation itself, the Xist gene has been proposed to have a role in these processes (McCarrey and Dilworth, 1992; Richler et al., 1992; Salido et al., 1992). The recent report by Marahrens et al., (1997) that the knockout males for this gene are fertile, has therefore come as a major surprise. Clearly, either Xist is not required for sex chromosome inactivation during spermatogenesis, or sex chromosome inactivation is not essential for spermatogenesis. Surprisingly the authors made no comment as to
the presence or absence of a sex vesicle in these knockout mice; this information is awaited with keen interest. In the meantime it would be of great interest to investigate further the sex 'vesicle' status of XYY-Y*X and XYY-Y*X males. This should not only shed light on the reasons for their sterility, but may also contribute to our understanding of the mechanisms involved in the inactivation of the sex chromosomes at pachytene in the male.

The conclusions from the analysis of XYY-Y*X and XYY*XY*X males are also of relevance to the spermatogenic failure present in XYY males. It is reasonable to propose that in XYY mice, one or more of the sex chromosomes fails partially or completely to become inactivated. What would fail to be inactivated depends on the requirements of sex chromosome inactivation itself. For example, if the X chromosome is the only requirement for inactivation, then a univalent Y chromosome or a YY bivalent will not form a sex body, but if both the X and Y are required, then only a univalent Y will remain active. On the other hand, if the spreading of a signal (such as the Xist RNA) is necessary for sex chromosome inactivation, and there is a limited amount of this signal, one could even envisage that in some cases, XYY trivalents may only become partially inactivated, due to the increase in chromatin that the extra Y will bring. As mentioned above, any of these possibilities could lead to the inappropriate expression of X or Y genes that may be deleterious to spermatogenesis and/or the activation of the meiotic checkpoint that monitors synapsis. Thus in XYY mice, both the incomplete sex chromosome synapsis and the failure of one of the sex chromosomes to become inactivated may be contributing to the spermatogenic failure.
CHAPTER 5.
IDENTIFICATION OF TWO GENETIC FACTORS THAT BY-PASS THE ACTION OF THE MEIOTIC CHECKPOINT THAT MONITORS SYNAPSIS/RECOMBINATION IN X\textsuperscript{Y*}O MICE.
5.1 INTRODUCTION

XY^*O males have a single sex chromosome comprising an X and a Y attached by a shared pseudoautosomal region (Eicher et al., 1991). XY^*O mice have been found to be sterile due to a meiotic arrest at metaphase I (MI). Their testis weights are less than half that of control animals and their epididymides are usually devoid of sperm. Pachytene analysis of these males revealed the expected univalent XY^* chromosome, and this very rarely achieves self synapsis (Burgoyne and Mahadevaiah, 1993). This univalent sex chromosome will activate the checkpoint that monitors synapsis/recombination and lead to the spermatocyte elimination that causes the spermatogenic impairment of XY^*O males. The fact that XY^*O males have a single sex chromosome, with one full complement of sex chromosome material, and therefore lacking the complications brought about by excess or absence of X or Y gene dosage, makes these mice a very good and simple model to study this checkpoint monitoring synapsis/recombination.

Our analysis of the mode of elimination of the spermatocytes with univalent sex chromosomes in XY^*O males, showed that these spermatocytes were eliminated by a p-53 independent apoptotic pathway. Those spermatocytes eliminated were predominantly at the first meiotic metaphase, and in contrast to the usually rapid elimination of apoptotic cells, the metaphase spermatocytes were found to accumulate in large numbers prior to their elimination (see attached manuscript). These observations are in agreement with the findings of a pile up at MI of cells with univalent sex chromosomes (Chapter 2).

A case of fertility in an XY^*O male has been referred to in a review (Eicher and Washburn, 1986), but no details were given. During the course of our analysis of the apoptotic elimination of spermatocytes in XY^*O males, an exceptional fertile XY^*O mouse was identified, and a breeding stock was subsequently established.
Three possible reasons could explain this fertility. In the first place, it is possible that in these mice, sufficient spermatocytes have the \( X^Y \) chromosome self synapsed, protecting these cells from elimination, to allow for fertility. Secondly, it is possible that a partial deletion of the PAR of these males has occurred, reducing the requirements for synapsis/recombination of this chromosome. Finally, it is possible that a heritable factor(s) arising through mutation, or present in the genetic background, has overcome the requirements of the checkpoint that monitors synapsis/recombination. The experiments described attempt to determine which of these causes explains the fertility of these \( X^Y \) mice.

5.2 MATERIALS AND METHODS

5.2.1 Production of \( X^Y \) males

The breeding crosses used to generate \( X^Y \) males are summarised in Figure 5.1 (Eicher et al., 1991). \( X^{Ta}Y^* \) males were mated to \( In(X)/X \) females (heterozygous for the X inversion \( In(X)1H- \) Evans and Phillips, 1975) or to \( XO \) females that were offspring of the original (or subsequent) fertile \( X^{Ta}Y^* \) males, in order to produce the \( X^{Ta}Y^* \) mice that were fertility tested. Fertile \( X^{Ta}Y^* \) males were mated to both these types of females to produce further \( X^{Ta}Y^* \) mice. The *Tabby* mutation, which affects the coat, was used to allow the visual identification of males carrying the \( X^{Ta}Y^* \) chromosome, thus allowing \( X^{Ta}Y^* \) males to be identified without resorting to karyotyping.

5.2.4 Sperm Counts.

See Chapter 2 and Appendix 1.

5.2.5 Synaptonemal Complex Analysis.

See Chapter 2 and Appendix 1.
(1) In(X)/X x XTaY* \\
\downarrow \\
XTaY*O \\

(2) XmO x XTaY* \\
\downarrow \\
XTaY*O \\

(3) In(X)/X x XTaY*O \\
\downarrow \\
XTaY*O XmO \\

(4) XmO x XTaY*O \\
\downarrow \\
XTaY*O XmO \\

Figure 5.1 Crosses set up to generate XTaY*O males.
5.3 RESULTS

5.3.1 Self synapsis in fertile \(X^{TaY^*}O\) males.

To ascertain whether self synapsis of the \(X^{TaY^*}\) chromosome was the reason for the fertility of the original \(X^{TaY^*}O\) male, pachytene synaptonemal complexes were analysed. Out of 50 cells, none showed any convincing signs of self synapsis (Figure 5.2). This observation ruled out self synapsis as a basis for the fertility.

5.3.2 Evidence for a heritable autosomal factor(s) responsible for the fertility of \(X^{TaY^*}O\) mice.

In order to test whether a heritable factor is responsible for the fertility of \(X^{TaY^*}O\) males, the fertility and sperm counts of \(X^{TaY^*}O\) mice from four different crosses were compared (Figure 5.1 and 5.3);

1. \(\text{In}(X)/X \times X^{TaY^*}\)
2. \(X^{mO}\) offspring of \(X^{TaY^*}O\) males \(X^{TaY^*}\)
3. \(\text{In}(X)/X \times \text{Fertile} \ X^{TaY^*}O\)
4. \(X^{mO}\) offspring of \(X^{TaY^*}O\) males \(\times\) Fertile \(X^{TaY^*}O\)

Three important conclusions can be drawn from these results. In the first place the \(X^{TaY^*}O\) offspring of fertile \(X^{TaY^*}O\) males (crosses 3 and 4) may themselves be fertile, with sperm counts and testis weights comparable to their fathers, or they may be sterile, with sperm counts ranging from zero to 100,000 sperm/caput. Since these males will have inherited an intact \(X^{TaY^*}\) chromosome from their fathers, the production of sterile \(X^{TaY^*}O\) offspring rules out the possibility that a deletion within the PAR, or any other factor linked to the \(X^{TaY^*}\) chromosome, is responsible for the fertility of their fathers (unless the factor is of very variable penetrance).

In the second place, it can be observed that when either \(X^{TaY^*}\) or \(X^{TaY^*}O\) males
are mated to the $X^mO$ offspring of fertile $X^{TaY*O}$ males (crosses 2 and 4) instead of being mated to the stock $In(X)/females$ (crosses 1 and 3) there is a highly significant increase in the proportion of fertile $X^{TaY*O}$ males produced (Table 5.1). This provides clear evidence for a genetic factor, or factors responsible for the fertility of $X^{TaY*O}$ males, that can be transmitted through the $X^mO$ females. Since these females have a single maternally-derived $X$ chromosome, this factor(s) must be autosomal.

In the third place, the observation that replacing the stock $X^{TaY*}$ males with fertile $X^{TaY*O}$ males also significantly increases the proportion of fertile $X^{TaY*O}$ offspring (Table 5.1) confirms the existence of this autosomal factor(s) and shows it can be paternally transmitted.

5.3.3 Evidence for the existence of two unlinked factors contributing to the fertility of $X^{TaY*O}$ males.

If the $X^{TaY*}$ males that are mated to $In(X)/X$ females are divided into two groups, the first encompassing those $X^{TaY*O}$ mice with sperm counts between 100,000 and 800,000 sperm/caput, and the second including $X^{TaY*O}$ males with sperm counts above 800,000 sperm/caput, and the sperm counts of their $X^{TaY*O}$ progeny are plotted (Figure 5.4), it is evident that two factors exist that affect the fertility of $X^{TaY*O}$ males.

The sperm counts of the $X^{TaY*O}$ progeny of the first group of males never go above the 800,000 limit. On the other hand the sperm counts of the $X^{TaY*O}$ offspring of the males in the second group show a much wider distribution, with counts ranging from 0 to over 1,500,000 sperm/caput (Figure 5.4). This shows that males with over 100,000 sperm/caput have two independently segregating factors contributing to their sperm counts - a major factor giving sperm counts in the 100,000-800,000 sperm/caput range and a minor factor that augments this to
over 800,000 sperm/caput. The range of sperm counts achieved by the minor factor alone have not yet been established, but the similarity in the distributions of sperm counts below 800,000 sperm/caput for the two sets of males suggests that the minor factor alone may have little effect.

5.3.4 Is the minor factor dominant or semidominant?

The observation that when $X^{Ta}Y^*$ males with 100,000-800,000 sperm/caput are mated to In(X)/X females over several generations, they never produce $X^{Ta}Y^*$ progeny with over 800,000 sperm/caput, indicates that the minor factor is not present in the In(X)/X stock. Since 7 out of the 13 fertile $X^{Ta}Y^*$ males produced by the cross: In(X)/X females x $X^{Ta}Y^*$ males with over 800,000 sperm/caput (who have to be carriers for this factor), have sperm counts above 800,000 sperm per caput (Figure 5.4) this factor must be dominant or semidominant.

The fact that from the $X^{Ta}Y^*$ x In(X)/X cross only the original fertile $X^{Ta}Y^*$ male (that had 1,600,000 sperm/caput) had a sperm count above 800,000 sperm/caput, indicates that the minor factor is probably a de novo event occurring in this male, and was therefore probably introduced into the pedigrees by the $X^mO$ offspring of this male.

5.3.5 Is the major factor dominant or semidominant?

The situation with the major factor is less clear. The two basic possibilities for this factor is that it is (a) dominant or (b) recessive. The sperm count of 100,000 sperm/caput has been taken as the lower limit for males considered to have a phenotype due to this factor. The arguments for and against both these possibilities are presented below.

(a) If the factor is dominant:
The cross between the stock mice In(X)/X x $X^{Ta}Y^*$ males only produce 8 out of 43 males with over 100,000 sperm/caput. If the factor was dominant this would
indicate that only very few of these stock mice could be carriers for this factor. Given that the cross $X^mO \times X^{TaY*}$ gives a higher proportion of fertile males than the cross $In(X)/X \times X^{TaY*}O$ (Table 5.1), it can be argued that this factor is present in some of the $X^{TaY*}$ males but not in the $In(X)/X$ females. Therefore the cross between $In(X)/X$ females and fertile $X^{TaY*}O$ males (that are known carriers) can be used to test if this factor is dominant. 37 out of 95 of the $X^{TaY*}O$ offspring from this cross were fertile or had over 100,000 sperm/caput. A chi squared test reveals this value is not significantly different from the 50% expected for a dominant factor ($X^2 = 2.3$, $P=0.1-0.25$). The deviation from 50% could be due to chance, the arbitrary nature of 100,000 sperm/caput as the lower limit for what is considered a carrier, and/or due to a partial lack of penetrance. Indeed evidence for lack of penetrance could be observed in some pedigrees. See for example pedigree 7, in which 16 out of 45 of the $X^{TaY*}O$ progeny of the $In(X)/X$ and $X^{TaY*}O$ mice are fertile (Figure 5.5). On the other hand pedigree 5, has 13 out of 23 of the $X^{TaY*}O$ offspring of the cross $In(X)/X \times X^{TaY*}O$ being fertile (Figure 5.6).

(b) If the factor was recessive:
- The cross between $In(X)/X$ and fertile $X^{TaY*}O$ males (that have to be homozygotes) could be used to determine the frequency of $In(X)/X$ carriers. As 37 out of 95 (39%) of the $X^{TaY*}O$ offspring from this cross were fertile or had over 100,000 sperm per caput, 78% of the $In(X)/X$ females would be predicted to be carriers for this factor.
- The cross between $X^mO$ offspring of the original fertile $X^{TaY*}O$ male (that would have to be heterozygotes as their mothers were $XX$ MF1 females) and $X^{TaY*}$ mice, can be used to determine the frequency of $X^{TaY*}$ carriers. Given that 14 out of 29 (48%) of the $X^{TaY*}O$ offspring from this cross were fertile or had over 100,000 sperm per caput, you would expect all, or at least nearly all, the $X^{TaY*}$ males to be homozygotes for this factor.
- If these predictions are tested on the cross In(X)/X x XTaY*, you would expect 38% of the XTaY/O progeny from this cross to be fertile or have over 100,000 sperm/caput, but only 10 out of 45 (22%) are. A chi square test shows that this difference is not significant \( X^2 = 2.95, P=0.05-0.1 \). The deviation from the expected ratio could be due to the same reasons as given for the dominant hypothesis.

At this stage neither of these models can be discarded, but the hypothesis that best seems to fit the data is that the major factor is dominant, as the deviation from expected is less for this model.
Figure 5.2. Synaptonemal Complex of the $X^{Ta}Y^*$ chromosome from the original fertile $X^{Ta}Y^*$O male showing no evidence of self pairing.
Figure 5.2. Synaptonemal Complex of the $X^{Ta}Y^*$ chromosome from the original fertile $X^{Ta}Y^*O$ male showing no evidence of self pairing.
Table 5.1. Numbers of fertile and sterile mice produced from each of the crosses set up to test for a heritable genetic effect being the cause for the fertility of the $X^{TaY*0}$ males. Those males that had sperm counts within the range that is expected to give fertility, but that had not bred by the time they were processed, were considered as fertile. Chi squared test indicates that the difference between crosses 1+3 and 2+4 is highly significant ($X^2=3.8$, $P=0.05$). The difference between crosses 1+2 and 3+4 is also highly significant ($X^2=4.2$, $P<0.05$).

<table>
<thead>
<tr>
<th>Cross</th>
<th>In(X)/X x XTaY*</th>
<th>Fertile</th>
<th>Sterile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>In(X)/X x XTaY*</td>
<td>8</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>X^mO x XTaY*</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>In(X)/X x XTaY*O</td>
<td>37</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>X^mO x XTaY*O</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure 5.3. Distributions of the sperm counts of the $X^{TaY^*}O$ males analysed from the following crosses:

1. In(X)/X x $XTaY^*$ (2 $XTaY^*O$ males were not included that were fertile as their sperm count data was not yet available).
2. $X^mO x X^{TaY^*}$
3. In(X)/X x $XTaY^*O$ (10 $XTaY^*O$ males were not included that were fertile as their sperm count data was not yet available).
4. $X^mO x X^{TaY^*}O$ (1 $XTaY^*O$ male was not included that was fertile, as it's sperm count was not yet available).

Shaded are those males that were fertile or had sperm counts within the range that is expected to give fertility.
1) \( \frac{\ln(X)}{X} \times X^{Ta} Y^{+} \)

2) \( X^{M} O \times X^{Ta} Y^{+} \)
3) \( \ln(X)/X \times X^{TaY^*}O \)

4) \( X^M O \times X^Y O \)
Figure 5.4. Distributions of the sperm counts of the $X^{TaY^*}O$ offspring of In(X)/X females and $X^{TaY^*}O$ males with either (a) 100,000-800,000 sperm/caput, or (b) over 800,000 sperm/caput. Note that 4 $X^{TaY^*}O$ males from the 100,000-800,000 sperm/caput and 6 $X^{TaY^*}O$ males from the over 800,000 sperm/caput were fertile but are not included as no sperm count data was available for them yet. Shaded are those males that were fertile or had sperm counts within the range that is expected to give fertility.
$X^{TaY^*}O$ offspring of $X^{TaY^*}O$ males
with 100,000-800,000 sperm

$X^{TaY^*}O$ offspring of $X^{TaY^*}O$ males
with >800,000 sperm
Figure 5.5. The $X^{TaY^*O}$ males were divided into pedigrees according to the identity of the $X^{TaY^*}$ founder males. Presented here are the sperm counts of the $X^{TaY^*O}$ males from Pedigree 7. (Box= male, Circle= female. Inside the boxes are the sperm counts of the $X^{TaY^*O}$ males $x10^{-3}$. F= Fertile, but sperm count data not available yet).
Figure 5.6. Sperm counts of the $X^T a Y^* O$ males from Pedigree 5. (Box= male, Circle= female. Inside the boxes are the sperm counts of the $X^T a Y^* O$ males $\times 10^{-3}$. F= Fertile, but sperm count data not available yet).
5.4 DISCUSSION

The identification of molecular components of the checkpoint that monitors synapsis/recombination is of vital importance for understanding the underlying mechanism. So far, in yeast, several proteins (see Introduction) have been identified and are currently being characterised, but in mammals the identification of these components has remained elusive. One way of identifying these components would be through the analysis of mouse mutants that can bypass this requirement for synapsis/recombination during meiosis. The discovery of fertile $X^{TaY*}O$ mice is therefore potentially of great importance.

Three possible causes were considered which might account for the fertility of the exceptional $X^{TaY*}O$ males:

(a) Sufficient spermatocytes could have the $X^{TaY*}$ chromosome self synapsed at pachytene to overcome the pairing requirements during this stage of meiosis. The analysis of SC formation in pachytene spermatocytes of a fertile $X^{TaY*}O$ males, demonstrated that this was not the case.

(b) It is possible that a deletion in the PAR of the $X^{TaY*}$ chromosome has reduced the capability of this chromosome to signal its unpaired status. The lack of sperm in some of the $X^{TaY*}O$ offspring of fertile $X^{TaY*}O$ mice and the fact that this fertility can be transmitted through the $X^mO$ offspring of fertile $X^{TaY*}O$ males, rules out this possibility.

(c) A heritable factor (or factors) arising through mutation, or present in the genetic background, could either reduce the efficiency of the checkpoint monitoring synapsis/recombination or overcome it's action. The fact that both the $X^mO$ and $X^{TaY*}O$ offspring of fertile $X^{TaY*}O$ mice can transmit this fertility to their $X^{TaY*}O$ progeny, demonstrates that a heritable autosomal factor(s) is
responsible for the fertility of $X^{Ta}Y^*O$ males.

The division of the $X^{Ta}Y^*O$ males produced by the In(X)/X $\times X^{Ta}Y^*O$ cross into males with 100,000-800,000 sperm/caput and males with over 800,000 sperm/caput, makes clear that two independent loci are contributing to the sperm counts of $X^{Ta}Y^*O$ males - a major factor giving sperm counts between 100,000 and 800,000 sperm/caput and a minor factor that augments this to over 800,000 sperm/caput.

The analysis of the segregation of the minor factor clearly indicates that it is dominant (or semidominant). As it is absent from the In(X)/X stock and only one male from the cross In(X)/X $\times X^{Ta}Y^*$ had a sperm count over 800,000, it seems likely that this factor is a de novo event in this male.

The analysis of the major factor is less simple. The hypothesis that best fits the data is that this factor is dominant, but the possibility of it being recessive cannot be discarded yet. The deviation from the 50% of fertile $X^{Ta}Y^*O$ progeny expected for a dominant factor in the cross In(X)/X $\times X^{Ta}Y^*O$, could be due to chance, the arbitrary nature of the lower sperm count limit of 100,000 sperm/caput used to classify males as carriers, and/or to a possible lack of penetrance of this factor. Whatever the mode of action of this factor, it is clear that it is present in at least one, if not both, of the stocks (In(X)/X females and $X^{Ta}Y^*$ males) used to produce the $X^{Ta}Y^*O$ mice. Therefore, this major factor can probably be attributed to something present in the genetic background of these mice.

These stocks of mice have a mixed genetic background, consisting principally of four components, MF1, C3H, 101 and 129. Previous studies have identified a chromosome 17 locus, $Hst 1$, linked to the H-2 complex (Forejt and Ivanyi, 1975), as a gene responsible for the sterility of interspecific F1 hybrids. The C3H strain
carries an allele at this locus which allows interspecific F1 males to be fertile. It has been shown that hybrid sterility is due to the spermatogenic block resulting from the failure to pair of the X and Y chromosomes (Matsuda et al., 1991), and therefore it is very likely to be due to the action of the checkpoint that monitors synapsis/recombination. Forejt (1976), found that a C3H allele linked to H-2, increases the testis weights and sperm counts of T(14;15)6Ca translocation heterozygotes. In a similar way, Mahadevaiah et al., (1988) found that male T(X;4)37H heterozygotes, which usually totally lack sperm, have some sperm when put on a partial C3H background. Thus, a C3H allele(s) linked to H-2 on chromosome 17, seems to be capable of attenuating the spermatogenic defect in both male translocation heterozygotes and interspecific F1 hybrids.

In the translocation heterozygote males, it has been suggested that part of the cause for their spermatogenic breakdown is the activation of the checkpoint that monitors synapsis/recombination by the unpaired segments of the translocated chromosome (Burgoyne and Baker, 1984). Therefore it seems possible that a C3H allele of a chromosome 17 gene (or genes), is overcoming, at least partially, the meiotic block caused by the activation of the checkpoint monitoring synapsis/recombination, in both translocation heterozygous males and in the interspecific hybrid mice. This attenuation of the action of the checkpoint that monitors synapsis/recombination by a C3H allele, combined with the fact that the XTaY*O mice analysed have C3H as part of their genetic background, opens up the possibility that a C3H allele is one of the factors (probably the major) that has been identified in this study. It would therefore be very interesting to test if there is any linkage between either of the two factors analysed and chromosome 17 loci in the vicinity of H-2. Experiments in the lab, are currently underway to test this.

In conclusion, two factors have been characterised that overcome, at least
partially the requirements of a meiotic checkpoint that monitors synapsis/recombination. It would be very interesting both to map these factors and to test the degree to which these factors can overcome the action of this meiotic checkpoint in other mouse models with unpaired sex chromosomes. The identification of these factors will also be of great value in determining to what extent this checkpoint monitors autosome asynapsis as well. Experiments to determine all these things are currently underway in the lab.

It seems unlikely that these factors act in the apoptotic pathway of elimination of the spermatocytes with unpaired sex chromosomes, as no other developmental abnormalities have been observed in these mice. It is possible that these factors could be involved in the signalling or in the recognition of the signal by the meiotic checkpoint. For this reason, further analysis of these factors should allow us to gain a very valuable insight not only into the circumstances in which this checkpoint acts, but also into the molecular mechanisms that constitute this meiotic checkpoint.
CHAPTER 6.

GENERAL DISCUSSION.
There is strong evidence for the existence of a meiotic checkpoint that monitors synapsis and/or recombination. The aim of the experiments described in this thesis was to resolve some of these questions that remained concerning the mode of action of this meiotic checkpoint, and thus gain a clearer understanding of the circumstances in which it acts.

The first question that was addressed was whether this meiotic checkpoint monitors sex chromosome synapsis, recombination or both. The most cogent arguments in favour of a recombination checkpoint in males are based on data from XY* mice (Hale et al., 1991; Hale, 1994; Burgoyne and Mahadevaiah, 1993). In Chapter 2, the comparison of the meiotic pairing and sperm output of XY* and XYSxr^ males established that the previous interpretation of the events occurring during the first meiotic prophase in these XY* mice is incompatible with the fertility of these males. The new interpretation of this data, namely that a reorientation of the sex chromosomes is occurring so as to allow for recombination to occur, not only is compatible with the fertility of XY* males, but also reopens the question of whether this checkpoint is monitoring recombination of the sex chromosomes during male meiosis or not.

The next two sets of experiments were designed to investigate further the reasons for the spermatogenic failure of XYY mice. As mentioned, XYY mice are sterile, and excess Y gene dosage, sex chromosome pairing failure, or a combination of both, have been put forward as the reasons for this sterility. In Chapters 3 and 4 an in depth analysis of the contribution of both these factors to the sterility of XYY mice has been carried out. In Chapter 3, the comparison of the levels of trivalent formation at pachytene in XYY*^ and XYY mice, demonstrates that sex chromosome synaptic failure is a major cause for the spermatogenic impairment of XYY mice. This study also provides the first solid evidence, that as proposed by Burgoyne and Biddle (1980), trivalent formation in
mice with three sex chromosomes, is the only meiotic configuration that allows for spermatocyte survival.

Chapter 4 describes an analysis of sex chromosome synapsis, testis weights and sperm output in $X^Y\cdot Y$, XYY$^*$, and XYY$^*\cdot Y^*$ mice. The analysis of XYY$^*\cdot Y^*$ mice indicates that in addition to the spermatogenic impairment resulting from incomplete sex chromosome synapsis, there is an additional factor related to the presence of an extra Y chromosome. The analysis of XYY$^*\cdot Y^*\cdot X$ males indicated that the spermatogenic failure in this mouse and the XYY$^*\cdot Y^*\cdot X$ males is due to the presence of an extra sex chromosome per se, rather than an increase in Y gene dosage. This opens up the possibility that in XYY males an additional reason for their spermatogenic impairment is the failure to inactivate one or more of their sex chromosomes.

In conclusion Chapters 3 and 4 give a clear insight into the causes of the infertility of XYY mice. These chapters also allow some of these conclusions to be extrapolated to XYY humans. These studies strongly suggest that both sex chromosome synaptic failure and the presence of an extra sex chromosome, that could interfere with sex chromosome inactivation, are likely causes for the sterility of XYY humans and therefore provide a plausible explanation for this sterility.

The studies carried out in Chapter 3 also allow some insight to be gained on the reasons for the fertility of some of the XYY humans. The analysis of XYy$^*$ males, showed that older males could become mosaic in their germ line. This mosaicism is due to the selective advantage of those germ cells that have lost a Y chromosome due to a mitotic nondisjunction event. It was established in this chapter, that if the level of mosaicism in the XYy$^*$ males is high enough, these mice will become fertile, but breed only from their euploid cell line. It is
supposed that similar occurs in humans.

One important general conclusion that can be drawn from Chapters 2, 3 and 4, is that there is a direct relationship in all genotypes of mouse, between sex chromosome synaptic failure and spermatogenic failure. Therefore plots like those of Chapter 4 (Figure 4.3) can be used to predict the expected sperm count for a given level of synapsis, and this will be of great value when analysing the causes for the sterility of both mice and humans.

Finally, Chapter 5 describes experiments designed to determine the genetic basis of the circumvention of the meiotic checkpoint that occurs in the exceptional fertile X\(^Y\)O males. This study established that two factors are responsible for this fertility. The first, or major factor, that is probably associated with the C3H or 101 genetic backgrounds, can produce between 100,000 and 800,000 sperm/caput in the usually sterile X\(^Y\)O mice. The second factor, acts as a modifier of the major factor, by producing over 800,000 sperm/caput in those males that already have the major factor, and is probably a \textit{de novo} mutation present in the first fertile X\(^Y\)O male. The identification of two factors that can bypass the checkpoint that monitors synapsis/recombination opens the way, perhaps for the first times in mammals, to gain insights into the molecular mechanism of this checkpoint.

The meiotic checkpoint that monitors the synapsis/recombination of the sex chromosomes is of great importance for a number of reasons. This checkpoint is vital to ensure that recombination between the sex chromosomes occurs, as this will allow for the proper segregation of these chromosomes. Proper segregation is essential not only to ensure a 1:1 ratio of males to females, but also to avoid the presence of aneuploid offspring. Organisms that have achiasmate chromosomes have needed to developed alternative mechanisms that ensure proper
segregation. For example the achiasmate disjunction in female *Drosophila* meiosis is regulated by the presence of centric heterochromatin (Karpen *et al.*, 1996, Dernburg *et al.*, 1996). Therefore this checkpoint could be viewed as one of the possible mechanisms that has evolved to ensure recombination of the sex chromosomes.

Although the object of this work was only to study the action of the checkpoint that is triggered by lack of sex chromosome synapsis/recombination, it seems probable that this checkpoint is just a reflection of a more general checkpoint that monitors synapsis/recombination of both sex chromosomes and autosomes. In yeast there is clear evidence for a checkpoint that monitors the synaptic/recombination status of all chromosomes (Li and Nicklas, 1995; Xu *et al.*, 1997). The localisation of some of the mouse homologues of the yeast proteins involved in this checkpoint, to the asynapsed, but not to the synapsed, segments of the chromosomes during zygotene and pachytene of mouse meiosis, indicates that this checkpoint probably also acts during mammalian meiosis (Keegan *et al.*, 1996). Further support for this idea comes from the analysis of sterile balanced chromosome translocations. Although it is possible that a combination of factors are involved in the sterility of some of these males, the recurrent feature of the presence of an unpaired chromosome segment in balanced translocation mice, is obviously, at the least contributing if not the main cause, for the meiotic breakdown in these males (De Boer, 1986). Both these sets of data strongly suggest the view that a general mechanism exists that monitors chromosome synapsis/recombination.

The nature of the signal that triggers the checkpoint that monitors sex chromosome synapsis/recombination is not clear. Miklos (1974) proposed that pairing sites distributed along the length of chromosomes need to be saturated by synapsis so as to ensure spermatocyte survival. McKee and Handel (1993)
have proposed that the double strand breaks (DSBs) that initiate recombination could be the molecular basis for these pairing sites. It can be speculated that if these DSBs are not repaired by recombination during pachytene, they will trigger the elimination of the cell. This model is attractive, as it would provide a simple mechanism for a checkpoint that would act both during female and male meiosis. It could also explain why the checkpoint that monitors synapsis/recombination is less efficient during female meiosis (Hunt et al., 1995), as the long arrest at MI in females could give a chance for the repair of these DSB to occur, and therefore suppress the signal that causes the elimination of this cell.

Proteins such as the previously described ATR, that is associated with the synapsed but not synapsed chromosomes, and ATM, that is associated with the synapsed but not the asynapsed chromosomes (Keegan et al., 1996), could act as molecules that could be involved in the recognition of the signal. A number of tools (mouse homologues of yeast proteins, etc...) are becoming readily available to test this model, and therefore the near future should see a great improvement in the understanding of the mechanisms that regulate meiosis.

With no doubt the analysis of the regulatory mechanisms that act during meiosis to ensure the fidelity of the transmission of the genetic material from one generation to the next, is entering a very exciting period. The work described here has opened up a new set of questions concerning the checkpoint that monitors synapsis/recombination. Answering these questions will allow further insight to be gained into both the action of this checkpoint and to causes of sterility in mammals in general.

In the first place it will be important to resolve if the mentioned checkpoint monitors synapsis, recombination or both. One approach will be to examine the fertility of novel mouse mutants that are defective only in recombination or only in synapsis. The wide range of knockout mice now becoming available should
provide these mutants. Another approach will be to compare the cellular localisation, in both fertile and sterile mice, of novel genes that have a proposed role in either synapsis or recombination, and attempt to relate this localisation to fertility. In a similar way, the characterisation of the molecular components of this checkpoint, and the study of the biochemical events that trigger their action, will allow one to analyse what exactly the checkpoint is monitoring.

Secondly, it will be of great interest to determine what is the cause for the sterility of XYY-*Y* mice and relate this to XYY mice. Analysis of sex vesicle formation, and what chromosomes are included in the sex vesicle of XYY-*Y* males, should allow us to investigate if failure of sex chromosome inactivation is involved in this sterility. If the answer to this question is yes, these mice will provide a very useful model to study the chromosomal requirements for sex vesicle formation, and also to investigate how failure of sex chromosome inactivation will lead to sterility.

Finally, it is going to be of great value to map and clone the genes that code for the two factors characterised in the analysis of the fertility of X*Y* males. These factors should provide a handle on the molecular components of the checkpoint that monitors synapsis/recombination and therefore allow an in depth understanding of this checkpoint to be obtained. In the shorter term, these factors can be introduced into other cases of chromosomally induced sterility, so as to assess the role of synaptic failure in these cases of sterility. For example by introducing these factors into autosome-autosome translocations should allow us to assess if this checkpoint also monitors autosome asynapsis. All these approaches should provide an invaluable understanding of the mechanisms that regulate meiosis and how these mechanisms arose.
APPENDIX 1.
1.1 Karyotyping of mice

Mice were karyotyped by at least one of three methods:

- Chromosome preparation from tail tip culture (Mahadevaiah et al 1993):

  Tail tips were taken between 2 and 10 days after birth. The tail was washed with 70% ethanol, sprayed with ethyl chloride anaesthetic spray and approximately 2mm removed from the tip. The tip was cut and sliced lengthways into about 8 fragments with a sterile scalpel. These were placed in Hepes buffered Dulbecco's Minimal Essential Medium (DMEM). Once all fragments were cut, they were placed cut-side-down on the base of a petri dish (Falcon, UK) and any remaining medium was removed. The tissue was allowed to dry slightly and stick to the bottom of the dish. Sterile complete medium (bicarbonate buffered DMEM + 10% Fetal Calf Serum + 1% Penicillin/Streptomycin + 1% glutamine) was then added to each dish and incubation was carried out at 37°C in 5% CO₂ for 6-7 days. The medium was renewed 40 hours before processing the cells.

  For processing the cells, 2 or 3 drops of 8 mg/l⁻¹ colcemid solution in DMEM were added to each dish. After 2 hours, the medium was removed and the cells were washed twice in Phosphate Buffered Saline (PBS). 2-3 ml of trypsin-EDTA stock solution (Robertson 1987) was then added to each dish and the cells were left at 37°C for 2 minutes. The trypsin was then inactivated with 1-2 mls of complete medium. The cells were then loosened by pipetting, spun at 1000rpm for 5 minutes and resuspended in 0.56% KCl. After 5 minutes the cells were spun and drained. Fixing was carried out using a 3:1 methanol/glacial acetic acid solution by adding one drop of fix to the cells and flicking the tube, this drop/flick procedure was carried out three times. After pelleting by centrifugation the cells were resuspended in 1ml fix. After 3 further washes with fix the cells were resuspended in 3 drops of fix. Two drops were then placed on a slide previously soaked in 2% acid alcohol and, once the fix had evaporated,
stained in 2% giemsa for 10 minutes. The karyotype was determined under a high power objective.

- Chromosome preparation from spleen (Mahadevaiah et al 1993).
  A 2-3 mm³ piece of spleen obtained by partial splenectomy under avertin anaesthesia was taken and placed in 1 ml 0.08 mg/l⁻¹ colcemid in Hepes buffered DMEM. The cells were dissociated by pipetting and incubated for 1 hour at 31°C before spinning for 5 minutes at 1000rpm and resuspending in 0.56% KCl. After 25 minutes in KCl the cells were pelleted and the pellet washed carefully with fix (3:1 methanol:glacial acetic acid) 4-5 times before resuspending in 1ml fix. Once this was carried out the cells were spun and resuspended in an appropriate amount of fix (depending on the number of cells). Two drops of cell suspension were then placed on a slide that was allowed to air dry and stained with 2% giemsa for 15 minutes. The karyotype was determined under a high power objective.

- Chromosome preparations from bone marrow (Mahadevaiah et al 1993).
  Both femurs of a freshly killed mouse were dissected and flushed with 0.5mls of 0.08 mg/l⁻¹ colcemid in Hepes buffered DMEM for each bone. Cells were then incubated for 1 hour at 31°C. Hypotonic treatment, fixing and staining was the same as for spleen cells. The karyotype was determined under a high power objective.

1.2 Synaptonemal Complex preparations for electron microscopy

The procedure used to prepare pachytene spreads for synaptonemal complex analysis was a modification (Mahadevaiah and Mittwoch, 1986) of that described by Moses and Poorman (1984). Two drops of mouse spermatocyte suspension in
RPM1 1640 medium (Gibco BRL) were placed on a plastic coated slide (0.45% plastic in chloroform) and incubated at 37°C, 5%CO₂ in a moist chamber. A few drops of hypotonic saline (0.5% NaCl) solution were then added to the slide and left for 5 min. The excess liquid was then removed and the cells fixed with 0.03% sodium dodecyl sulphate in 4% formaldehyde (pH 8.3-8.5) for 5 minutes. The slides were then immersed in 4% formaldehyde for 8 min, washed twice for 4 min. in 0.4% Photoflow and air dried. When dry, the slides were stained with silver nitrate (Howell and Black, 1980) with 2 drops of 2% gelatin and 2 drops 50% silver nitrate for 2 minutes at 57°C. The regions with a suitable density of cells were cut using a diamond marker, floated off in water and placed on a G-100 grid that could be analysed under an Phillips 300 electron microscope.

During the analysis of the XYY**, XYy⁻ and XYy⁻Y** males carried out in the initial set of experiments, the attempts to float off in water the regions with suitable densities of cells failed. For this reason SC analysis was carried out only for a small number of pachytene cells from these males. To overcome this problem, a modification of the procedure used by Messer et al., (1986) was used. Instead of using plastic coated slides, the spermatocyte suspension was placed straight on the slide. The procedure was then carried out as above until after the silver nitrate staining. Then, the slides were coated with plastic (0.45% plastic in chloroform). Once dry, the region of the slide with an adequate density of cells was cut with a diamond marker and covered with 10μl of 0.2 Normal hydrofluoric acid. After 10 seconds this region was floated off in water, and collected on a G-100 grid that was analysed under a Phillips 300 electron microscope.

Chromosomes were considered to be synapsed when all, or part of their axial elements were aligned. Cells were scored as early, mid or late pachytene according to the criteria of Moses and Poorman (1984). At early pachytene the
axial elements are thin and uniform, and the nucleoli are observed as individual units. At mid pachytene the axial elements are branched and the nucleoli are clustered. At late pachytene the axial elements have thick ends and the nucleoli are clumped, but more condensed than at mid pachytene.

1.3 Metaphase I chromosome preparations.

Air-dried slides of fixed testicular suspensions were prepared as described by Evans et al (1964). Part of a testis was placed in 1ml of 2.2% trisodium citrate, dissociated and spun for 5 minutes at 1000rpm. After draining the tube, the cells were resuspended in 2mls of 1% trisodium citrate. After 8 min the cells were fixed with 3:1 methanol/glacial acetic acid solution by adding one drop of fix to the cells and flicking the tube, this drop/flick procedure being repeated three times. After pelleting the fixed cells they were resuspended in 1ml fix. The cells were then washed with fix at least twice. Following a final spin the cells were resuspended in 6-9 drops of fix, dropped onto slides, air dried and stained in 2% giemsa for 5 minutes. Chromosome configurations were analysed under a high power objective.

1.4 Sperm Counts.

As a measure of sperm production, sperm counts were estimated for each caput epididymis by a procedure modified from that described by Searle and Beechey (1974). The epididymis was dissected out from freshly killed animals, and the caput was cut at the height of the third vein and placed in 0.1 mls of 1% trisodium citrate. The caput was then chopped up thoroughly and 1% trisodium citrate was added up to a total volume of 1 ml. Finally sperm counts from this suspension were carried out using a haemacytometer.

1.5 Immunofluorescence.

For immunodetection of Mlh1 (Baker et al., 1996), GS (Earnshaw and Rothfield,
1985) and A1 (Lammers et al., 1994) antibodies, a modification of the protocol by Moens et al., (1987) was used. Spermatocytes were surface spread by placing a drop of spermatocyte suspension in the Hepes buffered, CO₂ independent, MEM medium (Minimum Essential Medium- Gibco BRL), onto a slide. One drop of 0.003% liposol solution was then added to the spermatocytes and these were left for 10 min at room temperature. Subsequently 6 drops of 0.01% sodium dodecyl sulphate in 2% formaldehyde (pH 8.3-8.5) were added, and the slide left for 10 min at room temperature, washed twice with distilled water and air dried. Slides were then incubated for 30 min. at room temperature in PBT (IxPBS (Phosphate Saline Buffer), 0.1% Tween 20 and 0.15% BSA (Bovine Serum Albumin)) and then 30μl of antibody was added and the slides left overnight in a dark, humid chamber at room temperature. Mlh1 was used at a 1:500 dilution, GS at a 1:5000 dilution, and A1 in a 1:1000 dilution. The next morning the slides were washed three times in PBT for 5 min. and incubated for an hour with 30μl of the secondary antibody at room temperature. When labelling with Mlh1 and A1, Mlh1 was detected with goat anti-mouse-IgG conjugated with fluorescein isothiocyanate (FITC- Sigma) diluted 1:500, and A1 with goat anti-rabbit-IgG conjugated with tetramethylrhodamine B isothiocyanate (TRITC-Sigma) diluted 1:500. When labelling with GS and A1, GS was detected with goat anti-human-IgG conjugated with TRITC diluted 1:500, and A1 with anti-mouse-IgG conjugated with FITC diluted 1:500. The slides were then washed with PBT three more times for 5 min. and counter stained with 4',6-diaminino-2-phenylindole (DAPI) for 2 minutes. Preparations were digitally imaged using a Zeiss Axioskop (63-x 1.2NA Plan Neofluar oil-immersion objective). Each fluorochrome image was captured separately using an 8-bit source image-processing program using a computer-assisted cooled CCD camera (Photometrics CH 220).
REFERENCES


Heyting, C., Dietrich, A. J. J., Moens, P. B., Dettmers, R. J., Offenberg, H. H.,


chromosomes: evidence that infertility in XYY male mice is an effect of two Y chromosomes. Chromosoma (Berl.) 100, 293-299.


junctions as intermediates in meiotic recombination. Cell 83, 783-791.


Tease, C. (1990). Sex chromosome configurations in pachytene spermatocytes of


The "meiotic quality control" which acts to remove spermatocytes with incompletely synapsed chromosomes utilises a p53-independent apoptotic pathway

Teresa Odorisio¹, Tristan Rodriguez¹, Edward P. Evans², Alan R. Clarke³ & Paul S. Burgoyne¹
¹Laboratory of Developmental Genetics, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK, ²MRC Mammalian Genetics Unit, Harwell, Didcot, OX11 0RD, UK and ³Department of Pathology, University Medical School, Teviot Place, Edinburgh EH8 9AG, UK

Correspondence should be addressed to PSB
Tel: 0181 959 3666 x2549/2122
Fax: 0181 906 4477
e-mail: p-burgoy@nimr.mrc.ac.uk
Summary

Evidence is accumulating that meiosis is subject to 'quality controls'. One of these monitors chromosome synapsis - spermatocytes and oocytes with chromosomes which fail to attain full synapsis during meiotic prophase are eliminated. In this study we show that in the testis the spermatocytes are eliminated by apoptosis. However, in contrast to irradiation-induced spermatogonial apoptosis which is p53 dependent, the spermatocytes are eliminated via a p53-independent apoptotic pathway. In mice with a single unpaired sex chromosome, spermatocyte apoptosis occurs principally at the first meiotic metaphase. In contrast to the usually rapid removal of apoptotic cells, the metaphase spermatocytes accumulate in large numbers.

Chromosome synapsis starts at zygotene during the first meiotic division and is completed by pachytene when crossing over between homologous paired chromosomes takes place. Several lines of evidence indicate that full chromosome synapsis is essential for completion of meiosis, its failure leading to germ cell loss and sterility. The most compelling evidence relates to sex chromosome synapsis.

In the early seventies Miklos\(^1\) postulated the existence of a "meiotic quality control" monitoring synapsis at pachytene and this concept was extended by Burgoyne & Baker\(^2\) to include oogenesis. An analysis of gametogenic failure in male and female mice with sex chromosome anomalies which affect pairing\(^3\) or with varying degrees of autosomal asynapsis\(^4-6\) has provided extensive supporting evidence. In particular, XS\(x^3\)Y males [in which the X chromosome carries a small portion of the short arm of the Y chromosome including the sex determining gene, \(Sry^7\)]
have been a valuable model for studying the effect of sex chromosome asynapsis on spermatogenesis. These animals are sterile\(^8\), due to an almost complete spermatogenic block during the meiotic metaphases\(^9,10\). However, when the tiny \(Y^*X\) chromosome [comprising a pseudoautosomal region attached to a non-\(Y\) centromere, and lacking any \(Y\)-specific DNA\(^11\)] was provided as a pairing partner for the \(X_{Sxr}^a\) chromosome, the meiotic block was overcome\(^12\).

Despite the considerable body of evidence supporting the existence of a meiotic quality control, there is as yet no information regarding its molecular basis. In this study we show that the "meiotic quality control" acts via p53-independent apoptosis to eliminate spermatocytes in which there is incomplete chromosome synapsis at pachytene.

**Spermatogenesis in \(X^Y^*O\) mice**

As a model system for studying the mode of spermatocyte death in mice with incomplete synapsis during pachytene, we utilised \(X^Y^*O\) mice which have a single sex chromosome comprising an \(X\) and a \(Y\) attached by a shared pseudoautosomal region\(^11\). \(X^Y^*O\) mice are sterile due to meiotic arrest, their testis weight is less than half that of control animals and their epididymides are usually devoid of sperm\(^3\). There is some evidence that univalent chromosomes can avoid detection by the meiotic surveillance system by forming a self-synapsed hairpin structure\(^3,13\). However, analysis of synaptonemal complexes in \(X^Y^*O\) pachytene spermatocytes has shown that the univalent \(X^Y^*\) chromosome only rarely achieves any self-synapsis\(^3\) (Rodriguez and Burgoyne, unpublished observations), so there can be little or no protection from elimination by this means.

To avoid secondary effects on spermatogenesis associated with the
continuous elimination of germ cells within the seminiferous epithelium, we have used young adult animals (30-40 d.p.p.). In all six \( XY^*O \) mice analysed spermatogenesis was severely affected (Fig. 1) and in only one were any mature sperm detected within the tubule lumen. Just as in \( XSxr^aO \) mice\(^{10} \), the meiotic block occurs during the metaphase stages with the metaphase spermatocytes accumulating in large numbers within the seminiferous epithelium before being eliminated (Fig. 1D). Air-dried preparations of testis cell suspensions showed that, just as in \( XSxr^aO \) males, the accumulated metaphases are in fact first meiotic metaphases. The accumulation of arrested MI spermatocytes prior to their elimination results in a 11-fold increase in the ratio of MI to diakinesis compared to controls (Table 1).

The arrested metaphase I spermatocytes are eliminated via apoptosis in \( XY^*O \) mice

Acridine orange staining

We have used acridine orange vital staining of intact seminiferous tubules to check for apoptosis, since acridine orange specifically stains the DNA of apoptotic cells\(^{14} \). A small minority of strongly fluorescent nuclei were visible in the samples from XY control animals (Fig. 2A) and these nuclei were scattered throughout the seminiferous epithelium, except for occasional small groups of contiguous cells (Fig. 2C). In contrast, the seminiferous epithelium of \( XY^*O \) mice showed dramatic concentrations of stained nuclei in restricted portions of the tubules (Fig. 2B,D). This result gave a first indication that large numbers of cells, at a specific stage of spermatogenesis, are eliminated by apoptosis from the seminiferous epithelium of \( XY^*O \) mice.

DNA in situ end labelling analysis

216
To identify the cells undergoing apoptosis we used DNA \textit{in situ} end labelling (ISEL) which allows the visualisation of fragmented DNA in tissue sections. DNA cleaved during apoptosis has a single-stranded DNA tail and with this technique DNA polymerase is used to incorporate a biotinylated nucleotide when filling in the second strand, allowing subsequent visualisation with DAB via peroxidase-conjugated avidin\(^15\).

In sections from XY mouse testes a few cells were stained, the brown nuclei were scattered among the tubule sections, and corresponded to various stages of spermatogenesis (Fig. 3A). In the sections from X\(^Y*\)O mouse testis many more cells were stained, the vast majority of these were concentrated in a few tubule sections (Fig. 3B) and corresponded to metaphase spermatocytes (Fig. 3C,D).

\textit{Analysis of DNA fragmentation}

Further evidence of apoptosis in X\(^Y*\)O spermatogenesis was obtained by analysing the pattern of DNA cleavage, which during apoptosis occurs preferentially in inter-nucleosomal regions resulting in a 'ladder' of oligonucleosomal length DNA fragments\(^16\). Genomic DNA was extracted from the seminiferous epithelium of young adult (~2 month old) and 17.5 days \textit{post partum} X\(^Y*\)O and XY males (17.5dpp. is prior to the MI block so the seminiferous epithelium of X\(^Y*\)O and XY males has the same germ cell constitution). After end labelling the DNA with \(^{32}\text{P}-\text{dCTP} and running it on a gel, the DNA from X\(^Y*\)O mice of both ages showed the laddering characteristic of apoptotic cells while no such laddering was evident in the XY controls (Fig. 4). An unusual feature of this laddering was that, in addition to the expected periodicity of about 180bp, there was also a very clear band of ~270bp. This may be a manifestation of conformational changes in the nucleosomes associated with the process of synapsis, allowing altered access of nucleases to the DNA axis. In the lane

217
corresponding to the DNA from \( X^{Y*}O \) prepubertal mice it is evident that fragmentation of the DNA is less well advanced than in the adult; the shortest band of the ladder, corresponding to the single nucleosome-sized DNA fragment (approximately 180 bp) is barely visible.

**Apoptosis in \( XSxr^aO \) and T16H/Y males**

Histologically the spermatogenic block in \( XSxr^aO \) males is indistinguishable from that of \( X^{Y*}O \) males. Because \( XSxr^aO \) males have been used previously in providing evidence for the existence of the meiotic quality control, we also investigated the mode of spermatocyte death in these males. Acridine orange staining and ISEL revealed the same apoptotic elimination of metaphase spermatocytes as in \( X^{Y*}O \) males (not shown).

In T16H/Y males, which carry the reciprocal X-autosome translocation T16(X;16)H\(^4\), there is also meiotic failure, but in this case most spermatocytes are eliminated during the pachytene stage (Fig. 5A). It has been a matter of debate whether this loss is triggered by the disruption of synapsis associated with the translocation or whether it is due to interference with the inactivation of the X during meiotic prophase\(^2,17,18\). After staining the tubules with acridine orange a clear difference was evident between T16H/Y and XY samples, the former showing a much higher number of stained nuclei (Fig. 5B), but in contrast to \( X^{Y*}O \) and \( XSxr^aO \) testes, the stained nuclei were less densely packed and were distributed over longer lengths of the tubules. When the nuclear DNA was in situ end labelled it was apparent that the apoptotic cells were predominantly pachytene spermatocytes, probably late pachytene (Fig 5C). Thus it appears that apoptotic elimination is a feature of chromosomally induced meiotic failure whether it occurs at pachytene or
MI. However, in contrast to the 'piling up' of MI spermatocytes in $X^Y*O$ males, there is no accumulation of pachytene cells in T16H/Y males, suggesting that the dying cells are rapidly eliminated.

*p53 is not in the molecular pathway of the meiotic quality control mechanism*

Expression of the tumour suppressor gene *p53* is associated not only with the arrest of the cell cycle at **G**₁, but can also trigger apoptosis^{19-21}. The highest levels of *p53* expression have been found in pachytene spermatocytes^{22}; it therefore seemed possible that *p53* may be involved in the low level of apoptotic elimination of spermatocytes which occurs in normal testes and that *p53*-mediated apoptosis may also be responsible for the elimination of MI spermatocytes in $X^Y*O$ males. However, we first wished to establish that the *p53*- dependent apoptotic pathway can function in the testis. *p53*-dependent apoptosis is known to be triggered in some cell types by irradiation induced DNA damage^{23-27}; furthermore, irradiation is known to induce apoptosis in the testis, affecting spermatogonia in particular^{28,29}. We therefore analysed the apoptotic response of spermatogonia in *p53* null testes to 5Gy of whole body _-_ irradiation and compared this with controls. At 16h post irradiation there is a huge increase in the number of dying spermatogonia in XY testes and ISEL straining confirmed that the spermatogonia were being eliminated by apoptosis. In marked contrast there was almost no increase in spermatogonial apoptosis in *p53* null XY testes (Table 2 and Figure 6), so the apoptotic response to irradiation is clearly *p53* dependent.

We next generated *p53* null $X^Y*O$ mice to see if this prevented or reduced the apoptotic response to the presence of an unsynapsed sex chromosome. The three males produced were test mated but proved to be sterile. Their
testis weights (41-58mg) were in the normal range for $X^Y$O mice (38-64mg, 16 males) and the histological analysis of the testes showed that the lack of $p53$ had no effect on the spermatogenic block. Both the acridine orange and the ISEL analysis revealed the same apoptotic features as $p53$-positive $X^Y$O mice (not shown). Thus, the apoptotic elimination of spermatocytes in $X^Y$O testes is $p53$-independent.

**Discussion**

Conceptually the meiotic quality control can be divided into two components: 1) A system for signalling the presence of unpaired chromosome regions in pachytene cells, and 2) a mechanism which responds to the signal and causes the cells to die. In this study, by using $X^Y$O male mice as a model system, we have shown that the second component is acting via apoptosis.

How might unpaired sex chromosomes generate a signal which triggers apoptosis? We have been attracted to the possibility that the signal might be generated by unrepaired breaks in the DNA of pachytene cells. This is for three reasons. First, an early study$^{30}$ identified increased nicking of DNA during late pachytene as an early manifestation of the meiotic failure in males with translocation-associated synaptic errors. Second, studies of meiosis in yeast have led to the conclusion that double strand breaks in DNA are a feature of the 'homology search' prior to chromosome synapsis$^{31,32}$, and it is possible that in the absence of synapsis such DNA breaks may remain unrepaired$^{33}$. Third, DNA damage can trigger apoptosis.

Apoptosis triggered by DNA damage has been linked with upregulation of the tumour suppressor gene $p53$ and a pivotal role for $p53$ was
confirmed by the finding that thymocytes, keratinocytes and intestinal crypt cells from mice homozygous for a \( p53 \) null mutation are highly resistant to irradiation induced apoptosis\(^{23-27} \). Pachytene spermatocytes are the cells which express the highest levels of \( p53 \) protein in the testis\(^{22} \), which led the authors to suggest that the function of \( p53 \) at this particular stage of spermatogenesis is to allow time for the repair and reshuffling of DNA prior to the reductive divisions. Nevertheless, homozygous \( p53 \) null males can be fertile\(^{24,34} \). This led us to ask whether \( p53 \) expression in the testis might be a manifestation of the meiotic quality control process; in which case elimination of gene function, rather than making normal males sterile, might overcome the sterility of males with incomplete chromosomal synapsis.

The present results, showing that homozygosity for a null mutation for \( p53 \) did not overcome the meiotic block or apoptotic elimination of metaphase spermatocytes in \( X^Y \) \( O \) males, demonstrates that the apoptotic elimination of spermatocytes with incompletely synapsed chromosomes is \( p53 \)-independent; this is despite the fact that the \( p53 \)-dependent apoptotic pathway is utilised by spermatogonia following irradiation-induced DNA damage (our results). It thus now seems much less likely that unsynapsed chromosomes trigger apoptosis because of DNA damage. Indeed, the fact that we could already detect the DNA cleavage 'ladder' at 17.5dpp, makes it likely that the nicking of the DNA at late pachytene observed by Hotta et al.\(^{30} \) was simply a manifestation of the onset of apoptosis.

Our finding that \( p53 \) is not an essential component of the meiotic quality control re-opens the question of the function of \( p53 \) in pachytene spermatocytes. Rotter et al.\(^{35} \) have reported an increased incidence of multinucleated giant cells in the seminiferous epithelium of mice with
reduced p53 expression. In other situations such multinucleated cells in the testis are eliminated by apoptosis. This suggests that p53 may function in the testis to prevent some errors occurring in the meiotic process, and that in its absence these errors are detected by some other 'quality control' which eliminates them via apoptosis. Armstrong et al.\textsuperscript{34} have reported an increase in developmental abnormalities in p53-deficient mice which they suggest could result from increased transmission of genetic damage occurring during spermatogenesis. In this regard, it will be important to determine the fate of the p53 null spermatogonia which escape the normal process of irradiation induced apoptosis. We would predict that DNA damage leading to chromosomal rearrangements which disrupt synapsis, will result in elimination by the p53-independent pathway which monitors synapsis. However, not all rearrangements disrupt synapsis; insertions, duplications and deletions, in particular, can be accommodated by synaptic adjustment\textsuperscript{36}. Indeed, a number deletions, duplications and other gross chromosome anomalies have been shown to be compatible with fertility\textsuperscript{37}, so it would certainly be worth screening for chromosomal anomalies among the offspring (or early fetuses) of irradiated p53 null mice.

Cells undergoing apoptosis are usually removed very rapidly, either by macrophages or in some cases by neighbouring cells. Macrophages are excluded from the seminiferous epithelium and Sertoli cells substitute for macrophages in eliminating apoptotic cells\textsuperscript{28,38}. What was particularly striking about the apoptosis in the XY\textsuperscript{O} males was the delay in eliminating the apoptosing metaphase I spermatocytes, which consequently accumulated in large numbers. This was in marked contrast to the rapid elimination of pachytene cells in the males carrying the X-autosome translocation T(X;16)16H. As we have pointed out elsewhere\textsuperscript{3}
the piling up of metaphase I spermatocytes prior to their elimination, so strikingly apparent in the acridine orange stained tubule whole mounts in the present study, has important implications for the interpretation of meiotic data in mice with sex chromosome anomalies. In particular, it is relevant to the arguments used to support the view that during spermatogenesis there is a quality control which monitors recombination of the sex chromosomes, in addition to that which monitors synapsis\textsuperscript{39,40}. Studies of XYSX\textsuperscript{41,42} and XY\textsuperscript{*} males\textsuperscript{39}, which have relatively high levels of sex chromosome asynapsis at pachytene, have conclusively demonstrated (i) that the sex chromosome univalents seen at Diakinesis/MI are non-recombinant and (ii) that the cells with the univalents do not progress to MII; that is to say, they arrest at MI just like XY\textsuperscript{*}O spermatocytes. The crucial question is whether the lack of recombination is entirely due to a lack of synapsis at pachytene, or whether a proportion of the the non-recombinant cells did synapase but no chiasma was formed. Hale argues that the large increase in the frequency of sex chromosome univalence between pachytene and diakinesis/MI in XY\textsuperscript{*} males shows that a substantial proportion of the cells which were synapsed at pachytene, subsequently separate. This argument is undermined if, as demonstrated by the present results, MI spermatocytes with unpaired sex chromosomes accumulate before being eliminated. We have in fact looked at MI to diakinesis ratios in an XY\textsuperscript{*} male and there was a ~4-fold increase compared to the controls, showing that there is indeed a piling up of MI spermatocytes. This is discussed more fully elsewhere\textsuperscript{3}.

The delay in eliminating apoptotic metaphase spermatocytes does have some potential advantages for studies of apoptosis. It should, for example, make it easier to separate the molecular events triggering apoptosis from those involved in the self-destruction of the cell. Thus, it
may be possible to identify the molecular events which link chromosome asynapsis with activation of the apoptotic pathway, by identifying differences in gene expression between purified pachytene spermatocytes from $X^Y*O$ and $XY$ males.

**Methods**

**Mice**

In each experiment the $XY$ controls were matched for age with the sterile males.

$XY^*O$ males

To generate $X^Y*O$ males, females heterozygous for the $X$ inversion $\text{In}(X)\text{1H}$, which produce some nullo-$X$ eggs following crossing over within the inversion\(^{43}\), were mated to $XY^*$ males which generate the recombinant $XY^*$ chromosome\(^{11}\). About 1/7 of the male progeny had an $XY^*O$ genotype.

$p53$ null $XY$ and $X^Y*O$ males

Outbred heterozygous $p53$ null mice\(^{24}\) were crossed with $\text{In}(X)/\text{In}(X)$ females and $XY^*$ males to generate $p53$ null/+ $\text{In}(X)/X$ females and $p53$ null/+ $XY^*$ males. These were then mated together to generate homozygous $p53$ null mutant $X^Y*O$ males. $p53$ status was determined by PCR using the following primers: INTRON 7, caa aga gcg ttg ggc atg tg; EXON 6, gtg gtg gta cct tat gag cc; NEO, cat cgc ctt cta tgc cct tc. Primers INTRON 7 and EXON 6 amplify the wild-type allele giving a product of 642bp, while primers INTRON 7 and NEO give a diagnostic 510bp product from the targeted allele.

$XSxr^O$ males

In $XYSxr$ carrier males $Sxr$ is located distal to the pseudoautosomal region on the long arm of the $Y$ chromosome and is transferred to the $X$ chromosome by crossing over during male meiosis\(^{41}\). $Sxr^A$ is the original
(H-Y positive) version of Sxr. To generate X$Sxr^a$O males, XYSxr$^a$ males were mated to In(X)/X females.

**T16/Y males**

Males carrying the X-autosome translocation T(X;16)16H$^{44}$ were produced by mating heterozygous T16/X females (on a predominantly 129 inbred background) to 129 males. The normal X of the T16/X females carried the X-linked coat marker *Tabby*; their T16/Y sons were non-*Tabby* males.

**Karyotyping**

Air-dried metaphase spreads were obtained from bone marrow cells post mortem using standard procedures.

**Histology**

Testes were fixed overnight in freshly prepared 4% paraformaldehyde, dehydrated, cleared and embedded in paraffin wax using standard procedures. 5 mm sections were mounted on TESPA (3-aminopropyltriethoxysilane) (Sigma) coated slides. Some of the sections were stained with haematoxylin and eosin for the histological analysis, while other sections from the same testes were used for the *in situ* analysis of apoptotic cells.

**Air-dried preparations**

Two air-dried slides of fixed testicular cell suspensions were prepared from each of 6 $X^*O$ males and 6 XY controls and were stained with toluidine blue. Diakinesis and metaphase I spermatocytes, classified as to stage on the basis of the degree of condensation and 'opening out' of the bivalents were scored using a low power objective. Since the slides could not be scored 'blind' with respect to genotype (because the preparations
from the \(X^{Y*}0\) males lack sperm), a check was made for scoring bias by using mixtures of \(XY\) and \(X^{Y*}0\) testicular cells. Each diakinesis/MI plate from the mixtures was classified as to stage under low power and then for genotype under high power. The mean increase in the MI/Dia ratio in the \(4X^{Y*}0\) males assessed ‘blind’ from the mixtures was 10.4-fold, in close agreement with the estimate of 10.6-fold for the same males assessed separately and by a different observer.

**Preparation of seminiferous tubules and testis cell suspensions**

Adult testes were decapsulated and then treated with collagenase (0.25 mg/ml, Sigma-Type XI) for 15 min at 32°C in a shaking incubator, in HEPES buffered Dulbecco modified Eagle’s medium (DMEM). Decapsulated prepubertal (17.5dpp) testes were incubated in DMEM containing collagenase (0.5 mg/ml) and hyaluronidase (0.5 mg/ml) (Boehringer Mannheim) for 20 min at 32°C. Tubules were then washed and incubated with hyaluronidase (0.5 mg/ml) for a further 10 min. In all cases, after incubation, the seminiferous tubules were washed twice with the medium to remove the interstitial cells. Cell suspensions were obtained from the isolated seminiferous tubules by incubating at 32°C in DMEM containing 2.5 mg/ml trypsin (Gibco-BRL) and 50 g/ml DNAse (DN-25, Sigma) for 15 min. After incubation, trypsin digestion was stopped by adding 8% foetal calf serum and gently pipetting the suspension. Undigested tubule fragments were then allowed to settle for 2-3 min before removing the cell suspension for analysis.

**Analysis of apoptosis**

**Analysis of DNA laddering**

DNA was extracted according to Shi et al.\(^{46}\). The purified DNA was end-
labelled using Klenow polymerase (Promega) and analysed as described by Rösl\textsuperscript{16}.

*Acridine orange staining*

Seminiferous tubules or testis cell suspensions prepared as described above were incubated in DMEM containing 5 _g/ml of acridine orange (Sigma) at 32_C for 15 minutes, washed twice with PBS, placed on a slide, covered with a mounting medium for fluorescence (Vectastain, Vector) and a coverslip was gently pressed over them to spread the tissue or cell suspension. Samples were analysed on a fluorescence microscope using a rhodamine filter.

*DNA in situ end labelling analysis*

For *in situ* detection of apoptotic cells the procedure described by Wijsman et al.\textsuperscript{15} was followed with minor modifications. Briefly, sections were heated in 2 x SSC at 80_C for 20 minutes and then incubated with pepsin (Sigma) (4.5 units/ml) at 37_C for 15 minutes in HCl pH 2. The specimens were rinsed in a buffer containing 50 mM Tris-HCl pH 7.5, 5 mM MgCl\textsubscript{2}, 10 mM _-mercaptoethanol and 0.005% BSA (Fraction V, Sigma) for 5 minutes, dried and incubated in the same buffer containing 0.01 mM dATP, dCTP, dGTP (Pharmacia), 0.01 mM biotin-11-dUTP (Sigma), and 20 u/ml E. coli DNA polymerase I (Promega) for 5 minutes at room temperature (RT). Endogenous peroxidase was blocked by immersing the slides in PBS with 0.1% H\textsubscript{2}O\textsubscript{2} for 15 minutes. The slides were then incubated with horseradish peroxidase-conjugated avidin (Vector) diluted 1:100 in PBS containing 1% BSA and 0.5% Tween 20, for 30 minutes at RT, in a humid chamber. The staining was developed with diaminobenzidine (DAB) (Sigma) and sections were counterstained with methylgreen. Negative controls in which DNA polymerase was omitted from the nucleotide mixture were included in all experiments.

*Irradiation*
Outbred (a mixed 129 and SWR background) p53-null mice and wild type litter mates were given whole body \(-\)irradiation from a \(^{137}\)Cs source (\(-0.33\text{Gy min}^{-1}\) for 15 min) so that each animal received 5Gy. Two males of each genotype were processed at 16 and 42 hours post irradiation together with sham-irradiated controls. Testes were fixed in buffered formol and stained in haemotoxylin and eosin. Dying spermatogonia in stage II-V tubules were scored from 40 tubule cross sections per male. ISEL was used to confirm that the dying cells were apoptotic.

Acknowledgments

TO was the recipient of a European Communities HCM fellowship and TR an MRC studentship.

References


Figure 1. The spermatogenic block in X^{Y*}O males. Hematoxylin & eosin stained sections of: A. Stage IV XY tubule with round spermatid (rs) and elongating spermatid (es) layers internal to the pachytene (p) spermatocytes. B. Stage IV X^{Y*}O tubule showing the absence of the spermatid stages, reduced tubule diameter and multiple layers of pachytene cells (p). C. Stage XII XY tubule showing two rare meiotic metaphase spermatocytes (arrowed and enlarged inset). Meiotic metaphases are very brief and are difficult to find in control testes. D. Stage XII/I X^{Y*}O testis tubule with large numbers of arrested metaphase spermatocytes. Tubules with large numbers of meiotic metaphases appear to be at stage I (when the meiotic divisions should be complete) on the basis of spermatogonial morphology and most of the metaphases are more eosinophilic (see inset) than in controls suggesting that they have arrested and are dying. Air dried preparations (not shown) showed that the arrested metaphases are at the first meiotic metaphase.

Figure 2. Apoptosis in X^{Y*}O testes: acridine orange stained material. A. Whole tubule mount from XY testis showing low numbers of acridine orange positive cells (the diffuse staining is trapped dye-positive cells are intensely stained (see C & D). B. Whole tubule mount from X^{Y*}O testes showing the massive increase in acridine orange positive cells. Nevertheless, there are regions of tubule which are unaffected, suggesting that the apoptotic loss is restricted to specific stages of the spermatogenic cycle. C. Higher magnification of a group of contiguous acridine orange stained cells from an XY testis. These may be a group of spermatogonia or spermatocytes joined by intercellular bridges, since it has been shown that apoptosis often affects groups of conjoined cells. D. Higher magnification of a region of X^{Y*}O tubule with large numbers of
acridine orange positive cells.

Figure 3. Apoptosis in X<sup>Y*O</sup> testes: ISEL staining. A. Occasional ISEL-positive cells in a control XY testis section (arrows). The position of the cells suggests that spermatogonia and spermatocytes are affected. B. X<sup>Y*O</sup> testis section showing tubule sections with low levels of ISEL positive cells interspersed with tubule sections containing marked accumulations of ISEL positive cells. C. Higher magnification of a tubule with accumulated ISEL positive cells. D. High magnification of ISEL positive cells. In this case ISEL staining was reduced to allow the identification of the cell type more clearly. It is clear that these cells are accumulated metaphase spermatocytes (compare with inset in 1D).

Figure 4. Apoptosis in X<sup>Y*O</sup> testes: laddering of cleaved DNA. The DNA from the spermatogenic epithelium of control males shows little evidence of DNA cleavage. The DNA from the spermatogenic epithelium of 17.5day old X<sup>Y*O</sup> males already shows some cleavage even though there is no clear cytological evidence of spermatogenic failure at this age. A double nucleosome length fragment of ~360bp is clearly visible and there is a faint single nucleosome length fragment of 180bp. Unusually there is also a strong band of ~270bp, which would equate with 1.5 nucleosomes. In adult X<sup>Y*O</sup> testes the DNA cleavage is more advanced with single nucleosome length fragments forming a clear ~180bp band. The 270bp band is still present and is clearly out of synchrony with the ~180bp periodicity of the rest of the ladder.

Figure 5. The spermatogenic block and apoptosis in T16/Y testes. A. H & E stained tubule section showing the absence of postmeiotic stages. In contrast to X<sup>Y*O</sup> testes, metaphase spermatocytes are rarely seen, the
block being essentially complete by late pachytene. B. Acridine orange stained whole tubule mount showing the more widespread and lower density of stained cells in T16/Y testes as compared to X^{Y+}O testes. This reflects the fact that cells are eliminated at the pachytene stage which is present at nearly all stages of the spermatogenic cycle. C. ISEL stained tubule section showing apoptosing pachytene spermatocytes.

Figure 6. Irradiation induced spermatogonial loss is p53-dependent. A,B. Stage III tubules from a control male 16 hours post irradiation. The majority of the spermatogonia are eosinophilic with pyknotic nuclei (indicating cell death). C. Higher power view of dying spermatogonia. D. Stage III tubule from a p53-null male 16 hours post irradiation. No dying cells are seen.
Table 1. The ratio of metaphase I to diakinesis spermatocytes in $X^{Y*}O$ as compared to control males

<table>
<thead>
<tr>
<th></th>
<th>Metaphase I*</th>
<th>Diakinesis*</th>
<th>MI/Dia. ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY #1</td>
<td>13, 12</td>
<td>37, 38</td>
<td>0.33</td>
</tr>
<tr>
<td>#2</td>
<td>20, 19</td>
<td>30, 31</td>
<td>0.64</td>
</tr>
<tr>
<td>#3</td>
<td>9, 19</td>
<td>26, 30</td>
<td>0.50</td>
</tr>
<tr>
<td>#4</td>
<td>10, 12</td>
<td>23, 27</td>
<td>0.44</td>
</tr>
<tr>
<td>#5</td>
<td>16, 11</td>
<td>25, 26</td>
<td>0.52</td>
</tr>
<tr>
<td>#6</td>
<td>6, 7</td>
<td>27, 34</td>
<td>0.21</td>
</tr>
<tr>
<td>mean ± SE</td>
<td></td>
<td></td>
<td>0.44±0.06</td>
</tr>
<tr>
<td>$X^{Y*}O$ #1</td>
<td>33, 36</td>
<td>17, 14</td>
<td>2.23</td>
</tr>
<tr>
<td>#2</td>
<td>44, 45</td>
<td>6, 4</td>
<td>8.9</td>
</tr>
<tr>
<td>#3</td>
<td>54, 64</td>
<td>18, 14</td>
<td>3.69</td>
</tr>
<tr>
<td>#4</td>
<td>82, 77</td>
<td>18, 23</td>
<td>3.88</td>
</tr>
<tr>
<td>#5</td>
<td>85, 85</td>
<td>15, 15</td>
<td>5.67</td>
</tr>
<tr>
<td>#6</td>
<td>83, 80</td>
<td>17, 20</td>
<td>4.41</td>
</tr>
<tr>
<td>mean ± SE</td>
<td></td>
<td></td>
<td>4.80±1.26</td>
</tr>
</tbody>
</table>

* The duplicate numbers are for the duplicate slides from each male.
Table 2. Spermatogonial death following irradiation in p53-null and control males

<table>
<thead>
<tr>
<th></th>
<th>Dying spermatogonia in 20 tubule cross sections per slide-</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unirradiated</td>
<td>16h post irradiation</td>
<td>42h post irradiation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>slide 1</td>
<td>slide 2</td>
<td>total</td>
<td>slide 1</td>
</tr>
<tr>
<td>Control #1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td>Control #2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>138</td>
</tr>
<tr>
<td>p53-null #1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>p53-null #2</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

-Tubules at stages II-V only. These are the tubule stages previously shown to have apoptotic spermatogonia at 16 hours post irradiation in the rat and to have returned close to unirradiated levels by 42 hours post irradiation.\(^{29}\)