Non-homologous chromosome synapsis during mouse meiosis: consequences for male fertility and survival of progeny.

Niet-homologe chromosoom synapsis tijdens de meiose van de muis: gevolgen voor mannelijke vruchtbaarheid en overleving van nageslacht.

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Non-homologous chromosome synapsis during mouse meiosis: consequences for male fertility and survival of progeny

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Proefschrift

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Stellingen

1. Chromosomen hebben met betrekking tot hun synaptisch gedrag een geheugen.

Dit proefschrift

2. Homo- of heterozygotie voor de Robertsonische translocatie Rb(11.13)4Bnr (Rb4) heeft niet altijd een reducerend effect op de mannelijke vruchtbaarheid. Afhankelijk van de overige in het karyotype aanwezige chromosoomafwijkingen kan Rb4 ook stimulerend werken.

Dit proefschrift

3. Niet-homologe chromosoom synapsis in afwezigheid van meiotische recombinatie leidt niet tot dominante of recessieve vormen van chromosoom instabiliteit. In aanwezigheid van recombinatie mogelijk wel.

Dit proefschrift

4. Klassieke chromosoom mutanten kunnen een vergrootglas funktie vervullen bij meiotisch cytologisch onderzoek.

Plug *et al.*, Nature Genetics, in press

5. Publiceerbaarheid van gegevens bepaalt niet alleen het plezier in wetenschappelijk onderzoek. Het kan dit wel versterken.

6. Moleculair biologen leven in zwart/wit, cytologen in kleur.

7. Sonore koorzang is het zoeken naar harmonie tussen het individu en het collectief.

8. Helaas zijn veel (vooraanstaande) wetenschappers geen goede koorzangers.

9. Nalatige hooggeplaatsten zijn ware alchemisten: het lukt hen vaak het vuil aan hun handen om te zetten in goud.

10. Email maakt onmogelijke relaties mogelijk.

11. Het betere is de vijand van het goede.

Stellingen behorende bij het proefschrift
"Non-homologous chromosome synapsis during mouse meiosis:
consequences for male fertility and survival of progeny"

Wageningen, 21 november 1997 Antoine H.F.M. Peters

Voor mijn vader

Contents

Chapter 1

General Introduction

Introduction

In most eukaryotic organisms the sexual reproductive cycle is characterized by an alternation of diploid and haploid generations of cells. The transition from diploid to haploid phase occurs at meiosis. The diploid state is restored by fusion of two haploid cells (gametes) at fertilization.

A diploid cell contains two versions of each chromosome, referred to as homologues, one derived from each parent. In the mitotic cell cycle, DNA replication and chromosome condensation are followed by equational segregation of sister chromatids so that the two daughter cells will each inherit the same chromosome complement as the mother cell (fig. 1). In meiosis, a single round of DNA replication is followed by two successive rounds of nuclear division, referred to as meiosis I and II (fig. 1). After premeiotic S-phase, during the prophase of meiosis I, chromosomes condense, homologues pair and non-sister chromatids of homologues recombine with each other. Then, at anaphase of meiosis I, homologues move to opposite poles so that the number of chromosomes per nucleus is reduced from the diploid to the haploid number. At meiosis II, sister chromatids segregate as during mitosis. Meiosis thus yields four haploid cells, each containing a different and novel assortment of genes.

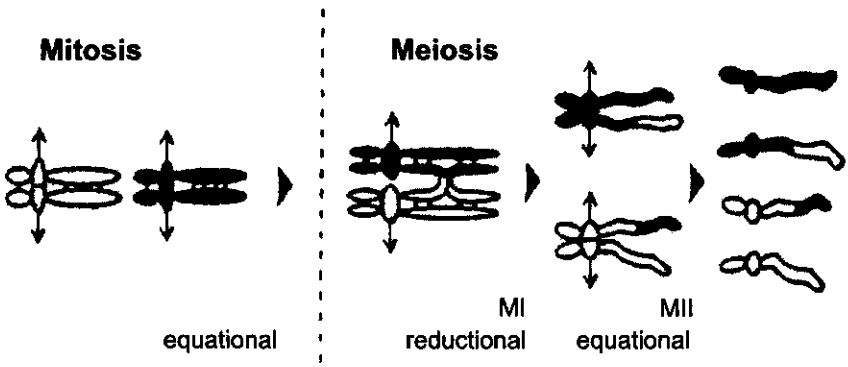

Figure 1: Mitosis and meiosis (from Kleckner, 1996)

Part I of the introduction focusses on two meiosis specific structures which have been the classical objects of cytological research on prophase I of meiosis: synaptonemal complexes (SC) and recombination nodules (RN). Subsequently, recent findings on meiosis in yeast and mammals are described. In Part II, cytological and genetic data on meiosis in mouse heterozygotes for structural chromosome aberrations are described which form the basis of this thesis.

Part I

Synaptonemal complexes

The SC is a proteinaceous, zipper like structure that holds the homologues in close apposition along their entire length during meiotic prophase (von Wettstein *et al.*, 1984). Following DNA replication in premeiotic S-phase, the chromosomes start to condense during the meiotic prophase I at the leptotene stage and each pair of sister chromatids develops a common proteinaceous axis called an axial element (fig. 2). The chromatin itself is organized in loops which are attached to the axial element at their base (Weith and Traut, 1980). In the subsequent zygotene stage, the axial elements become interconnected by numerous thin transverse filaments and a longitudinal structure, called the central element, which develops between the axial elements (Schmekel *et al.*, 1993; Schmekel and Daneholt, 1995). The connecting process and the resulting tripartite structure are called synapsis and SC, respectively (Moses, 1968; von Wettstein *et al.*, 1984). The axial elements within an SC are referred to as lateral elements. During pachytene, all homologues display the SC structure from telomere to telomere and symbolize the bivalents. In early diplotene, the SC starts to disintegrate. In mammals the transverse filaments disappear so that two axial elements per chromosome pair remain. At the end of diplotene and the onset of diakinesis, the axial elements have also disappeared. During the successive zygotene and pachytene substages of meiotic prophase I recombination between non-sister chromatids takes place. During the subsequent diakinesis stage, the bivalents condense further and the scaffolds of the individual sister chromatids become discernible. Yet, adhesion between sister chromatids remains intact, aiding in the delineation of chiasmata which are the cytological representations of crossing over between homologous non-sister chromatids. At prometaphase, the nuclear envelope disappears while the centromeres become attached to the spindle. Concomitantly, the bivalents orient themselves in preparation of chromosome disjunction. Until disjunction at metaphase I, the homologues remain attached to each other by the chiasmata.

Recombination nodules

Recombination nodules (RNs) are spherical to ellipsoidal proteinaceous structures approximately 100 nm in diameter that become closely associated with elements of the SC, before and after completion of this structure during prophase I (Carpenter, 1975; von Wettstein *et al.*, 1984; Stack *et al.*, 1993; Stack and Roelofs, 1996). In many eukaryotes, two types of RNs can be distinguished: early and late ones.

In plants, numerous early RNs are observed along axial elements at the leptotene stage. At zygotene, early RNs are often observed at sites of convergence between synapsing axial elements of homologous chromosomes and in association with completed SCs (Albini and Jones, 1987; Anderson and Stack, 1988; Zickler *et al.*, 1992). When synapsis is complete at

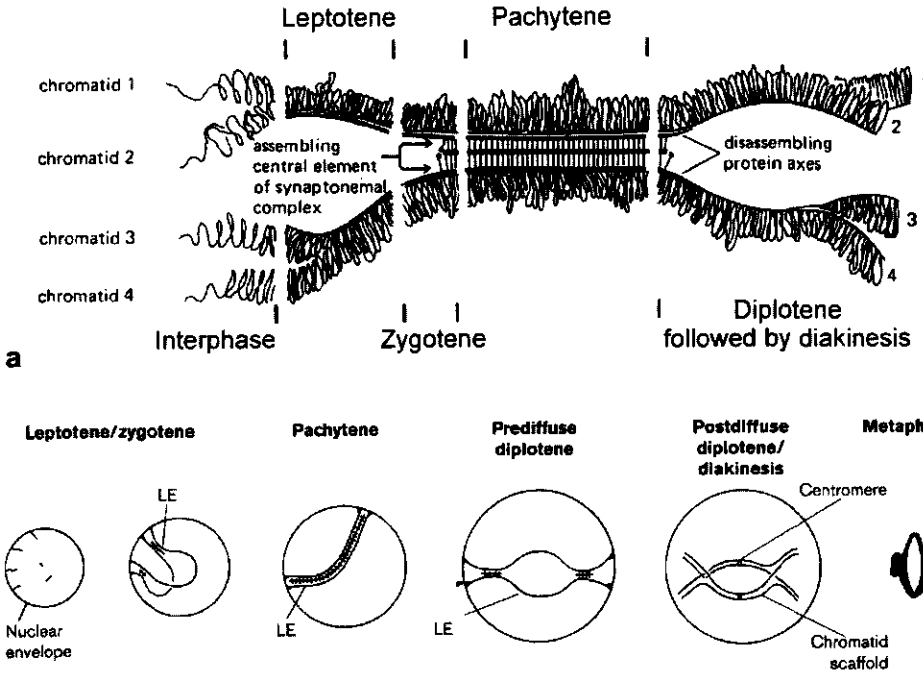

Figure 2: a A schematic representation of the successive stages of meiotic prophase as defined on the basis of morphological changes of the SC: leptotene (proteinaceous axes start to form along homologous chromosomes), zygotene (start of synapsis of homologues), pachytene (synapsis is completed and the SC extends from telomere to telomere), diplotene (SC disassembles) and diakinesis (SCs are completely disassembled)(from Alberts *et al.*, 1989). **b** Schematic diagram showing the events in a single chromosome pair in a meiotic prophase nucleus. The circles represent the nuclear envelope. In leptotene/zygotene, pachytene and prediffuse diplotene, the axial cores/LEs of the SC are represented (indicated by LE). The axial cores/LEs are probably localized between the sister chromatids (not shown). In the postdiffuse diplotene nucleus, the scaffolds (longitudinal supporting structures) of the sister chromatids are shown. In leptotene phase, the assembly of the axial cores begins mainly at the nuclear envelope. In the zygotene phase, axial cores are connected by transversal filaments to form SCs, which are represented by zipper-like stretches. Synapsis is complete in the pachytene phase. The telomeric ends of the axial cores are attached to the nuclear envelope and in the zygotene phase these ends are clustered in a so-called bouquet arrangement. During pachytene, the telomeric ends have spread out but remain attached to the nuclear envelope. In the prediffuse diplotene phase, the SCs are disassembled and in postdiffuse diplotene/ diakinesis phase, the axial cores/LEs have completely disappeared whereas the scaffolds of the sister chromatids become discernible. In addition to this, the telomeres are detached from the nuclear envelope. In metaphase I, the nuclear envelope has disassembled and the chromosomes have condensed further. Moreover, the chromosomes are deformed by the attachment of the centromeres to the spindle (which is not shown in this figure). Due to the compaction of the chromosomes the scaffolds of the sisterchromatids are no longer discernible (from Heyting, 1996).

early pachytene, the number of early RNs gradually decreases so that from mid through late pachytene no early RNs are left. The number and distribution of early RNs along the bivalents does not correlate with the distribution of crossovers. It has been suggested that early RNs are involved in the homology search and that they leave gene conversions as footprints of their activity (Carpenter, 1987, 1994).

Late RNs appear on the central element of SCs during early pachytene and persist into early diplotene. The frequency and distribution of the late RNs correlates with those of reciprocal exchange events and chiasmata in organisms like *Sordaria, Drosophila* and tomato (Zickler *et al.*, 1992; Carpenter, 1994; Sherman and Stack, 1995). Late RNs are therefore presumed to mark the sites of reciprocal crossing over. Moreover, in *Sordaria* (Zickler *et al.*, 1992) and tomato (Havekes *et al.* 1994), the position of late nodules almost completely coincides with the SC initiation sites. A re-examination of the mammalian data on the observed numbers of late RNs and chiasmata however, revealed a severe deficiency in RNs in male mice and human and in female marsupials near chromosome ends and other recombinational hotspots or positions of localized chiasmata (Ashley, 1994). Apparently, there is a RN-independent pathway for reciprocal recombination in these organisms that operates specifically at recombinational hotspots (Ashley, 1994).

Chromosome pairing, synapsis and meiotic recombination in yeast and mammals: temporal considerations

During recent years, the use of a combination of genetic, molecular and cytological techniques has revealed an overwhelming amount of data on the process of homologous chromosome pairing, synapsis and recombination during meiosis in the budding yeast *Saccharomyces cerevisiae* (see reviews: Roeder, 1995; Kleckner, 1996). The often surprising results have necessitated to abandon the idea that pairing of the homologous chromosomes depends upon SC formation and that recombination is exclusively initiated in the context of the tripartite SC.

In yeast, homologues are paired via multiple interstitial interactions in premeiotic cells and likely also in vegetative cells (Weiner and Kleckner, 1994). Intimate interstitial interactions are lost during premeiotic S phase, though homologues may remain substantially colocalized. Then interactions reappear, independent of and probably prior to initiation of recombination (Weiner and Kleckner, 1994, Loidl *et al.*, 1994, Nag *et al.*, 1995). Most or all meiotic recombination is initiated by double-strand breaks (DSBs) (Game *et al.*, 1989; Nicolas *et al.*, 1989; Sun *et al.*, 1989; Cao *et al.*, 1990; Wu and Lichten, 1994), which are catalyzed by the protein Spo11 (Keeney *et al.*, 1997). In yeast, almost all DSBs occur in intergenic regions that contain transcription promoters (Wu and Lichten, 1994). Transcription is not required for DSB formation (White *et al.*, 1992) and transcription through the promoter can interfere with recombination hot spot activity (Rocco *et al.*, 1992) . 5' strand termini of the DBSs are rapidly

resected, leaving 3'single stranded tails suitable for strand invasion and polymerase extension. DSB formation precedes the initiation of SC assembly (Padmore *et al.*, 1991) which is approximately concomitant with double Holliday junction formation (Schwacha and Kleckner, 1995). Double Holliday junctions persist until the end of pachytene (Schwacha and Kleckner, 1995). Mature crossover and non-crossover (gene conversion) products are formed at about the time when the SC disappears (Padmore *et al.*, 1991), but their appearance is not dependent upon SC disassembly (Xu *et al.*, 1995).

For mouse and human, it has recently been shown by Scherthan *et al.* (1996) that synapsis is also preceded by chromosome pairing, at least of the telomeres. These authors studied the early steps of meiotic chromosome pairing and synapsis by performing fluorescence in situ hybridization (FISH) with chromosome-specific probes in combination with immunocytochemical staining of SC components in mouse and human testis tissue sections. In spermatogonia, the premeiotic homologous chromosomes occupy compacted predominantly separate domains. The meiotic pairing process starts after premeiotic S-phase by the transition of centromeres to the nuclear envelope. Slightly later, the telomeres also become attached to the nuclear envelope. A comparable movement of centromeres and telomeres has been found in the meiotic prophase of *Schizosaccharomyces pombe* (Chikashige *et al.*, 1994; Kohli, 1994), an organism which does not form SCs (Bähler *et al.*, 1993; Kohli and Bähler, 1994). The chromosomal domains then develop into long thin cords while the centromeres of the (sub)metacentric chromosomes move away from the nuclear membrane and the telomeres cluster at one site of the nuclear envelope. The resulting chromosomal bouquet arrangement, which is a common feature of meiotic prophase (von Wettstein *et al.*, 1984), is thought to increase the probability of homologues finding each other.

The assembly of axial elements starts at about the time of tight telomere clustering and predominantly at the nuclear periphery, near the telomeric ends. Initiation of synapsis also begins primarily in the nuclear periphery, before complete axial elements have been assembled (Offenberg *et al.*, 1991). In other organisms (Loidl, 1994; Hasenkampf, 1984, Albini and Jones, 1987), complete axial elements are formed and aligned along the entire length of the homologues before the first SC segments appear. Presynaptic alignment of homologous chromosomes is probably a common step in the pairing process of meiosis though it can not be observed as such in SC preparations of species in which the assembly and alignment of axial elements is rapidly followed by synapsis.

In eukaryotes other than yeast, it has not yet been possible to analyze the meiotic recombinational pathway by means of physical analyses of meiotic DNA. However, by use of immunocytochemical techniques, proteins have been identified which are presumably involved in meiotic recombination. For example, in several eukaryotes it has been shown that proteins with sequence similarity to the bacterial repair/recombination enzyme RecA are

present during early meiotic prophase: in yeast: Dmc-1 and Rad51 (Bishop, 1994; Rockmill *et al.*, 1995); in Lily: Lim15 and Rad51 (Terasawa *et al.*, 1995) and in mouse: Rad51 (Ashley *et al.*, 1995). Bishop (1994) estimated that the timepoint of cytological appearance of antibodies against Rad51 and Dmc1, the yeast homologue of Lim15, coincides with the appearance of biochemically detectable DSBs in yeast DNA. This timing is consistent with the known function of RecA in mediating homology recognition, strand invasion and heteroduplex formation (see Radding, 1991; Kowalczykowski *et al.*, 1994 for reviews). Moreover, as the timing is in concordance with the classical model of gene conversion which is thought to occur at the time of synaptic initiation (Smithies and Powers, 1986; Carpenter, 1987), Bishop (1994) proposed that both Dmc1 and Rad51 are components of early RNs. Using electron microscopic immunogold localization in spreads of zygotene and early pachytene SCs from Lily, Anderson *et al.*(1997) confirmed that Lim15 and/or Rad51 indeed are components of early RNs. Recent studies on meiosis in mouse null mutants of the mismatch repair enzyme Mlh1 indicated that Mlh1 is involved in mammalian meiotic reciprocal crossover, possibly as a component of late RNs (Baker *et al.*, 1996; Edelmann *et al.*, 1996).

Taken together, similar to the situation in yeast, assembly of the SC in higher eukaryotes is preceded by chromosome pairing. The question how and when meiotic recombination is initiated and recombinational intermediates are processed in higher eukaryotes is the hot topic of present-day research on mammalian meiosis.

Part II

Non-homologous synapsis and meiotic recombination in mice with chromosome rearrangements

In mammals it has been argued that the meiotic homology search initially depends on chromatin configuration (Chandley, 1986; Ashley, 1988). The karyogram of mitotic chromosomes displays different types of bands which are a reflection of the differential packaging of chromatin loops along the chromosomal scaffold (Saitoh and Laemmli, 1994). G-bands contain highly condensed chromatin and generally replicate late in S-phase. The R-bands have less condensed chromatin structures and are generally early replicating. Late replicating, highly condensed DNA is transcriptionally relatively inactive. Genes are known to be concentrated predominantly in the R-bands.

Based on cytological observations of the synaptic behaviour of heterozygotes for several different X-autosomal and autosomal-autosomal reciprocal translocations, Ashley (1988) suggested that the chromatin composition in the areas of partner exchange (R-bands versus G-bands) was decisive for the occurrence of either homologous or non-homologous

synapsis around the translocation breakpoints during the zygotene to early pachytene stages. According to this model, meiotic synapsis of R-bands is restricted to homologous sequences. In contrast, G-bands do not recognize homology and synapse non-homologously with either G- or R-bands during zygotene which has been confirmed by several investigations (Ashley and Cacheiro, 1990; Ashley 1990; Winking *et al.*, 1993; Gabriel-Robez and Rumpler 1994).

Ashley's analyses also revealed a correlation between the chromatin configuration and the occurrence of suppression or non-suppression of crossing over in translocation heterozygotes. Translocations with both breakpoints in R-bands show no suppression of crossing over around the breakpoints while translocations with one breakpoints in a G-band do (Ashley, 1988). In a fluorescence antibody labeling study, Plug *et al.*(1996) showed that the distribution of Rad51 foci on the chromatin of fully synapsed bivalents at early pachytene corresponds to an R-band pattern of mitotic chromosomes. Considering that Rad51 is a component of early RNs in Lily (Anderson *et al.*, 1997), this suggests that early RNs are preferentially located in R-bands of mammalian meiotic chromosomes.

For several different paracentric inversions and a tandem duplication, all characterized by having both breakpoints in R-bands, synapsis was absent or severely delayed in regions near the breakpoints during the late zygotene/early pachytene stage of heterozygous carriers (Moses, 1977; Poorman *et al.*, 1981a, 1981b; Moses and Poorman, 1981; Moses *et al.*, 1982, 1984). In the paracentric inversion heterozygotes, the inverted segments of the inversions formed synapsed loop SC configurations during early meiotic prophase stages (Moses *et al.*, 1982). At late pachytene however, bivalents heterozygous for the chromosome aberration could not be identified. From these studies it can be concluded that during the progression of pachytene, the demand for homology is relieved and desynapsis and subsequent synapsis of non-homologous segments, a process referred to as synaptic adjustment, results in the formation of synaptonemal complexes, which are indistinguishable from SCs of fully homologously synapsed bivalents. For several paracentric inversion heterozygotes, the frequencies of anaphase bridges were about half the ones which were expected on the basis of the genetic length of the inversions (Moses *et al.*, 1982). These findings were explained as the result of a lower level of initiation of meiotic recombination. However, synaptic adjustment could also disturb the progression of meiotic recombination, thereby reducing the frequency of anaphase bridges and inducing mutations.

In summary, the discussed data indicate that R-bands are preferrentially involved in both initiation of homologous synapsis and reciprocal recombination. Depending on the chromatin constitution near the breakpoints, some chromosome abnormalities show non-homologous synapsis from the zygotene stage onwards. In other chromosome aberrations, during progression of the pachytene stage, the initial state of homologous synapsis is replaced by non-homologous synapsis via the synaptic adjustment mechanism to produce a presumably

more stable SC structure. The genetic consequences of non-homologous synapsis are, however, unknown.

Meiotic recombination and removal of mutational load.

One consequence of meiotic recombination is the breaking up of parental gene combinations and the production of new ones in the offspring, thereby increasing the genetic variation among the progeny. In many mouse reciprocal translocations however, crossing over is suppressed or absent in the vicinity of translocation breakpoints in heterozygous carriers (Beechey and Evans, 1996). Population genetics predict that non-recombining sequences should degenerate due to the gradual accumulation of deleterious mutations (Muller, 1964; Felsenstein, 1974; Haigh, 1978; Rice, 1987; Lynch and Gabriel, 1990; Strachan and Read, 1996). In small populations, deleterious mutations may reach fixation due to random sampling of gametes (genetic drift) resulting in a decrease in fitness of the population. Thus if reciprocal translocations are maintained via heterozygous carriers, recessive deleterious mutations could hypothetically accumulate in regions near breakpoints due to the suppression of recombination and automatic fixation of the mutations because of selection for the translocation in a small breeding nucleus.

Examples of intergenerational chromosomal instability in humans and mice.

Molecular mutation analyses of several human diseases show that the recombination process also results in the production of deleterious mutations. Several examples of mutations in single copy genes, microsatellites and minisatellites are discussed below.

Unequal homologous recombination has been described as the cause of human disorders like haemophilia A (Lakich *et al.*, 1993; Naylor *et al.*, 1993) and the Hunter syndrome (Bondeson *et al.*, 1995; Lagerstedt *et al.*, 1997), and has also been shown to be involved in X-Y translocations (Rouyer *et al.*, 1987; Yen *et al.*, 1991; Guioli *et al.*, 1992; Weil *et al.*, 1994). The Hunter syndrome, a X-linked recessive disorder is caused by mutation of the iduronate-2-sulfatase (IDS) gene. One class of mutations is most likely caused by an inappropiate intrachromosomal recombination event during male meiosis between the IDS gene and the 20 kb distally located putative pseudogene IDS-2. This results in disruption of the IDS gene and inversion of the intervening DNA (Bondeson *et al.*, 1995). Based on sequence analysis of the inversion junctions, Lagerstedt *et al.* (1997) suggested the involvement of double strand breaks as the initiating event of the mutagenic process. A similar mechanism might account for the inversions found for the X-linked factor VIII gene leading to severe haemophilia A (Lakich *et al.*, 1993; Naylor *et al.*, 1993). As both genes are positioned in the distal part of the X chromosome in a region that is generally unsynapsed during male meiosis, the state of intrachromosomal synapsis likely influences the recombination process.

The increasing severity of an inherited disease during intergenerational transmission, known as genetic anticipation, is the major characteristic of the growing number of trinucleotide repeat expansion disorders. At least ten inherited neurodegenerative/-neuromuscular human diseases are caused by this class of "dynamic mutations" (Warren, 1996). Recently, transgenic mice carrying expanded arrays of the myotonic dystrophy (DM) or the Huntingon's disease (HD) trinucleotide repeats showed intergenerational instability as well (Monckton *et al.*, 1997; Gourdon *et al.*, 1997; Mangiarini *et al.*, 1997). Replication slippage models have been proposed to explain the somatic and germline repeat expansion observed in these illnesses. In DM, gene conversion events have been reported to play a role in contraction of the expanded triplet repeats during transmission (O'Hoy *et al.*, 1993; Hunter *et al.*, 1993). Gene conversion events have also been detected in the fragile X syndrome (Ouweland, *et al.*, 1994). Single sperm analysis of the CAG repeat in the gene for Machado-Joseph disease revealed segregation distortion of expanded alleles and suggestive evidence for inter-allelelic interactions related to intergenerational instability (Takiyama *et al.*, 1997).

Germline instability has been reported for several different human minisatellite loci with mutation rates up to 13% in the male germline (CEB1 minisatellite)(Jeffreys *et al.*, 1991; Jeffreys *et al.*, 1994; Vergnaud *et al.*, 1991). For the CEB1 minisatellite, sister chromatid exchanges as well as conversion-like events have been implicated in the mutation processes (Buard and Vergnaud, 1994). For the MS31A and MS32 loci, inter-allelic complex transfers of repeat units result in astonishing complex rearrangements in a single mutation event most likely occurring during meiosis (Jeffreys *et al.*, 1994). Various base substitutional polymorphisms exist near the upstream end of the repeat array of the MS32 minisatellite. One polymorphic variant appears to block mutation by suppressing initiation in the male germline but not in blood (Monckton *et al.*, 1994; Jeffreys and Neumann, 1997). This suggests that instability is not an intrinsic property of the repeat array, but instead is conferred upon the array by flanking cis-acting recombination/mutation regulators (Jeffreys and Neumann, 1997; Buard and Jeffreys, 1997), possibly by regulating the accessibility of (meiotic) chromatin.

These examples of chromosomal instability in single copy genes, trinucleotide repeat microsatellites and minisatellites illustrate the relation between (meiotic) recombination and the generation of mutations. In this light, it would be interesting to investigate the role of the state of meiotic chromosome synapsis (homologous versus non-homologous synapsis; asynapsis) on meiotic recombination and chromosome (in)stability.

Synapsis and mammalian fertlity in mice with chromosome rearrangements

In many mammalian species the presence of a sub-selection of structural chromosomal aberrations correlates with impairment of gametogenesis. In mice, heterozygosity for reciprocal translocations characterized by one breakpoint at the very proximal and one at the very distal end of the chromosomes is often correlated with synaptic failure and association of the meiotic multi- or univalent with the sex chromosomes (Forejt, 1974; Forejt and Gregorovà, 1977; de Boer and Searle, 1980; Forejt *et al.*, 1981; de Boer *et al.*, 1986). Depending on the translocation, germ cell death as early as pachytene until the later stages of spermiogenesis and a (partial) block at metaphase I is observed. This can result in reduced numbers of sperm or a complete spermatogenic arrest (Cattanach, 1975; de Boer and Searle, 1980; de Boer and Speed, 1982; de Boer *et al.*, 1986; de Boer and de Jong, 1989). Oocytes with the same chromosomal abnormalities display a similar synaptic behaviour which is correlated with a decrease of oocyte numbers and a reduction of the reproductive lifespan (Lyon and Hawker, 1973; Burgoyne and Baker, 1985; Burgoyne *et al.*, 1985; Setterfield *et al.*, 1988a, 1988b).

Two hypotheses regarding the mechanism for chromosomally-derived impairment of male gametogenesis have dominated the literature for years. Miklos (1974) as well as Burgoyne and Baker (1984) labelled unsaturation of "pairing sites" of synapsing chromosomes during pachytene of male and female meiotic prophase as the primary cause for germ cell death. On the other hand, Lifschytz and Lindsey (1972) and Forejt *et al.*, (1981) proposed that the interaction between the unsynapsed segments of autosomes and the X-chromosome causes the lethal pertubation of spermatocytes because of either the distortion of X inactivation or inappropiate autosomal gene expression (reviewed by Forejt, 1996). Comparison of the synaptic behaviour of many different reciprocal and Robertsonian translocations in mouse and human in relation to male fertility failed to resolve the dichotomy between the two hypotheses (de Boer and de Jong, 1989; Gabriel-Robez and Rumpler, 1996; Forejt, 1996).

Scope of this thesis.

This thesis addresses to the following question: what are the consequences of the occurrence of non-homologous synapsis and/or suppression of meiotic recombination over many successive generations for male fertility and viability of the progeny ?

To assess the consequences of non-homologous synapsis and suppression of meiotic recombination over many successive generations for male fertility, we used mice which are heterozygous for two near identical reciprocal translocations, T(1;13)70H and T(1;13)1Wa, as

a model system. The T70H and T1Wa breakpoints are positioned about 10 cM apart from each other on chromosome 1 and are cytologically indistinguishable at the distal end of chromosome 13. During meiotic prophase and diakinesis, the four different translocation chromosomes of the T70H/T1Wa karyotype produce two differently sized heteromorphic bivalents in both sexes (Wauben-Penris *et al.*, 1983; de Boer *et al.*, 1986; chapter 3). The chromosome 1 and 13 regions positioned between the T70H and T1Wa breakpoints (Δ1 and Δ13 segments) are excluded from crossing over as meiotic quadrivalents have never been encountered in T70H/T1Wa heterozygotes (Wauben-Penris *et al.*, 1983; de Boer *et al.*, 1986; Peters et al., 1997b, chapter 3). Introduction of the Robertsonian translocation Rb(11.13)4Bnr (Rb4) into the double heterozygous system at the late seventies enabled us to incorporate this genetic modification as a factor in our studies.

We compared fertility of (Rb4)T70H/(Rb4)T1Wa males in which the (Rb4)T1Wa translocation chromosomes had either no, an one-generation or a multi-generation history of non-homologous synapsis in heteromorphic bivalents during previous meioses (partially dependent on the presence of Rb4Bnr; chapter 3, 7). To estimate the degree of male fertility we used testis weights, epididymal sperm counts and the capacity to produce offspring as parameters. Male fertility estimates were compared with the synaptic behaviour of the two heteromorphic bivalents during meiotic prophase. To be able to analyze nuclei from the early meiotic prophase stages, we developed a drying down technique which results in high yields of nuclei of all meiotic stages in both males and females from only small amounts of tissue (chapter 2). Preparations are suitable for SC analysis by normal light and electron microscopy (chapters 2, 3 and 7) as well as for fluorescence immunocytochemistry using a variety of antibodies and *in situ* hybridization (chapters 2, 8; Plug, 1997).

To assess the consequences of the occurrence of non-homologous synapsis and/or suppression of meiotic recombination over many successive generations for viability of the progeny, we compared the (Rb4)T70H/(Rb4)T1Wa system (chapter 6) with systems characterized by heterozygosity for either the (Rb4)T70H or (Rb4)T1Wa translocations only (chapters 5 and 6 respectively). Death profiles were established for the first 5 days of life and between day 5 and weaning (at day 20). Body weights were recorded at days 28-42.

Mapping of several microsatellites in the Δ1 segment between the chromosome 1 breakpoints of T70H and T1Wa (chapter 4) extended our study in several ways.
a) We used these microsatellites to estimate the recombination frequency, distal of the T70H breakpoint in T70H and Rb4T70H heterozygous females (chapter 5). To correlate the level of meiotic recombination with chromosome behaviour at meiotic prophase I, we analyzed

translocation multivalent SC configurations of T70H/+ males (and females) and of Rb4T70H/Rb4+ males (chapter 5).

b) The crossover reduction of the Δ1 segment in the Rb4T70H/Rb4T1Wa karyotype and in Rb4T70H/Rb4+ females enabled us to use the various microsatellite haplotypes for genotyping of offspring from backcrosses between (Rb4)T70H/(Rb4)T1Wa double heterozygotes and (Rb4)T70H homozygotes, that were used for viability and fertility measurements.

c) Finally, the vulnerability of microsatellite sequences for mutation in a situation of a disturbed homology search and subsequent non-homologous synapsis was analyzed by the use of the (Rb4)T70H/(Rb4)T1Wa system.

Chapter 2

A drying down technique for spreading of mammalian meiocytes from the male and female germline.

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Introduction.

Over the last 25 years several different techniques have been used for the preparation of meiotic cells displaying the synaptonemal complexes and adherent chromatin. The Counce and Meyer technique (1973) of spreading cells upon a hypotonic solution and transferring them to a slide or grid has proved its value in many studies on mammalian meiotic chromosome pairing.

The shortcomings of most of these surface spreading techniques are the low yield of nuclei and especially the underrepresentation of nuclei from the early meiotic prophase stages. To obtain a better representation of meiotic prophase we used the drying down technique of Speed (1982) as a framework and optimized both the release of the meiotic cells from the adherent tissue as well as the spreading of the cells themselves. The technique results in high yields of nuclei of all meiotic stages in both males and females from only small amounts of tissue. The preservation and accessibility of the proteinaceous nuclear components are excellent as has been shown by several immunolocalization studies (Ashley *et al.*, 1995; Baker *et al.*, 199; Plug *et al.*, 1996). The spreads are also suitable for combined immuno-cytochemistry and fluorescence *in situ* hybridization (FISH) as result of a high accessibility of the chromatin.

Methodology.

Spreading of spermatocytes and oocytes on glass slides.

Mouse spermatocytes can be obtained from adult and juvenile males. Mice were killed by cervical dislocation. Testes were dissected, weighted and quickly placed in phosphate buffered saline (PBS) pH7.4 at room temperature. The tunica albuginea was removed and adhering extratubular tissue was removed by rinsing the seminiferous tubules with PBS. Washed tubules were kept in fresh PBS at room temperature until further use. Some tubules were placed in a hypotonic extraction buffer containing 30 mM tris, 50 mM sucrose, 17 mM trisodium citrate dihydrate, 5 mM EDTA, 0.5 mM DTT and 0.5 mM PMSF pH8.2 for 30 to 60 min. Subsequently a suspension was made in 40 µl 100 mM sucrose pH8.2 (pH set by using NaOH) on a clean (boiled in milliQ water) glass slide. First the tubules, approximately 1 inch length, were torn to pieces between the tips of two fine watchmaker forceps in 20 µl sucrose solution. Thereafter the volume was increased to 40 µl and a slightly cloudy suspension was made by use of a 10 µl finnpipet. The tubular remnants were removed and the remaining suspension was divided between two new clean glass slides which had been dipped just before in a freshly made and filtered (0.2 µm) 1% paraformaldehyde (PFA) pH9.2 solution (pH set by

using 10 mM sodium borate pH9.2 buffer solution) containing 0.15 % Triton X-100. The cell suspension was placed at the upper right corner of the slide and was slowly dispersed first in a horizontal direction, thereafter in a vertical direction whilst exposing the cells to the fixative. For each new pair of slides the suspension in sucrose was freshly made. Nuclei were dried for at least two hrs in a closed box with high humidity. Finally the slides were washed twice for 2 min in 0.4% photoflo (Kodak) and dried at room temperature. For SC analysis by electron microscopy, slides were stored at room temperature and stained within a few days after preparation. For immunocytology and/or FISH, slides were stored at -20°C or -80°C until further use.

Mouse prophase oocytes were obtained from female fetuses, killed by decapitation. The spreading of oocytes occurred according to the spermatocyte protocol with some minor modifications. Incubation in the hypobuffer took place for 20 to 40 min. For each slide a cell suspension from one ovary was made in 10 µl sucrose solution and was dispersed only at the center of the wetted slide.

Preparations for electron microscopy.

For SC configuration analysis using electron microscopy, cells can be spread either directly onto slides coated with Falcon plastic or onto glass slides. The plastic coated slides should be glow discharged for 1 min at 30 mA and 10^{-1} mbar at least 1 hr before spreading.

Both types of freshly made preparations (on plastic or glass) were stained with 50% $AgNO_3$ under nylon mesh for 1 hr at 60°C and then for 1 hr at 50°C (Kodama *et al.*, 1980). Slides were washed 4 times for 5 min each in double demineralised water and air dried. Following staining of the spreads on plastic-coated slides, selected nuclei were covered by coppergrids. The plastic coat was detached from the glass using 0.3% hydrofluoric acid, floated off onto water and transferred to the grids. The silver stained glass slides were coated with a Falcon plastic film using a 1% Falcon solution made in chloroform. Selected nuclei were covered by coppergrids and transferred to the plastic using 2.5 % hydrofluoric acid (Messier *et al.*, 1986).

Immunocytology with subsequent FISH.

The immunocytochemical detection procedure was a modification (Ashley *et al.*, 1995) of that described by Moens *et al.* (1987). The SCs were stained with a rabbit polyclonal anti-SC antiserum (serum 175; 1:200 dilution) which reacts specifically with the 30,000- and 33,000-M_r SC components encoded by the SCP3 gene (Lammers *et al.*, 1994). These components are present in the axial and lateral elements of the SC as shown by immunogold labeling (Moens *et al.*, 1987). The polyclonal antiserum was detected with goat-anti-rabbit-IgG conjugated with fluoresceinisothiocyanate (FITC, Sigma). For *in situ* hybridization we used a 40 kb

genomic probe of the gene St2, also named T1 (St2/T1), which has been located on chromosome 1 (Klemenz *et al.*, 1989; Tominaga *et al.*, 1991). Following the immunostaining of the SCs, the probe (labeled with biotin-16-dUTP by nick translation) was hybridized and detected with one layer of rhodamine-labeled avidin according to the protocol of Lichter *et al.* (1990) with modifications. Briefly, after RNAse treatment and dehydration, the slides were denatured for 3 min in 70% formamide and 2x SSC pH7.0 at 70°C followed by 3 washes in ice cold 2x SSC pH7.0 and dehydration on ice. The probe was denatured for 10 min at 80°C in a mixture containing 50% formamide, 2x SSC pH7.0, 10% dextran sulfate, 1% Tween, 50* C_0t1 DNA and herringsperm DNA and prehybridized for 30 min at 37°C. Hybridization occurred at 37°C for 48 hrs. After detection slides were stained with 4,6-diamidino-2-phenylindole (DAPI) and mounted in antifade (2% 1,4-diazobicyclo-(2,2,2)-octane (DABCO), 20 mM Tris pH8, 90% glycerol). Examination and digital imaging was performed as described by Ashley *et al.* (1995).

Results and discussion.

The three basic elements of the drying down technique described here, are the incubation of the gonadal tissue in a hypotonic extraction buffer, suspension of the tissue in 0.1 M sucrose and subsequently spreading of the cells on a thin layer of PFA containing Triton X-100. As a result of these modifications numerous nuclei from all meiotic prophase stages are spread on the slide, clearly showing the axial elements and SCs (fig. 1). Also nuclei in pre-meiotic S-phase and at metaphase I & II can be identified (Plug *et al.*, 1996).

The hypotonic extraction treatment especially increases the yield of spread nuclei in female material. The cells are fixed and simultaneously spread during the drying down step. Triton X-100 retains the nuclei on the glass surface and enhances the spreading of the nuclear chromatin and the SCs within a cell. High cell concentration should be avoided as this inhibits the effective spreading of the nuclear contents. The sharpness of the SCs and the contrast between the SCs and adherent chromatin can be varied by changing the humidity and time of drying. Rapid drying caused by a variable and/or too low humidity results in contraction of the chromatin onto the SCs. Protracted drying leads to a low contrast between the SCs and the chromatin making these preparations less useful for analysis by electron microscopy.

Figures 1 and 2 show a nucleus of a mouse double heterozygous for the T(1;13)70H and T(1;13)1Wa reciprocal translocations which have their breakpoints on the same two chromosomes. The four different translocation chromosomes in these mice form two heteromorphic bivalents during meiosis with one copy of the chromosome 1 region between the two breakpoints in each bivalent (de Boer *et al.*, 1986). During zygotene this chromosome 1 region can be seen as a loop in the large bivalent (fig. 1b) and as a length difference between

the unpaired small translocation chromosomes (fig. 1a). The gene St2/T1 is located in this region. Preliminary results show that the hybridization signal of the 40 kb St2/T1 probe is seen as two dots adjacent to each other on both heteromorphic bivalents in only 40% of the spermatocytes (n=15). The other nuclei harbour predominately only three signals of which the single signal is often larger in size. Using a different 40 kb cosmid probe, Heng *et al.* (1994) also found approximately the same percentage for the individual detection of sisterchromatids in meiotic cell preparations.

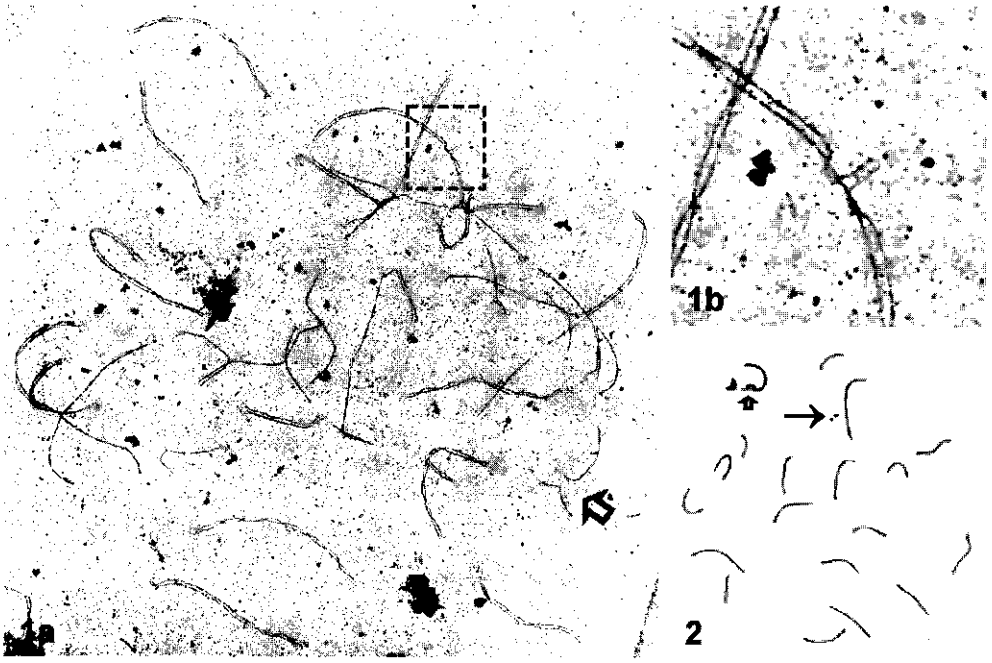

Figure 1: a Silver stained zygotene nucleus of a T(1;13)70H/T(1;13)1Wa female, showing asynchrony in meiotic pairing. The chromosome 1 region of the 13^{1}H chromosome forms a loop in the large 13^{1} heteromorphic bivalent (rectangle). An enlargement of this loop is given in **b**. Both small 1^{13} translocation chromosomes are univalents (open arrow).

Figure 2: Digital image of a pachytene nucleus of a T(1;13)70H/T(1;13)1Wa male with the SCs stained by the polyclonal anti-SC antiserum in grey and the 40 kb genomic St2/T1 probe in black. The St2/T1 signal on the 1^{13}Wa chromosome in the small heteromorphic bivalent is located over the XY bivalent (open arrow). The small heteromorphic bivalent is positioned on the left side of the XY bivalent. The large fully paired heteromorphic bivalent shows two separated signals of the 13^{1}H sisterchromatids (larger arrow).

Acknowledgements.

We thank Dr. Christa Heyting for making available the polyclonal anti-SC antiserum and Dr. Roman Klemenz for the genomic probe of St2/T1. The technical assistance of Danny Voorhamme is highly appreciated. Terry Ashley is gratefully acknowledged for the use of the microscope and computer facilities.

Chapter 3

Meiosis in carriers of heteromorphic bivalents: sex differences and implications for male fertility

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Summary.

Mice that are double heterozygous for the semi-identical T(1;13)70H and T(1;13)1Wa reciprocal translocations display a great variation in male fertility. The synaptic behaviour of the different translocation chromosomes of adult males was therefore studied in relation to this parameter. Juvenile males and embryonic females (16 and 18 days old) were included for comparison. In agreement with the minor differences in the translocation breakpoint positions, two differently sized heteromorphic bivalents are formed in meiotic prophase of both sexes (a quadrivalent was never encountered). Synaptonemal complex (SC) configurations of both bivalents in either sex are characterized by a high degree of non-homologous synapsis at zygotene - early pachytene. The rate of synaptic adjustment during pachytene is dependent on the size of the heteromorphic bivalent and varies between the sexes. Differences in SC configuration and morphology of the small heteromorphic bivalent in particular exist between the sexes and between animals. In males this correlates with different degrees of fertility. Normal SC morphology in a fully synapsed small heteromorphic bivalent is an important determinant of succesfull meiosis and spermatogenesis. Moreover, aberrant synapsis favours the "unsaturated pairing site" model of Miklos (1974) as the primary cause for male sterility.

Introduction.

The relationship between chromosome synapsis and fertility has attracted scientific interest for a long time (Gillies, 1989). In the mouse, heterozygosity for reciprocal translocations with one breakpoint at the very proximal and one breakpoint at the very distal end of chromosomes is often associated with synaptic failure and association of the meiotic multivalent or univalent with the sex chromosome bivalent (Forejt 1974, Forejt and Gregorovà 1977, de Boer and Searle 1980, Forejt *et al.*, 1981, de Boer *et al.*, 1986). This results in germ cell death as early as pachytene or during the later stages of spermiogenesis resulting in male sterility. Oocytes with the same chromosomal aberrations suffer from similar synaptic problems resulting in a decrease of oocyte numbers (Burgoyne and Baker 1985, Burgoyne *et al.*, 1985, Setterfield *et al.*, 1988a and 1988b).

Two hypotheses have been given as causes for the male sterility associated with the chromosome abnormalities in *Drosophila* and mice. Lifschytz and Lindsley (1972) postulated that association of autosomal segments with the sex chromosomes caused a disturbance of X-inactivation resulting in male sterility. This hypothesis is still a matter of debate (reviewed by Forejt 1996). Alternatively, Miklos (1974) suggested that the presence of "unsaturated

synaptic sites" in sex chromosomes was the cause of male sterility. In line with the second hypothesis, Ashley *et al.*, (1994) concluded from a cytological study using fluorescence in situ hybridisation techniques that primary spermatocytes, suffering from asynapsis and a lack recombination between the sex chromosomes during meiotic prophase, undergo a developmental arrest at first meiotic metaphase.

In 1986, de Boer *et al.* reported on the extensive variation in male fertility existing in a mouse stock, double heterozygous for two semi-identical reciprocal translocations (T(1;13)70H/ T(1;13)1Wa). Male fertility of this hybrid has subsequently changed over different breeding generations of the parental stocks. The breakpoints of the T70H and T1Wa translocations are positioned close to one another on chromosome 1 and are cytologically indistinguishable at the distal end of chromosome 13 (fig. 1a). Therefore this karyotype is characterised by having two large and two small semi-identical translocation chromosomes always producing two differently sized heteromorphic bivalents during meiotic prophase of both sexes (fig. 1b; Wauben-Penris *et al.*, 1983). The region between the breakpoints on chromosome 1 is approximately 10 cM and can be recognized during late-zygotene as an unsynapsed "insertion-like" region in both bivalents.

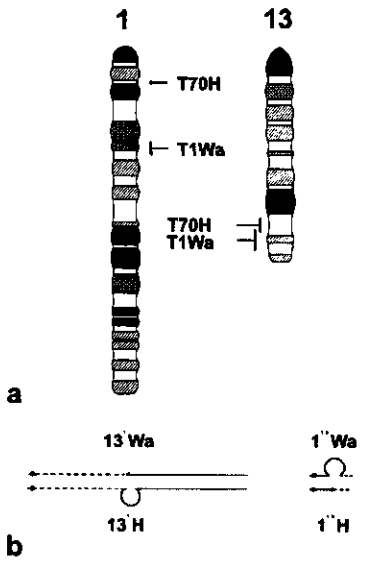


Figure 1: **a** Position of the T(1;13)70H and T(1;13)1Wa translocation breakpoints along the G-banded mitotic chromosomes 1 and 13. **b** Meiotic synapsis diagram of the double heterozygous T(1;13)70H/T(1;13)1Wa mouse karyotype showing complete homologous synapsis in two heteromorphic bivalents. The ectopic homologous chromosome 1 segments are depicted by a loop. Chromosome 13 segments are ripped and centromere positions are given by a dot.

Males are usually sterile in each breeding generation but some breeding-pairs produce a variable portion of fertile sons. We have utilised this variance to define cytological characteristics of synapsis of the heteromorphic bivalents at the SC level during zygotene and pachytene, which correlate well with sperm count and testis weight.

The second part of the work focused on the synaptic behaviour of the two differently sized heteromorphic bivalents during the pachytene stage of double heterozygous males (adult and juvenile). In addition embryonic females (at day 16 and 18 of gestation) were studied to analyse the development of synapsis and to define differences between the sexes. Finally, we compared the meiotic cell distribution during the successive meiotic prophase substages at days 16 and 18 of gestation in ovaries of wild type (+/+) and double heterozygous (F1) females to determine the effect of the presence of heteromorphic bivalents on the progression of oocytes through meiotic prophase.

Materials and methods.

Animals.

Both parental translocation stocks are maintained by crossing homozygous translocation (T/T) males with heterozygous (T/+) females. These T/+ females are produced by crossing T/T males (m) with Swiss random bred +/+ females (f). In this way, genetic variation is maintained at the level of the Swiss random bred stock except for the regions around the breakpoints where crossing-over is reduced or absent (unpublished observations).

Males used for measuring fertility were from 3 kinds of crosses: T70H/T70H (f) x T1Wa/+ (m) performed in 1982, T70H/T70H (f) x T1Wa/T1Wa (m) and the reciprocal cross , both performed in 1991, and T70H/T70H (f) x T1Wa/T1Wa (m) performed in 1996. SCs were studied from meiocytes of the last cross.

Fertility estimates.

The capacity of males to produce offspring was tested by mating them to two NMRI (Han) virgin females as described by de Boer *et al.* (1986). Both epididymal sperm counts and testis weights were also determined (de Boer *et al.*, 1986). Sperm morphology was checked in males which were used for SC analysis (de Boer *et al.*, 1986).

Synaptonemal complex analyses.

Spermatocytes were spread and processed using the drying down technique of Peters *et al.* (1997a, chapter 2). Briefly, nuclei were firstly spread on glass slides and subsequently silver stained. After coating the glass slides with falcon plastic, cells on plastic were transferred to

copper grids using hydrofluoric acid (Messier *et al.*, 1986). Over 250 nuclei from five adult males (between 3 and 6 months old) with different testis weights and sperm counts and 80 nuclei from 3 juvenile males (19 days old) were analysed. Classification of the nuclei into the substages of meiotic prophase (zygotene, pachytene and diplotene) was carried out according to previous studies (Moses 1980, Dietrich and de Boer 1983, Guitart *et al.*, 1985) . Pachytene was substaged based on the degree of synapsis and the morphology of the sex chromosomes, and on the morphology of the attachment plaques (APs) of the autosomal chromosomes (Tres 1977, Moses 1980, Dietrich and de Boer 1983, Moses *et al.*, 1984). Pachytene spermatocytes with unsynapsed straight sex chromosome axial elements (sometimes showing the typical little hairpin at their ends) and nuclei with XY-synapsis upto 10 % of the length of the Y-chromosome in the absence of APs at the autosomal telomeric ends, were classified as *early*. Spermatocytes with XY-synapsis varying from 10% to the maximum, both ascending and decending (the difference decided by the morphology of the APs) were classified as *middle* and pachytenes with less than 10 % synapsis of the XY plus pronounced APs were named *late*.

Female meiocytes from Swiss +/+ and F1 hybrid mice were obtained from fetuses at day 16 and day 18 of gestation (vaginal plug formation at day 0 of development). +/+ females were from a cross between homozygous parents. A total of 24 female foetuses were analysed, distributed as follows (with number of litters in brackets): +/+ day 16: 5 (2) ; day 18: 6 (2) ; F1 day 16: 7 (3) ; day 18: 6 (2). Nuclei were directly spread on falcon plastic-coated slides and processed using the same sedimentation technique as for spermatocytes (Peters *et al.*, 1997a, chapter 2). This technique was slightly modified to visualize the meiotic chromatin (fig. 10) by omitting the paraformaldehyde from the "spreading and drying down solution". The criteria of Speed (1982) and Dietrich and Mulder (1983) were used for substaging the female meiotic prophase. Most nuclei were examined at a Jeol 1200 EKII and some were viewed on a Philips EM 208 at 80 kV.

Results.

Fertility of double heterozygous T(1;13)70H/ T(1;13)1Wa males is not constant during 15 years of breeding.

Table 1 gives a summary of the male fertility indices of the T70H/T1Wa offspring from the crosses T70H/T70H x T1Wa/+ in 1982 and T70H/T70H x T1Wa/T1Wa in 1991 and 1996. Table 2 presents the family dependency of male fertility as assessed by inspecting uterine contents at day 13 of pregnancy and/or sperm counts. Fertility is not constant in this stock. Compared to the 1982 and 1996 crosses, only a few sons from the 1991 matings were capable

of producing offspring (tables 1 and 2). Moreover, these males had both a lower mean testis weight and mean sperm count (table 1).

Table 1: Fertility indices of T70H/T1Wa double heterozygous males from 1982, 1991, 1996.

Karyotype	*No. Males*	*No. Fertile Males (%)* [a]	*No. Implants* [b] *mean ± sd*	*% Large moles* [b]	*% Small moles* [b]	*Testis Weight (mg) mean ± sd*	*Sperm Count* [c] *mean ± sd*
+/+ [d]	40	40 (100)	13.6 ± 2.4	0.3	4.3	-	417 ± 71
1982	25	9 (36)	5.7 ± 6.1	0.9	31.5	-	57.4 ± 84.8
1991	43	5 (12)	9.9 ± 3.3	0	20.8	45.7 ± 22.9	43.9 ± 115.8
1996	32	15 (47)	10.8 ± 5.0	0.6	30.2	67.8 ± 30.2	72.5 ± 97.0

sd = standard deviation
a: A male is declared fertile when at least one conceptus, expressed as a decidual reaction, is produced after two matings.
b: Results from uterine inspection of +/+ females mated to fertile +/+ and double heterozygous males at day 13 of pregnancy (vaginal plug formation at day 0 of development).
c: Actual haemocytometer counts summed over the left and right caput epididymis.
d: From de Boer *et al.* (1986).

Another difference shown in table 1 is the increase in the percentage of fertilized oocytes in F1 hybrids from later generations versus the 1982 series. Despite this increase, the percentage of small moles, caused by the early embryonic death of the segregating deletion carriers of the chromosome 1 region between the T70H and T1Wa breakpoints, was relatively constant over the different generations. Finally, fertility was family restricted in all breeding generations. Moreover, some families in 1996 produced only fertile sons, a situation not encountered in previous breeding generations (table 2).

Table 2: Number of fertile males [a] per number of tested males in different families.

	Families						
1982:	0/2	0/3	0/3	1/3	1/5	5/7	
1991:	0/39	0/31	0/21	1/12	4/20	3/11	
1996:	0/4	0/4	0/4	1/4	2/4	6/6	6/6

[a] A male is declared fertile when at least one conceptus, expressed as a decidual reaction, is produced after two matings

Synaptonemal complex configurations in relation to male fertility.

The small heteromorphic (1^{13}) bivalent displays several synaptic configurations during pachytene (fig. 2). Successful non-homologous synapsis of the excess of chromatin from the 1^{13}Wa chromosome into the small bivalent results in a "symmetrical" horse-shoe type configuration with the outer lateral element (LE) longer than the inner one (fig. 2a). Complete synapsis sometimes formed an "asymmetrical" horse-shoe configuration with the longer LE, representing the 1^{13}Wa genome, being thickened (more intensely silver staining over its entire length; fig. 5b) or containing pseudo-axes (fig. 2b). In cases in which synapsis is restricted to both or one homologous chromosome end(s), loop (fig. 2c) or fork (fig. 2d) configurations were found. Univalents were rarely seen (fig. 2e). Sometimes, non homologous synapsis of the small translocation chromosomes with one or both sex chromosomes occurred (fig. 2f). The large heteromorphic bivalent shows synaptic problems in only a minority of pachytene spermatocytes. As these synaptic irregularities do not correlate with fertility, this bivalent was not considered as a possible cause of sterility.

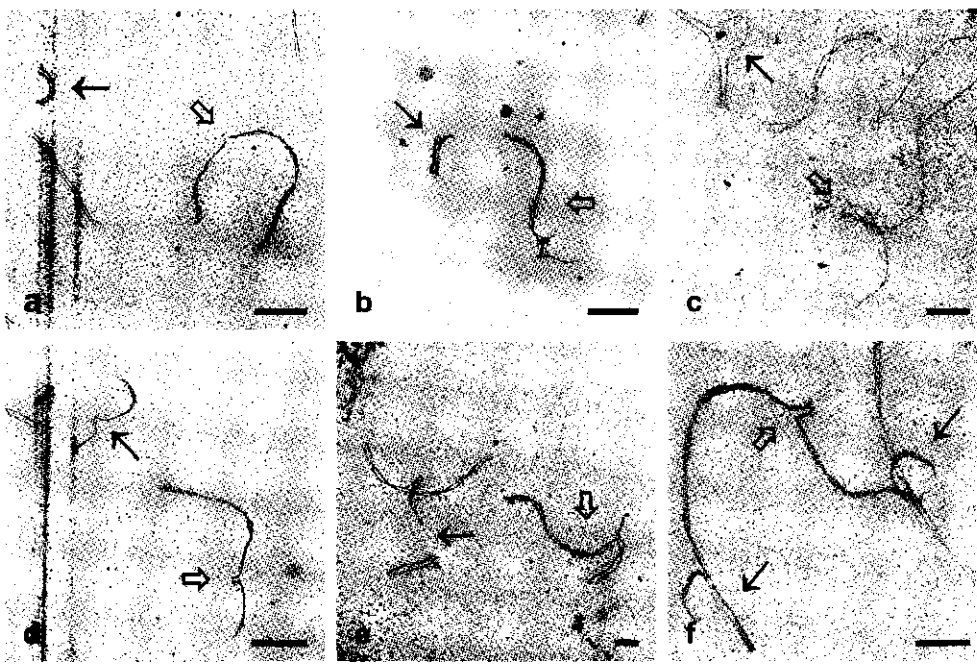
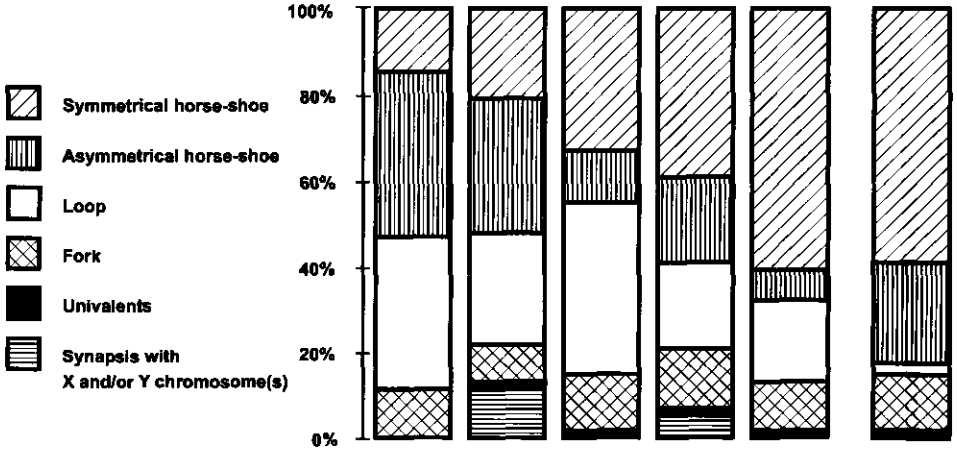


Figure 2: Early (**a**, **d**), middle (**b**, **c**, **e**) and late (**f**) pachytene nuclei, showing silver stained SCs of the different synaptic configurations of the small heteromorphic bivalent (arrow), and of the sex bivalent (double arrow). Complete synapsis in a symmetrical (**a**) or asymmetrical (**b**) horse-shoe configuration. The latter shows many pseudo-axes. Restriction of synapsis to two or one homologous chromosome end(s) in loop (**c**) or fork (**d**) configurations. **e** Hairpin (bottom) and pseudo-axis (upper) formation by the univalents of the 1^{13}Wa and 1^{13}H chromosomes respectively. **f** Non-homologous synapsis of both 1^{13} chromosomes with the sex chromosomes. Scale bar = 1 μm.

A: Fertility indices of the adult and juvenile males.

Mouse	A	B	C	D	E	Juvenile
Age (month)	6	4.5	4.5	3.5	6	19 days
n Fertile littermates/total tested littermates	0/4	1/4	6/6	6/6	6/6	1/4, 2/4, 2/4
Mean testis weight (mg)	42	43.7	77.2	102.7	128.9	22.2
Epididymal sperm count	0	0	69	74	244	nd
% Abnormal sperm (n cells)	nd	nd	82 (104)	77 (111)	33 (120)	nd
n Pachytene nuclei	47	47	52	56	53	68

B: Percentages of the different synaptic configurations of the small heteromorphic bivalent.



C: Percentages of the different synaptic configurations of the small heteromorphic bivalent positioned close to (+) or separated from (-) the sex bivalent.

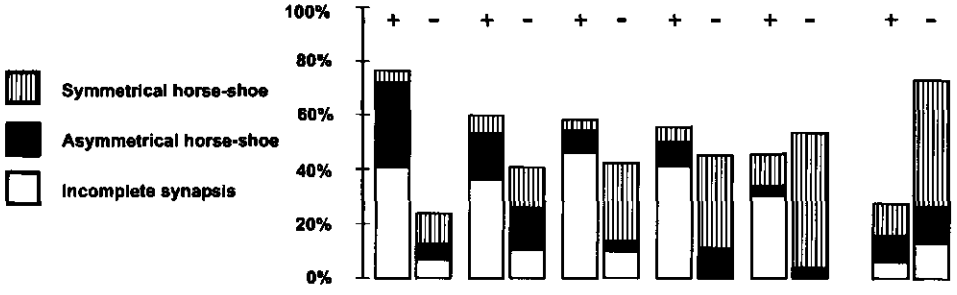


Figure 3: Fertility indices and synaptic behaviour of the small heteromorphic bivalent in T70H/T1Wa adult and juvenile males.

Figure 3 was prepared to correlate the synaptic behaviour of the 1^{13} bivalents with the fertility status of a male as defined by testis weight and sperm count. Males A and B had low testis weights and no sperm. Males C and D had higher testis weights and some sperm, predominantly with minor head abnormalities. Mouse E had the highest testis weights and sperm counts, although lower than +/+ mice (see table 1). A significant positive correlation was found between the fully synapsed symmetrical horse-shoe configuration (fig. 2a) and the degree of fertility (fig. 3b; Spearman rank correlation coefficient (R_{sp}) = 0.975; $P < 0.05$). Asymmetry within the fully synapsed bivalents was predominantly caused by pseudo-axes in the sterile males and by thickening of the outer LE in the more fertile adult and juvenile males. The level of complete synapsis was higher in all juvenile versus adult males (fig. 3b; last column) although some variation in the presence or absence of a thickened LE existed (data not shown).

In a similar manner to other reciprocal and insertional translocation heterozygotes, unsynapsed ends of the 1^{13} bivalent were almost always positioned close to the sex chromosomes (fig. 3c; Forejt *et al.*, 1981, de Boer *et al.*, 1986, Setterfield *et al.*, 1988a, de Boer and de Jong 1989) and approximately half of them possessed thickened axial elements and/or pseudo-axes (data not shown). For the fully synapsed 1^{13} bivalent, clearly more asymmetric than symmetric configurations were positioned near the XY bivalent. This results in a higher rate of association of the 1^{13} bivalent (all configurations) with the sex chromosomes in sterile males (R_{sp} = 0.975; $P < 0.05$). However, males B, C and D did not differ greatly in this respect. In the juvenile males, fewer small heteromorphic bivalents were associated with the sex chromosomes.

Synapsis of heteromorphic bivalents over subsequent meiotic stages in males.

For the small 1^{13} bivalent, the degree of non-homologous synapsis only slightly increased during the progression of pachytene in all juvenile and adult males except in male A, in which a small decrease was observed (data not shown). The long 13^{1} marker bivalent contained a loop or asymmetrical twists of one of the LEs in 15.5% of the early and middle pachytene stages of the adults (n=97; figs. 4b, 4c). During late pachytene, only asymmetrical twists were found in 5.6% of the nuclei (n=158) which suggests the occurrence of synaptic adjustment as described in mice heterozygous for a tandem duplication (Moses and Poorman, 1981). The remaining 85% of the large heteromorphic bivalents must have synapsed non-homologously during zygotene. In juvenile males, fewer bivalents with delayed synapsis were observed (2.9%; n=69). The axial/lateral elements of the ectopical homologous chromosome 1 regions on both heteromorphic bivalents (fig. 1) were positioned near one another in 3 out 255 (1.2%) pachytene spermatocytes of adult males suggesting homologous chromatin recognition, without SC formation (fig. 5c).

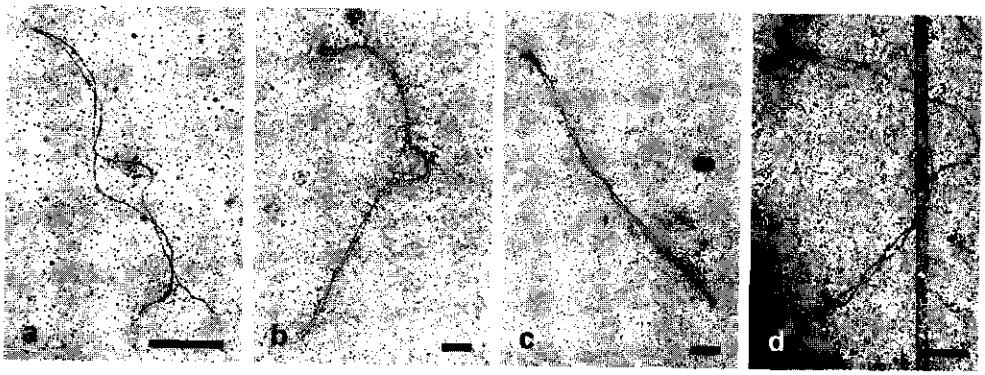


Figure 4: The large heteromorphic bivalent shows delayed synapsis of the chromosomal regions between the T70H and T1Wa breakpoints for the 13^{1} chromosomes in zygotene (**a**) and early pachytene (**b**) spermotocyte nuclei. **c** Asymmetrical twist formation in the process of synaptic adjustment. **d** Reappearence of the length difference between the axial elements of the 13^{1} bivalent in a diplotene spermatocyte. Scale bar = 1 μm.

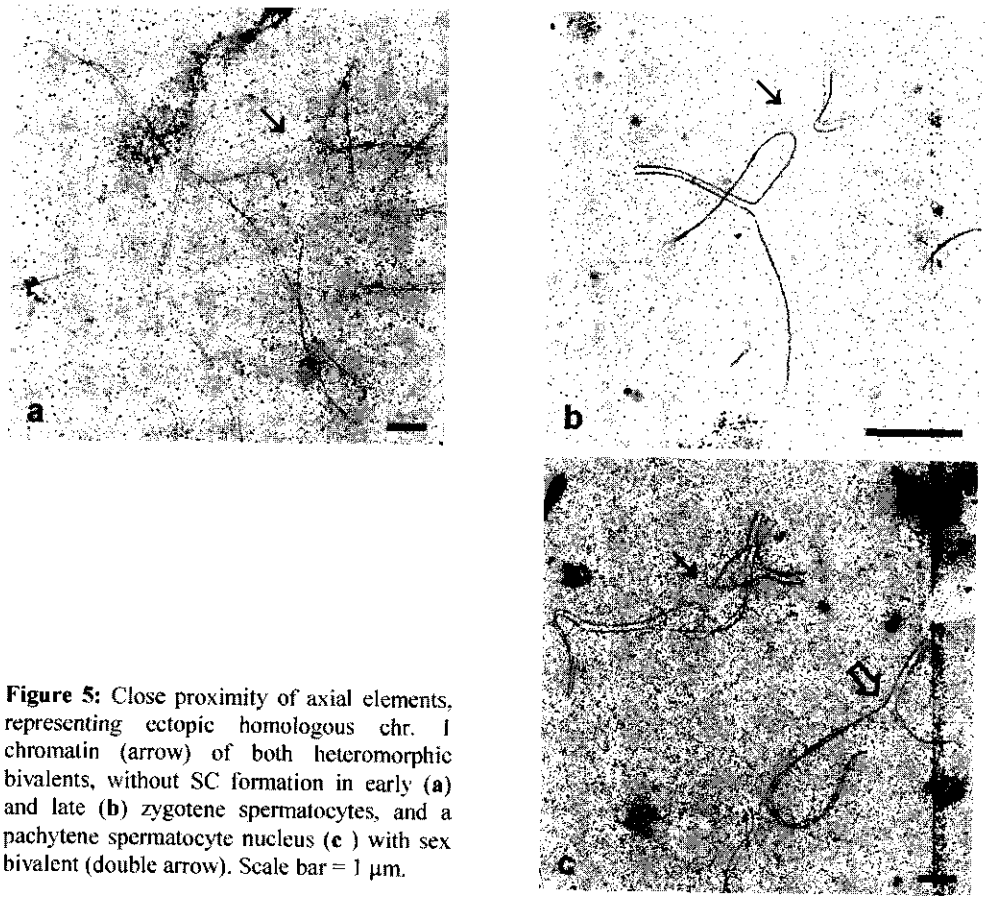


Figure 5: Close proximity of axial elements, representing ectopic homologous chr. 1 chromatin (arrow) of both heteromorphic bivalents, without SC formation in early (**a**) and late (**b**) zygotene spermatocytes, and a pachytene spermatocyte nucleus (**c**) with sex bivalent (double arrow). Scale bar = 1 μm.

At the same time as the pachytene nuclei were analysed, small numbers of zygotene nuclei (from juvenile males) and diplotene nuclei (from adult males) were analysed. At zygotene, the small heteromorphic bivalent could be found either as aligned axial elements, or with a completed SC at one of its telomeric ends. This bivalent is not one of the earliest ones to synapse, but the suggestion of a synaptic delay can not be justified from our observations. In contrast, only the "insertion-like" region of the large bivalent suffered from a synaptic delay during zygotene (88%; n=9; fig. 4a). Contact between ectopic homologous chromatin was seen in 3 out of 13 (23%) zygotene cells of juvenile males (figs. 5a, 5b) which suggests a more frequent occurrence of this type of homologous interaction during early meiotic prophase stages. Figure 5b shows that this chromatin contact does not necessarily severely influence the SC formation within the small heteromorphic bivalent.

In diplotene nuclei, 3 desynapsing large bivalents showed an axial loop (n=7; fig. 4d). Apparently, in the course of and/or after desynapsis of the previously non-homologously synapsed SC, a reorganization of the axial elements occurs during diplotene. The small bivalent was easily recognized according to the same principle of length difference.

Chromosome synapsis and meiotic progression of oocytes in fetal ovaries of +/+ and T70H/T1Wa females.

In females, thickening of the axial or lateral element of the 1^{13} bivalent was never that conspicious as in males. On the other hand, only in females can the T70H-T1Wa chromosome 1 segment form a fully synapsed hairpin configuration in both heteromorphic bivalents (fig. 6a). Moreover, only in oocytes can the LE of the 1^{13}Wa chromosome form an asymmetrical twist leading to a fully synapsed configuration. Assuming that the majority of pachytene cells of females at day 16 of gestation are in the early and at day 18 in the late pachytene stage, the mean frequency of early non-homologous synapsis for the 1^{13} bivalent in all day 16 oocytes is approximately 50% although some variation exists between these day 16 females (two left columns in fig. 7). Subsequently synaptic adjustment significantly increased the percentage of full synapsis for the 1^{13} bivalent of four 18 days old females (third column in fig. 7). At the same time, desynapsis probably resulted in a high level of 1^{13} univalents in two 18 days old females (fourth column in fig. 7) which coincided with a higher rate of association with the NOR carrying chromosome 19 (fig. 6b) as compared to the other day 18 females (20.6% versus 5.1%).

For the large heteromorphic bivalent, two females showed a low degree of delayed synapsis at day 16 (fig. 8), similar to the situation in the males (± 15% at early and middle pachytene). However, synapsis in all other females was clearly delayed at both successive days of gestation (40% and 20% respectively; fig. 8). The presence of more large bivalents with a loop in both groups of day 16 females compared to day 18 females points at the

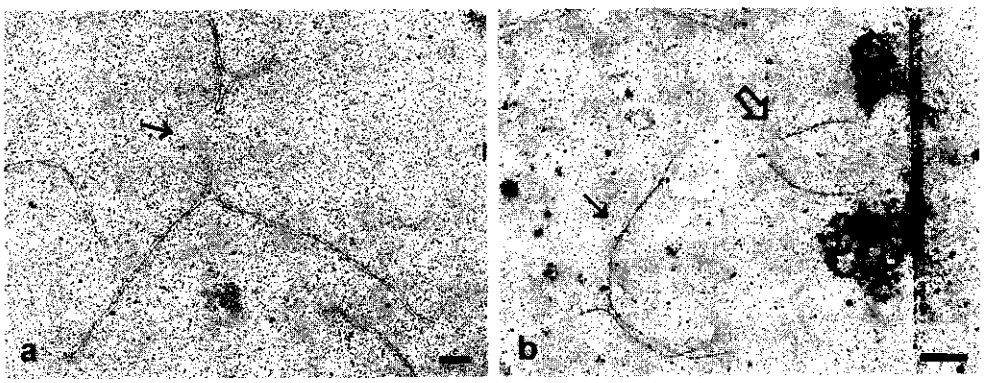
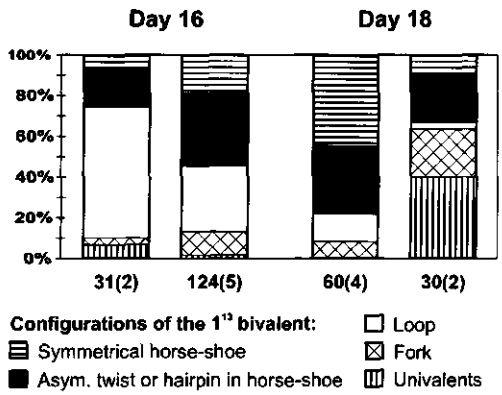


Figure 6: a Pachytene oocyte showing close proximity of ectopic homologous lateral element segments without ectopic SC formation (arrow) and non-homologous synapsis of these segments into a hairpin like SC within the heteromorphic bivalents. **b** Premature desynapsis in bivalent 19, recognized by its length and NOR material (double arrow), and the closely positioned partially unsynapsed 1^{13} bivalent (arrow) in a pachytene oocyte. Scale bar = 1 μm.



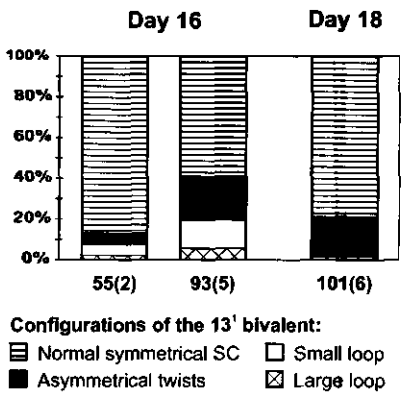


Figure 7 (left): Percentages of different synaptic configurations of the small heteromorphic bivalent in pachytene nuclei of T70H/T1Wa females at days 16 and 18 of gestation. Each column represents summed data of females with similar distributions of SC configurations for the 1^{13} bivalent. The total number of analysed nuclei is given below each column with the number of animals in brackets. The difference in the summed percentages of both horse-shoe versus other configurations between different columns is at day 16: $\chi^2_1 = 7.24$; $P < 0.005$, at day 18: $\chi^2_1 = 15.56$; $P < 0.0005$, and between both columns at day 16 (all females) and left column at day 18 (4 females) $\chi^2_1 = 8.55$; $P < 0.005$.

Figure 8 (right): Percentages of different synaptic configurations of the large heteromorphic bivalent in pachytene nuclei of T70H/T1Wa females at days 16 and 18 of gestation. Each column represents summed data of females with similar distributions of SC configurations for the 13^1 bivalent. The total number of analysed nuclei is given below each column with the number of animals between brackets. The difference in the percentages of normal versus other synaptic configurations between the two columns at day 16 is: $\chi^2_1 = 392$; $P < 0.0005$.

occurrence of synaptic adjustment (fig. 8). Similarily to males, homologous contact between both bivalents, characterized by close proximity facing of the axial elements of the ectopic chromosome 1 regions without forming a SC, was found in 3 out 156 (1.9%) pachytene cells at day 16 of gestation (fig. 6a).

For general synaptic failures like asynaptic regions, univalents, non-homologous synapsis, interlocks and broken SCs, we found only a small increase at the pachytene stage (F1 versus +/+: at day 16: 15% versus 8%; at day 18: 10% versus 3%). More striking was the increased desynapsis of chromosome 19 in the double heterozygotes (18% (range 0-39%; day 16) and 24% (range 7-75%; day18) versus 8% at both days for +/+ (range 0-20%; both days)).

To assess a possible delay in the progression of meiotic prophase in F1 versus +/+ females as a result of the aberrant synaptic behaviour of the translocation and other bivalents, the distribution of oocytes over the subsuquent stages at day 16 and 18 of gestation was analysed (fig. 9). In contrast to our expectation, significantly more nuclei had entered the pachytene stage in F1 females at day 16 compared to +/+ females ($\chi^2_3 = 8.69$; $P < 0.05$).

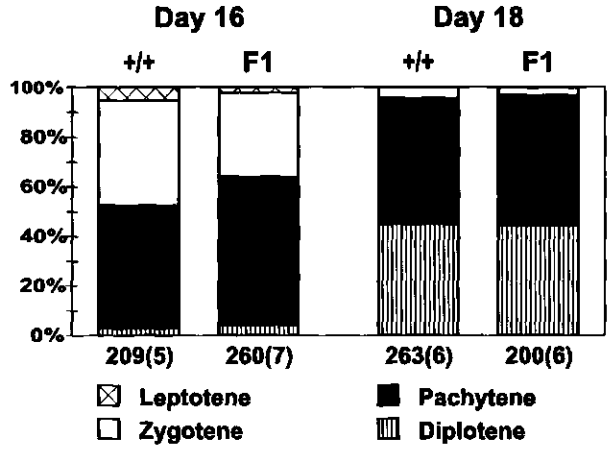


Figure 9: Distribution of foetal oocytes over successive meiotic prophase stages in +/+ and T70H/T1Wa mice at days 16 and 18 of gestation. The total number of analysed nuclei is given below each column with the number of animals between brackets. The difference in distribution at day 16 is: $\chi^2_3 = 8.69$; $P < 0.05$.

Discussion.

Meiotic synapsis and male fertility

Since the first report on the variation in male fertility in T(1;13)70H/T(1;13)1Wa mice (de Boer *et al.,,* 1986), this parameter has been shown to be variable over subsequent generations. Fertility is restricted to maximally half of the males within a population and in a family dependent manner. This is most likely due to the random bred nature of the Swiss stock.

In 1986, de Boer and co-workers also reported on the variation in male fertility of the analogous T(2;8)26H/T(2;8)2Wa stock, which is also characterized by two large and two small translocation chromosomes and likewise one large and one small heteromorphic bivalent during meiosis. From the SC analysis, especially of one fertile and one sterile T26H/T2Wa male, fertility seemed to be correlated with a high frequency of fully synapsed configurations for the small 8^2T26H/8^2T2Wa heteromorphic bivalent. Moreover, these SCs had a normal symmetrical architecture of the lateral elements and were not associated with the sex chromosomes (de Boer *et al.,* 1986). The relationship between aberrant synapsis of a bivalent and its association with the sex chromosomes is also illustrated by SC analyses of sand rat pachytene spermatocytes. In this case, succesfull completion of synapsis of an inversion bivalent by synaptic adjustment, results in the dissociation of the now fully synapsed bivalent from the sex chromosomes (Ashley *et al.,* 1981).

Since Lifschytz and Lindsey (1972) and Miklos (1974) postulated their models for male sterility, many different reciprocal and Robertsonian translocations in mouse and human have been unsuccesfully used to validate one or the other hypothesis (Forejt *et al.*, 1981, de Boer *et al.,* 1986, Gabriel-Robez and Rumpler 1996). In the T70H/T1Wa system, male fertility is positively correlated with the percentage of nuclei with a fully synapsed 1^{13} bivalent having a normal symmetrical SC architecture (figs. 2a, 3b). This suggests that any departure from the symmetrical tripartite SC structure (unsynapsed segments (figs. 2c-2f) or asymmetry of opposite LE segments (fig. 2b) has a deleterious effect on male meiosis and spermatogenesis. According to Miklos' hypothesis, these aberrently synapsed bivalents might harbour "unsaturated" synaptic sites. In contrast, male fertility is also negatively correlated with the degree of association of the 1^{13} bivalent with the XY bivalent (fig. 3c) which supports the hypothesis of Lifeschytz and Lindsey. However, the degree of this "1^{13} - XY association" did not vary greatly between males with different testis weight (fig. 3c; males B, C and D). Therefore, this observation points to aberrant synapsis as the primary cause of male sterility and favours the "unsaturated pairing site" model of Miklos. Association of aberrantly synapsed bivalents with the sex bivalent may additionally impair male fertility.

De Boer and Speed (1982) suggested that defective spermatocytes with aberrant synapsis could be rescued by healthy spermatocytes. This could occur through a functional

metabolic cooperation between interconnected spermatocytes and spermatids (Dym fig. Fawcett 1971, Braun *et al.,* 1989) and might explain why relative minor differences in the degree of synapsis and the association with the sex chromosomes leads to divergent testis weights and even more divergent sperm counts (compare males C, D fig. E in fig. 3).

Remarkably, the degree of synapsis of both heteromorphic bivalents was higher and the level of association of the 1^{13} bivalent with the XY chromosomes lower in pachytene spermatocytes of juvenile males. This finding is difficult to explain. The shorter cycle of the spermatogenic epithelium in juvenile males (Kluin *et al.,* 1982) might influence synaptic behaviour, just as differences in gene expression during the initiation of spermatogenesis at puberty and its maintenance during adulthood (Zhao *et al.,* 1996).

Recently Rad51, a RecA homologue, has been proposed to be a component of early recombination nodules in a variety of organisms (Bishop 1994, Ashley *et al.,* 1995, Terasawa *et al.,* 1995, Plug *et al.,* 1996). Antibody localization of the single-strand DNA binding protein RPA suggests that RPA is a component of early nodules as well (Plug *et al.,* submitted). Moreover, the cell cycle checkpoint proteins Atm, which is mutated in the inherited human disease ataxia-telangiectasia, and Atr, the human homologue of the yeast rad3 gene, have been shown to have a meiotic function (Barlow *et al.,* 1996, Keegan *et al.,* 1996, Xu *et al.,* 1996). Therefore, the abnormal localization of Rad51, RPA and Atm on partially unsynapsed and fully synapsed small heteromorphic bivalents during meiotic prophase may yield new insights on the discussion of male sterility (Plug *et al.,* submitted). This may also link potential chromosome damage in these bivalents with the p53 apoptosis pathway (Hawley fig. Friend, 1996).

Thickening of one lateral element in a SC.

In some spermatocytes of male mice, carrying the partially overlapping inversions In(1)1Rk and In(1)12Rk, homologous synapsis was confined to the central region of the double heterozygous inversion bivalent (Borodin *et al* 1992). Synapsis between the non-homologous proximal and distal chromosomal ends, which are unequal in length, resulted in thickening of a different lateral element at each end of this bivalent (Borodin *et al* 1992). In contrast, thickening of axes in regions of non-homologous synapsis around breakpoints in translocation quadrivalents has to our knowledge never been reported. Similarly, thickening does not occur in the large adjusted heteromorphic bivalents of Dp(7)1Rl, T70H/T1Wa, T26H/T2Wa and D1Lub1Hsr (Moses fig. Poorman 1981, de Boer *et al.,* 1986, Winking *et al.,* 1993, this study). Therefore, in heteromorphic bivalents formed from synapsing partners with major chromosomal length differences, thickening of the larger lateral element could reflect the relatively high torsional stress, imposed on the bivalent in the process of chromatin reorganization during synapsis. To illustrate this point in oocytes, where in normal bivalents

the meiotic lampbrush chromosome organization has been retained, the chromatin configuration is altered for the unsynapsed 1^{13} chromosomes (fig. 10). Finally, thickening of one lateral element has been reported in what were described as normal autosomal bivalents in XO females (Speed, 1986). This phenomenon developed over meiotic age of both normal ánd degenerating pachytene XO oocytes. Therefore, as thickening of the 1^{13}Wa lateral element was found in spermatocytes from early pachytene onwards, the differential staining of the lateral elements of autosomal bivalents in XO oocytes might be related to forms of stress other than originating from torsion.

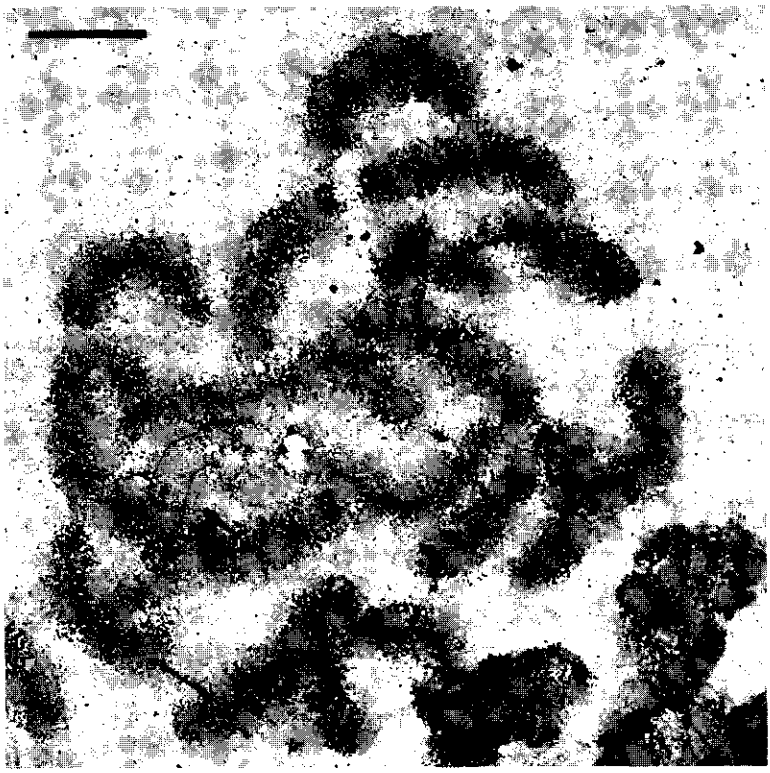


Figure 10: Pachytene oocyte revealing a lampbrush like chromatin organization in the normally synapsed bivalents. In comparison, the meiotic chromatin of both unsynapsed 1^{13} bivalents (arrow), each having a longer LE than anticipated upon mitotic length, is differently organized. Scale bar = 1 μm.

Non-homologous synapsis in heteromorphic bivalents of T70H/T1Wa males and females.

Restriction of synapsis to homologous regions during zygotene and the subsequent release of the demand for homology during pachytene, allowing for non-homologous synapsis, has been termed synaptic adjustment (Poorman *et al.*, 1981, Moses *et al.*, 1981). Ashley (1988)

suggested that chromatin composition (R-bands versus G-bands) was important for non-homologous synapsis during zygotene - early pachytene and subsequent crossing-over. This has been confirmed by several investigations (Ashley fig. Cacheiro 1990, Ashley 1990, Winking et al 1993, Gabriel-Robez fig. Rumpler 1994).

This work shows that the small 1^{13} and especially the large 13^{1} heteromorphic bivalents undergo extensive non-homologous synapsis during zygotene - early pachytene in male as well as in female double heterozygous mice. This is in agreement with the hypothesis of Ashley (1988) as the breakpoints on chromosome 1 of both translocations are positioned near the G-dark bands 1A5 and 1C1.1 (see fig. 1). Synaptic adjustment subsequently takes place in the large heteromorphic bivalent of both sexes as is clear from the morphological changes which accompany meiotic progression. The reduction in number of 13^{1} bivalents with three chiasmata at diakinesis-metaphase I in T70H/T1Wa females (versus T70H/T70H; Wauben-Penris *et al.*, 1983) is probably related to the delayed synapsis of the "insertion-like" region. The development of synapsis in the 1^{13} bivalent during pachytene differs between females since both synaptic adjustment and desynapsis were observed. In males, the degree and the symmetry of synapsis in the majority of the small heteromorphic bivalents appear to be defined at the zygotene stage as only a minor progression in synapsis was observed over the successive pachytene substages. Figures 5b and 6a reveal that contact between ectopically positioned homologous chromatin and (early) non-homologous synapsis of the corresponding axial elements within the heteromorphic bivalents can occur simultaneously in both sexes.

In contrast to several other chromosomal abnormalities (Mittwoch fig. Mahadevaiah, 1992), T70H/T1Wa females only showed a minor increase in the frequency of synaptic problems in bivalents not involved in the chromosomal aberration. Genetic background could play a role, as the random bred Swiss stock has shown a low level of general synaptic problems in a previous study (Speed and Chandley, 1983). Nevertheless, a small increase in anaphase I non-disjunction for bivalents other than the heteromorphic bivalents has been described (Wauben-Penris *et al.*, 1983). Desynapsis of chromosome 19 was higher in most double heterozygous mice and increased during pachytene, as has been reported previously (Speed 1982, Speed fig. Chandley 1983). Finally, delayed synapsis of the heteromorphic bivalents appears to have no deleterious effect on the overall meiotic progression (fig. 9).

Synapsis in and between heteromorphic bivalents of different "insertion" type mutants

The Is(7;1)40H insertion is ± 38 cM of chromosome 7 and in previous studies (Searle *et al.*, 1983, Mahadevaiah *et al.*, 1984) many quadrivalents were found in both sexes, although at different rates. In Cattanach's insertion Is(7;X)1Ct, the translocated segment located near the pseudo-autosomal region is of approximately equal size and chromatin composition (G and R-bands) as the chromosome 1 segment between the T70H and T1Wa breakpoints. In

spermatocytes, quadrivalents were formed in almost 40% of the nuclei and extensive ectopic asynaptic contact was frequently observed (Ashley 1984). In contrast, in both male and female T70H/T1Wa meiocytes, asynaptic ectopical homologous chromatin contact was only found in 23% and 1-2% of the nuclei at the zygotene and pachytene stages respectively and quadrivalents were not observed. This suggests that an autosomal telomere close to the "insertion" region directs the synaptic process towards non-homologous synapsis. Moreover, the relatively small size of the "insertion-like" region in the large 13^{1} bivalent results in a very high frequency of complete non-homologous synapsis in these bivalents at early pachytene. Formation of quadrivalents is presumably therefore not favoured in the T70H/T1Wa mice. Examination of female meiosis in Cattanach's insertion mice would be elucidative.

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Chapter 4

Chromosomal assignment of the microsatellites D1Mit4, -52, -20, -121, -122 and the collagen 9a1 locus (Col9a1) distal from the T(1;13)70H breakpoint and of the loci for the crystallin protein (Cryg), the cAMP responsive element binding protein (Creb1) and the myosin light chain protein (Mylf) distal from the T(1;13)1Wa breakpoint proximally on mouse chromosome 1.

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Abstract

We gave localized the gene encoding the alpha 1 chain of type IX procollagen protein (Col9a1) protein and the microsatellites D1Mit4, D1Mit52, D1Mit20, D1Mit121 and D1Mit122 in the region between the T(1;13)70H and the more distally positioned T(1;13)1Wa breakpoints on mouse chromosome 1 by dose-mapping. We determined the chromosomal position of these markers by quantitative Southern blot and PCR analysis of DNA from mice with 2 or 3 copies of this region. Fluorescence in situ hybridisation experiments confined the T1Wa breakpoint (in 1C1.2 or 1C2) and accordingly the whole 1C1 subband to the very small genetic region between D1Mit18 and Cryg on mouse chromosome 1.

The reciprocal translocations utilized in this study, T(1;13)70H (Searle *et al.*, 1971) and T(1;13)1Wa (de Boer *et al.*, 1977) have their breakpoints in close vicinity on the chromosomes (chr.) 1 and 13 (figs. 1a, 1c). Backcrosses of mice double heterozygous for these translocations with T(1;13)70H homozygotes produce one class of unbalanced offspring, trisomic for the chr. 1 and possibly monosomic or trisomic for the small chr. 13 region between the two different translocation breakpoints (bottom of fig 2a). The phenotype of these duplication mice, bred on the Swiss random bred genetic background, is affected. To determine the genetic position of the breakpoints and to identify molecular markers for segregation analysis of the double heterozygous T70H/T1Wa (F1) parents, we localized several genes and microsatellites relative to the T70H and T1Wa breakpoints on chr.1 by fluorescence in situ hybridization (FISH) and by dose-mapping using quantitative southern blot and quantitative polymerase chain reaction (PCR) approaches.

On the basis of the SSLP map of the Whitehead Institute/MIT CAST intercross (Dietrich *et al.*, 1994) and the mouse chromosome atlas map (Lyon *et al.*, 1996) we selected 9 microsatellites, presumably from the chr. 1 T70H-T1Wa interval, for mapping. Genomic DNA was isolated from ear biopsies using the Puregene DNA isolation kit (Genta Systems, Research Triangle Park, NC) following the manufacturer's protocol. Microsatellites were amplified as described by Crooijmans and colleagues (1994). Fifty nanogram genomic DNA was amplified in a 20 µl solution which contained 0.2 Units Tth DNA polymerase (HT Biotechnology) and 0.16 µM oligonucleotide primers (purchased from Research Genetics (Huntsville, AL)). One primer was [^{32}P]-endlabeled. Reactions were performed in a Perkin-Elmer/Cetus 9600 thermal cycler for 23 cycles (each cycle: 45 s at 94°C; 2 min at 53°C; 2 min at 72°C) with an initial denaturation of 5 min at 94°C and a final extension for 10 min. at 72°C. D1Mit69, D1Mit70, D1Mit72 and D1Mit73 were not polymorphic in the T70H and T1Wa stocks and were therefore not further analyzed. On the other hand, D1Mit121 and D1Mit122 (fig. 1c) could be mapped between the two translocation breakpoints by the presence of three different alleles within the karyotype. This is in agreement with the physical

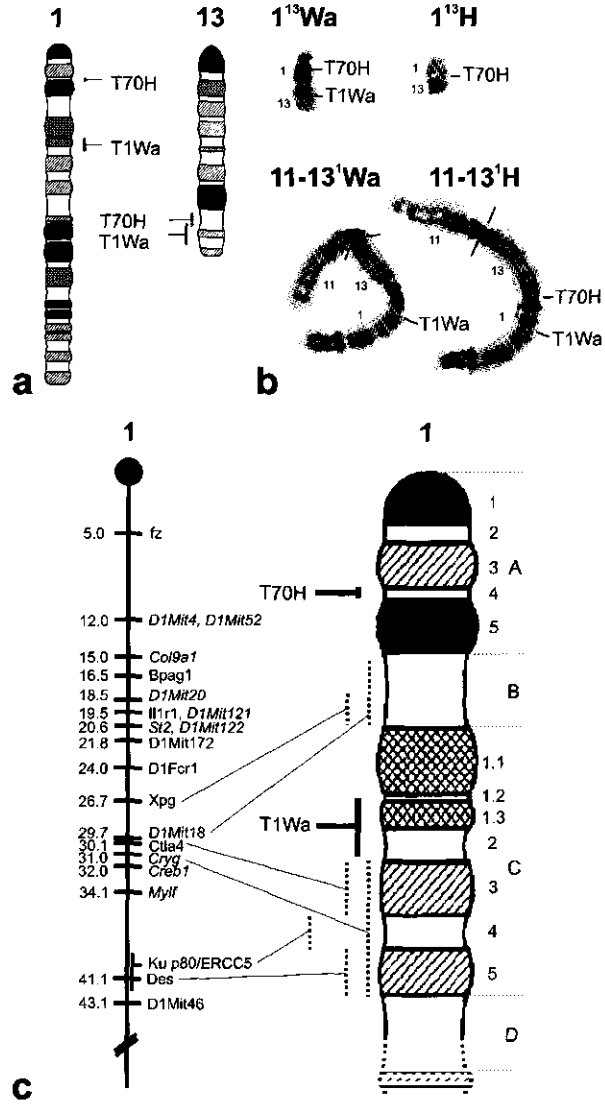

Figure 1: **a** Position of the translocation breakpoints of T(1;13)70H in 1A4 and 13D1 (2) and of T(1;13)1Wa in 1C1.2 and 13D2.2 or in 1C2 and 13D1. **b** G-banded T70H and T1Wa translocation chromosomes. In this particular example, chromosomes 13^1 are fused to chr. 11 via the Rb(11.13)4Bnr Robertsonian translocation. (**c**) Genetic and physical map of chr. 1 based on the fifth mouse chr. 1 committee report (18) and references in the tekst. The T1Wa breakpoint region (1C1.2 - 1C2) is depicted as a bar.

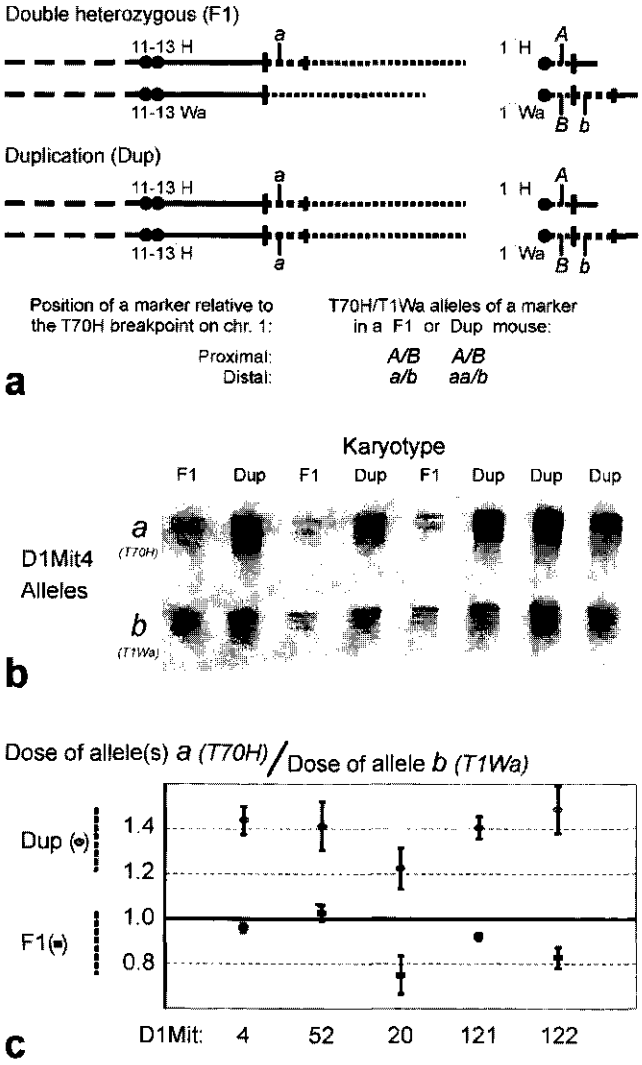

Figure 2: a Concept of dose-mapping: duplication mice reveal the position of a microsatellite relatively to the T(1;13)70H and T(1;13)1Wa breakpoints (see tekst for explanation). Chromosomes 13^{1} are fused to chr. 11 via the Rb(11.13)4Bnr Robertsonian translocation, for reasons not relevant to this mapping study (see chapter 5). **b** D1Mit4 pattern showing a more dark allele a of the T70H genome in duplication (dup) versus double heterozygous (F1) animals. **c** Ratio (mean and SE) of allele(s) a versus allele b of different microsatellite markers in dup (n = ± 6) versus F1 (n = 4) mice. The larger allele a belongs to the T70H genome and shorter allele b to the T1Wa genome for all five microsatellites.

localization of the St2 gene (Tominaga, 1989) which contains D1Mit122 in its 7th intron, between the two translocation breakpoints (Peters *et al.*, 1997a; chapter 2). In F1 mice, D1Mit4, D1Mit52 and D1Mit20 (fig. 1c) displayed two alleles each segregating with one of the translocation genomes. However, the tight cosegregation of an allele with a translocation genome is not informative for the position of the marker relative to the breakpoint. Therefore, we determined the ratio between the T70H and T1Wa alleles for these microsatellites in duplication versus F1 animals by quantitative PCR analyses (fig. 2).

The concept of this approach is that if a microsatellite of chr. 1 is mapping proximal to the T70H breakpoint, equal amounts of product of the T70H allele (depicted as an "A" in fig. 2a) and the T1Wa allele ("B" in fig. 2a) should be produced in both F1 and duplication genotypes. In case of a position distal to T70H on chr.1, the ratio of the T70H to T1Wa alleles should be one in F1 animals ("a/b" in fig. 2a) versus two in duplication mice ("aa/b" in fig. 2a). Experimentally, the incorporated radioactivity of each allele, including the shadow bands, was quantified by phosphor-imager analysis (ImageQuant, version 3.3, Molecular Dynamics Ltd., Kensing, England). The ratio of the alleles "a" to "b" was significantly higher in duplication versus F1 mice for all three markers (D1Mit4, D1Mit52 and D1Mit20) and resembled the ones found for the positive controls D1Mit121 and D1Mit122 (fig. 2c; for each marker: $P < 0.003$; $n_{F1} = 4$; $n_{dup} = 7$). This implies that all three markers are localized distal to the T70H and proximal to the T1Wa breakpoint.

For D1Mit20 and D1Mit122, the differences between the expected and observed ratios of the T70H to T1Wa alleles in the F1 and animals (fig. 2c) are at least partially caused by overlapping of shadow bands of the upper T70H allele "a" with the top band of the lower T1Wa allele "b". The differences between the expected and observed ratios (2 versus 1.4; fig. 2c) for the other three microsatellites might be related to a higher amplification rate of the shorter allele b .

We also mapped the gene Col9a1, often used as a reference locus in interspecific backcross mapping studies, relative to the T70H breakpoint on chr. 1. Quantitative southern blot analysis revealed a 1.49 times more intense signal in duplication versus F1 mice ($P < 0.01$; $n_{F1} = 9$; $n_{dup} = 6$) for the Col9a1 probe compared with the control probe of myosin light chain (Mylf) which is located distal to the T1Wa breakpoint (see below). In these experiments, 10 µg of fully HindIII digested and separated genomic DNA of different F1 and littermates was transferred to Hybond^{TM}-N+ nylon membranes (Amersham, England) according to manufacturer's protocol. Blots were firstly hybridized (Sambrook *et al.*, 1989) with a [^{32}P]-random prime labeled (Rediprime Random Prime labellingskit, Amersham, England) 444 bp cDNA fragment of Col9a1 (Metsaranta *et al.*, 1991) and subsequently with a 8 kb genomic fragment encoding the first exon and most of the first intron of the MLC1f gene (= Mylf locus; pers. com. Benoît Robert). The amount of bound radioactivity was determined

by phosphorimager analysis. In control experiments using a 1582 bp HincII cDNA fragment of the St2 gene (Tominaga., 1989) and the genomic fragment of Mylf as probes, we obtained similar results (data not shown). The Col9a1 locus is therefore also distal to the T70H breakpoint.

The breakpoint of T70H in the R band 1A4 (de Boer and van Gijsen, 1974) was originally mapped between the loci fz and ln, at about one-third of the distance from fz (Searle *et al.*, 1971). This corresponds to the genetic position of D1Mit172 in fig. 1c (Seldin, 1996) or of Il1r1 on the mouse chromosome atlas map (Lyon *et al.*, 1996). The present study places T70H proximal to D1Mit4 and D1Mit52 (fig. 1c) indicating that the genetical distance between fz and T70H was overestimated in the original backcross analysis (Searle *et al.*, 1971).

Using G-band karyotype analysis the T1Wa breakpoint was localized in the 1C1.2 or 1C2 subband (figs. 1a, 1b). The genes for the crystallin proteins (Cryg; Moormann *et al.*, 1985), the cAMP responsive element binding protein (Creb1; Cole *et al.*, 1992) and Mylf were located on chr. 1 distal to T1Wa by FISH (Suijkerbuijk *et al.*, 1992) on metaphases of peripheral blood lymphocytes of homozygous T1Wa mice. Single and double signals of all three genes were observed predominantly on the large T1Wa translocation chromosomes in 70-80% of the analysed metaphases ($n \geq 20$; data not shown). We observed no difference in labelling of the small T1Wa translocation chromosomes in comparison with other non-translocation chromosomes. These results are in agreement with the chromosomal localizations of Ctla4 in 1C3 (Brunet *et al.*, 1987), Cryg in 1C3-1C5 (Zneimer *et al.*, 1988), Xrcc5 in 1C4 (Koike *et al.*, 1996) and of Des in 1C5 (Malo *et al.*, 1993). Xrcc5 was recently mapped just proximal of D1Mit46 (Koike *et al.*, 1996). Together with the chromosomal localizations of Xpg, mapped between D1Mit20 and D1Mit18, in the distal segment of 1B (Harada *et al.*, 1995) and of D1Mit18 in 1B (Mongelard *et al.*, 1996), these localization studies confine the G-dark 1C1 subband to a very small genetic region (fig. 1c).

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Chapter 5

Inaccurate meiotic chromosome synapsis around the points of partner exchange in the reciprocal translocation T(1;13)70H quadrivalents reduces the viability of homozygous translocation carriers.

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Abstract

Exclusion of sequences from recombination is thought to entail accumulation of deleterious mutations (Strachan and Read, 1996). In many mouse reciprocal translocations, crossing-over is suppressed or absent in the vicinity of translocation breakpoints (Beechey and Evans, 1996). In this paper we investigate whether absence of crossing-over in chromosome segments near the T(1;13)70H translocation breakpoints in T70H heterozygotes reduces the viability of T70H homozygotes after multi-generational transmission of the translocation chromosomes via heterozygous karyotypes. Analysis of offspring that was produced during ~25 successive generations of backcrosses between T70H homozygous (T/T) males and T70H heterozygous (T/+) females revealed a significant deficit of T/T karyotypes compared with T/+ karyotypes. We observed a similar but lower degree of transmission distortion in an analogous backcross stock which additionally is homozygous for the Robertsonian translocation Rb4(11.13)4Bnr (abbreviation: Rb4). Comparison of litterizes of contemporary crosses between T70 homozygotes and between T70HT70H males and +/+ females revealed that the deficit of T/T offspring is not restricted to T/T x T/+ crosses only, indicating that an increased prenatal lethality is the major cause of the shortage of T/T segregants.

Using microsatellites, we show that in T/+ females of both backcross stocks the introgression of alleles from the "+" genome into the translocated chromosome 1 segment near the T70H breakpoint is suppressed in a distance dependent manner. This effect is more prominent in the presence of Rb4. In translocation multivalent configurations in T70H/+ and Rb4T70H/Rb4+ males we frequently observed non-homologous chromosome synapsis of segments near the T70H breakpoints. Homologous and non-homologous synapsis of these segments was clearly more delayed during the zygotene and pachytene stages in the presence of Rb4.

To summarize, the levels of meiotic recombination and non-homologous chromosomes synapsis in segments near the T70H breakpoints are postively correlated with the increased prenatal lethality of the T/T segregants in comparison with T/+ segregants. In addition, we did not observe an increase in the transmission distortion over successive backcross generations in both stocks. This suggests that accumulation of recessive lethal mutations over many successive generations due to suppression of recombination around the T70H breakpoint only is not the cause of the low survival of T/T progeny. We propose that meiotic recombination and/or non-homologous synapsis play an active role in the acquisition of recessive lethal mutations of genetic or epigenetic nature in segments near the T70H breakpoints during meiosis of T/+ females. Accumulation of these mutations over short periods of successive backcross generations would explain the shortage of the T70H homozygotes in the progeny of both T/T x T/+ backcross stocks.

Introduction

Several population genetic models predict that non-recombining sequences should degenerate by the accumulation of deleterious mutations (Muller, 1964; Felsenstein, 1974; Haigh, 1978; Rice, 1987; Lynch and Gabriel, 1990). A high mutation rate and a decreasing number of succesfully reproducing individuals accelerate this degenerative process with each generation. Furthermore these studies suggest that recombination can substantially slow down or even stop the process of genetic decay by breaking up complicating deleterious linkage arrangements (Felsenstein, 1974; Haigh, 1978; Rice, 1987; Lynch and Gabriel, 1990). In many mouse reciprocal translocation heterozygotes, crossing-over is suppressed or absent in the vicinity of translocation breakpoints (Beechey and Evans, 1996). Therefore, absence of crossing-over in these segments during many successive backcross generations of translocation heterozygotes in finite populations would theoretically lead to the accumulation of detrimental and/or lethal mutations. To test this hypothesis, we analysed the survival rates of mice homozygous for the T(1;13)70H translocation (T/T) relative to contemporary T70H heterozygotes (T/+). The T70H translocation chromosomes which are present in bot0h karyotypes have been transmitted for many generations via heterozygous karyotypes. The non-translocation chromosomes of these T70H heterozygotes ("+") originated from the Swiss random bred (+/+) stock each subsequent generation (see below).

The T(1;13)70H translocation karyogram is characterized by a very large 13^{1}H and very small 1^{13}H translocation chromosome (figs. 1a, 1b). In crosses between T70H homozygous males and heterozygous females, homozygous progeny can easily be distinguished from the T70H heterozygotes by their fuzzy hair coat. This is due to the presence of the recessive fuzzy (fz) locus (Dickie and Wooley, 1950) on the chromosome 1 region of the 1^{13}H chromosome which is tightly linked to the translocation breakpoint (fig. 1; Searle *et al.*, 1971; de Boer et al., 1983). In addition to balanced gametes, a very small percentage of unbalanced gametes is produced during meiosis of T70H/+ females which can give rise to viable offspring trisomic for the small translocation chromosome. These translocation trisomics have a smooth hair coat and often display skull malformations (de Boer, 1973; Wauben-Penris and Prins, 1983).

After the discovery of the T70H translocation in the early sixties (Lyon *et al.*, 1964), it was kept on the C3H/HeH x 101/H background in an unknown manner (fig. 2). Since 1972, the T70H translocation chromosomes were transmitted for 20 generations in crosses between T70H heterozygous males and +/+ females of the Swiss random bred background (T/+ x +/+ stock). During the following 22 to 25 generations (1980-1997), these translocation chromosomes have been transmitted in crosses between T70H homozygous males and T70H/+ females (T/T x T/+ stock; fig. 2). The T70H translocation has also been maintained in

a similar backcross stock in which both types of parents are homozygous for the Rb(11.13)4Bnr (Rb4) translocation (fig. 2). Consequently, the T70H translocation chromosomes have been alternatively transmitted by T70H homozygous males and heterozygous females within each backcross stocks either in the "absence" or "presence" of homozygosity for Rb4. In contrast, the normal chromosomes 1 and 13 have been originating

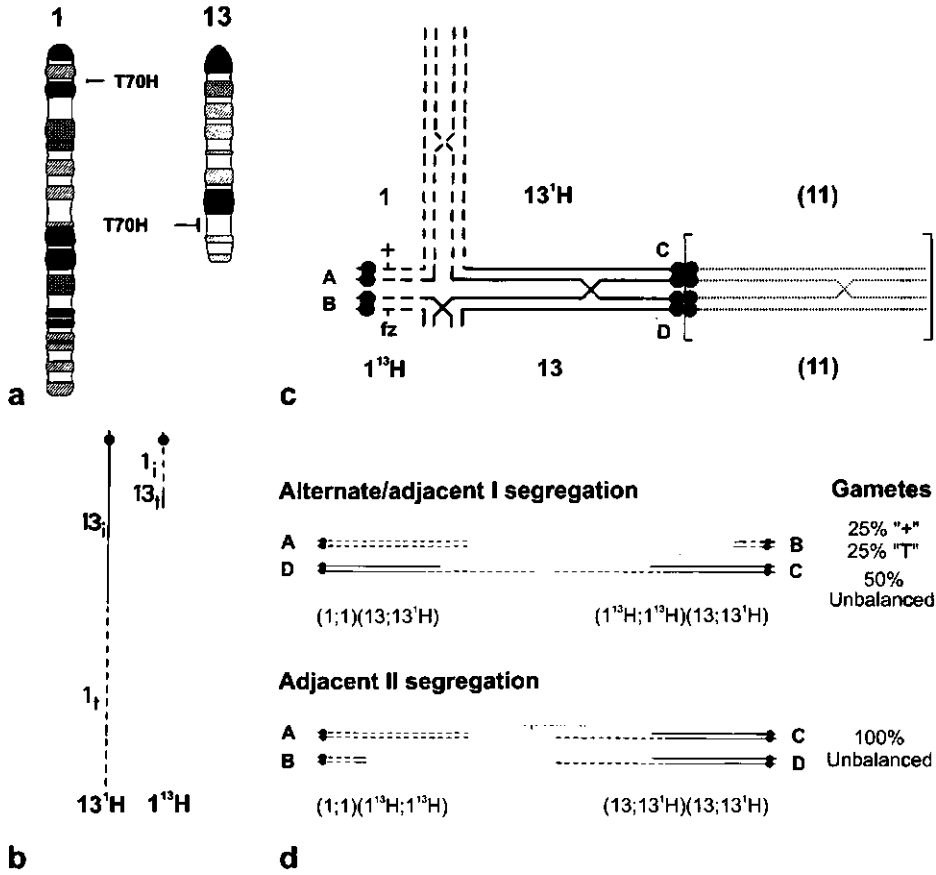


Figure 1: **a** Position of the T(1;13)70H translocation breakpoints along the G-banded mitotic chromosomes 1 and 13. **b** The T(1;13)70H translocation chromosomes. The segments originating from chromosome 1 are indicated by dashed lines. **c** Pachytene pairing diagram of the T(1;13)70H/+ involved chromosomes. No chiasma is present in segment 1_i and two chiasmata are depicted in segment 1_t. The centromeres are designated A, B, C and D. The wildtype (+) and fuzzy (fz) loci are depicted on the chromosomes 1 and $1^{13}H$ respectively. In the Rb4T70H/Rb4+ karyotype, chromosomes 11 are fused to the chromosomes 13 and 13^1H, and are represented by dotted lines between brackets. **d** Possible two by two segregation products of the quadrivalent (lacking Rb4) shown in panel **c**. Percentages of balanced (+ and T) and unbalanced gametes are given.

each generation from +/+ or Rb4/Rb4 Swiss random bred stocks. Thus, if recessive lethal mutations would accumulate on the (Rb4)T70H chromosomes, a deficit of fuzzy segregants having the (Rb4)T70H homozygous karyotype is expected in the progeny of crosses between (Rb4)T70H homozygotes and heterozygotes (T/T x T/+ crosses).

~1960 X-ray induction of T70H translocation; maintained on the C3H/HeH x 101/H background.

1972 Introduction of T70H into the Swiss random bred stock; maintained
- in T70H/+ (male) x +/+ (female) crosses
- for 20 generations
- in more than 5 pedigrees.

1976 Introduction of Rb4Bnr into the Swiss random bred stock; maintained in the following crosses:
Rb4-A stock: Rb4/Rb4 (m) x Rb4/+ (f))
Rb4-B stock: Rb4/Rb4 (m) x +/+ (f)

1980 Introduction of Rb4Bnr into the T70H stock
Since then, T70H and Rb4T70H have been maintained as follows:

T70H-A stock:
T70H/T70H (m) x T70H/+ (f)
T70H-B stock:
T70H/T70H (m) x +/+ (f)
- for 22 generations
- in 5 pedigrees
- according to a family rotation scheme (fig. 3)

Rb4T70H-A stock:
Rb4T70H/Rb4T70H (m) x Rb4T70H/Rb4+ (f)
RB4T70H-B stock:
Rb4T70H/Rb4T70H (m) x Rb4+/Rb4+ (f)
- for 25 generations
- in 5 pedigrees
- according to a family rotation scheme (fig. 3)

Figure 2: History of the T70H-A, Rb4-A and Rb4T70H-A translocation stocks. The T(1;13)70H and the Rb(11.13)4Bnr translocations were introduced in the Swiss random bred stock in 1972 and 1976 respectively. The T70H translocation was first kept in crosses between T70H/+ males and +/+ females. Each generation, an embryonic lethal test (Carter *et al.*, 1955) was used to select the semi-sterile T/+ males. In 1980, the Rb4 translocation (Gropp *et al.*, 1970) was bred into the T70H stock (Nijhoff and de Boer, 1979). Since then, both the T70H and Rb4T70H translocation have been maintained in crosses between (Rb4)T70H homozygous males and (Rb4)T70H/(Rb4)+ females according to a family rotation scheme (T70H-A and Rb4T70H-A stocks; see fig. 3). Rb4 has been maintained using a similar family rotation scheme (Rb4-A stock; see fig. 3). In any of these crosses, fully fertile translocation homozygous (T/T) males were selected by peripheral blood lymphocyte karyotyping (de Boer *et al.*, 1977) or an embryonic lethality test (Carter *et al.*, 1955). Translocation heterozygous (T/+) females were generated by crossing T/T males with Swiss random bred females (or Rb4/Rb4 females in case of the Rb4-T70H combination).

In this paper we analysed the progeny which was produced during the last 20 to 25 backcross generations in both T/T x T/+ backcross stocks (1980-1997): we observed a significant shortage of fuzzy (Rb4)T70H homozygous offspring in both stocks. This deficit was most severe in the stock lacking Rb4. We did not observe an increase in the transmission distortion over successive backcross generations in both stocks. To relate chromosome synapsis within the meiotic translocation quadrivalent with the transmission distortion, we

have analysed the synaptic behaviour of the chromosome segments near the T70H breakpoints in the meiotic quadrivalents of T70H/+ males and females and Rb4T70H/Rb4+ males at the pachytene stage. Moreover, we have used the microsatellites D1Mit4, D1Mit20 and D1Mit122, which are located about 8.6 to 15.6 cM distal of the T70H breakpoint on chromosome 1 (chapter 4; Seldin, 1996), to determine the rate of introgression of new alleles from the wildtype genome into the translocation genome in both T/T x T/+ backcross stocks (with and without Rb4). Finally, we discuss the significant deficit of homozygous T70H offspring relative to T70H heterozygous offspring in terms of a possible active role of homologous and non-homologous chromosome synapsis and of meiotic recombination on the acquisition and accumulation of deleterious mutations in the chomosome segments near the T70H breakpoints of the T70H translocations chromosomes.

Materials and Methods

Meiosis in the T(1;13)70H/+ and Rb(11.13)4Bnr,T(1;13)70H/Rb(11.13)4Bnr,+ karyotypes.
The breakpoints of the T(1;13)70H translocation are in the proximal part of chromosome 1 (in subband A4) and at the distal end of chromosome 13 (in subband D1; fig. 1a; de Boer and Gijsen, 1974). In both male and female T(1;13)70H heterozygotes, pairing and synapsis between the homologous segments of the 13^{1} and 1^{13} translocation and the 1 and 13 normal chromosomes (fig. 1b) produce a synaptonemal complex (SC) quadrivalent configuration at the pachytene stage (fig. 1c). A SC is usually formed in all four segments of the quadrivalent (de Boer *et al.*, 1986). In almost 100% of the T70H/+ male and female meiocytes one chiasma is present in the interstitial segment of chromosome 13 (13_i) (fig. 1c; de Boer, 1976; de Boer *et al.*, 1983; Wauben-Penris and Prins, 1983). Therefore, 25% of the gametes have the "+" genotype, 25% have the translocation genotype and 50% are unbalanced after either alternate or adjacent I segregation (fig. 1d). Adjacent II segregation and non-disjunction of non-translocation chromosomes on the contrary produce only unbalanced gametes (fig. 1d). Equational separation of the small 1^{13}H translocation chromosome at metaphase I results among others in balanced "+" and translocation gametes. Furthermore, several unbalanced chromosome combinations are produced of which only the "+, 1^{13}H" gamete can produce viable trisomic offspring (de Boer, 1976). However, viability of these trisomics is reduced and approximately half of them can easily be recognized by skull malformations (de Boer, 1973).

On the basis of quantitative chromosome segregation analysis of secondary oocytes from T70H/+ females (Wauben-Penris and Prins, 1983) the following percentages of segregating karyotypes are expected after meiosis in the progeny of the T70H/T70H (male) x T70H/+ (female) (T/T x T/+) cross: 49.56% of the homozygous and heterozygous segregants

each and maximally 0.88% of the translocation trisomics provided that segregation at anaphase II is not distorted.

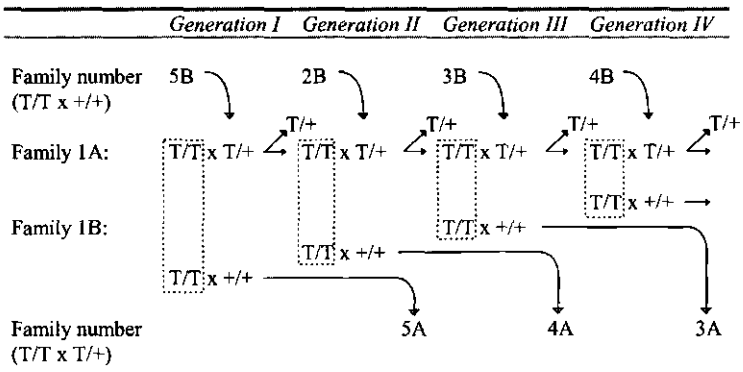
Analyses of meiotic chromosome segregation of Rb4T70H/Rb4+ females have never been performed. In T70H/+ and Rb4T70H/Rb4+ males however, the chiasma frequencies in the 1_i segment are comparable (3.6% and 3.1% respectively) and one chiasma is almost always present in the 13_i segment (98.8% and 87.5% respectively). Also, chromosome 11 which is fused to the 13^1 chromosome in the Rb4 containing karyotype contains usually at least one chiasma (97%; de Boer, 1976; Nijhoff, 1981). Moreover, the rates of numerical "3:1" non-disjunction and equational separation of the 1^{13}H chromosome are comparable for both karyotypes (de Boer, 1976, 1986). It is therefore not expected that meiotic segregation of "+" and "T" gametes in Rb4T70H/Rb4+ females significantly differs from that in T70H/+ females, and that almost equal percentages of fuzzy and smooth offspring are produced in crosses between Rb4T70H homozygotes and heterozygotes.

History of translocation stocks and littersize data.

The X-ray induced reciprocal translocation T(1;13)70H (Lyon *et al.*, 1964) was kept for many generations on the C3H/HeH x 101/H background before it was introduced into the Swiss random bred (Cpb: SE(S)) background in 1972 (fig. 2). On the Swiss background, the T70H and the Robertsonian Rb(11.13)4Bnr translocations have been maintained according to an outbreeding program as shown and described in figures 2 and 3. Consequently, genetic variation has been maintained at the level of the Swiss random bred background in these translocation stocks except for the regions around the breakpoints where crossing-over is reduced or absent (see results; Cattanach *et al.*, 1972; Philips *et al.*, 1980; Davisson and Akeson, 1993).

In addition to the T/T x T/+ and T/T x +/+ crosses, crosses between (Rb4)T70H homozygotes have also been performed (table 1; fig. 3c: T70H-C stock; fig. 3d: Rb4T70H-C stock).

The littersize data of the T70H-A, T70H-B, T70H-C and Rb4T70H-B stocks (table 1) refer to the number of live plus dead pubs from the first three pregancies of a single female. Most "breeding pairs" of the Rb4T0H-A and -C stocks consisted of two females and one male. Accordingly, the littersize data of these stocks refer to the first 6 pregnancies, 3 pregnancies per female. Based on the date of birth of the successive litters and the size of the individual litters, we declared a litter to be generated by one or two females. The number of pubs and litters were adjusted accordingly. Most litters seemed to be produced by one female.



a

b

Family number of T/T x T/+ crosses	Family number of T/T x +/+ crosses			
	Generation			
	I	*II*	*III*	*IV*
1A	5B	2B	3B	4B
2A	1B	3B	4B	5B
3A	2B	4B	5B	1B
4A	3B	5B	1B	2B
5A	4B	1B	2B	3B

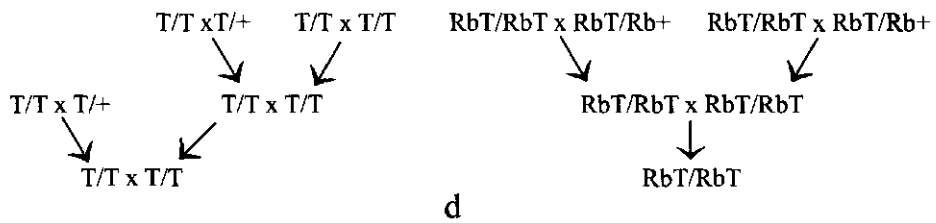


Figure 3: Outbreeding scheme (**a**) of reciprocal and Robertsonian translocation production stocks used in this study. "T" and "+" denote the translocation and Swiss random bred genomes respectively used for maintaining the T(1;13)70H, T(1;13)1Wa and Rb(11.13)4Bnr stocks. Likewise, the Rb4T70H and Rb4 genomes of the Rb(11.13)4Bnr-T(1;13)70H stock are represented by "T" and "+"respectively. Each new generation, a T/T male belonging to a specific T/T x T/+ family line (coded 1A to 5A; (Rb4)T70H-A stocks) is crossed with one or two T/+ females from the T/T x +/+ families with a different family number (coded 1B to 5B; (Rb4)T70H-B stocks; numbers are based on the family number of the T/T father). The same T/T male or T/T brothers are used to sire families 1A and 1B (symbolized by stripped rectangles). A family rotation scheme, as presented in **b** is used to vary the origin of the T/+ females over subsequent T/T x T/+ generations. Panels **c** and **d** show the breeding schemes of the translocation homozygote production stocks T70H-C and Rb4T70H-C respectively (T/T x T/T crosses in table 1). **c** In the T70H-C stock, fully fertile T70H homozygous male segregants of the T70H-A stock (T/T x T/+ cross; table 1) were mated with T70H homozygous females which were generated by homozygous parents (T/T x T/T cross). These T70H homozygous parents were also randomized according to a family rotation scheme. **d** In RbT70H-C stock, Rb4T70H homozygotes were produced in intercrosses between fuzzy segregants from different breeding pairs of the Rb4T70H-A stock (RbT/RbT x RbT/Rb+ cross). These fuzzy presumed Rb4T70H homozygous parents were only karyotyped (by peripheral blood lymphocyte karyotyping) if the mean littersize of their offspring was low (suggesting one of the parents being RbT/Rb+).

Table 1: Summary of names and characteristics of crosses.

Stock Code	*Cross*			*General*	*Origin of Parent*	
	male		*female*	*Cross Code*	*male (n mice)*	*female (n mice)*
T70H-A	T70H/T70H	x	T70H/+	T/T x T/+	T70H-A (1)	T70H-B (1)
T70H-B	T70H/T70H	x	+/+	-	T70H-A (1)	Swiss +/+ (1)
T70H-C	T70H/T70H	x	T70H/T70H	T/T x T/T	T70H-A (1)	T70H-C (1 or 2)
Rb4T70H-A	Rb4T70H/Rb4T70H	x	Rb4T70H/Rb4+	T/T x T/+	Rb4T70H-A (1)	Rb4T70H-B (2)
Rb4T70H-B	Rb4T70H/Rb4T70H	x	Rb4+/Rb4+	-	Rb4T70H-A (1)	Rb4-A (1)
Rb4T70H-C	Rb4T70H/Rb4T70H	x	Rb4T70H/Rb4T70H	T/T x T/T	Rb4T70H-A (1)	Rb4T70H-A (1 or 2)
Rb4-A	Rb4+/Rb4+	x	Rb4+/++		Rb4-A (1)	Rb4-B (1 or 2)
Rb4-B	Rb4+/Rb4+	x	++/++		Rb4-A (1)	Swiss +/+ (1)

T70H/+ hybrids of Mus musculus and Mus musculus molossinus.

In 1977, T70H/+ hybrids were produced by crossing four males of the Japanse house mouse *Mus musculus molosinnus* (a gift from the late Prof. A. Gropp, Lübeck) with T70H homozygous females which had been maintained on the Swiss random bred background (de Boer and Nijhoff, 1981). The first backcross generation was produced by 27 hybrid T70H/+ females and 20 Swiss T70H/T70H males.

Chromosome 1 microsatellite analysis.

Microsatellite markers D1Mit4, D1Mit20, D1Mit121 and D1Mit122 (Research Genetics, Huntsville, AL), were amplified and analysed as described in chapter 4 (table 6). The microsatellites are located distal of the T70H breakpoint in the given order (chapter 4; Seldin, 1996) Analysed homozygous T70H and Rb4T70H mice were derived from the 1992 populations of the T70H-A and Rb4T70H-A stocks respectively. Contemporary Swiss random bred mice were used as controls to determine the introgression rate of microsatellite alleles into the translocation stocks.

Male fertility estimates of T70H and Rb4T70H homozygotes from the T/T x T/T backcross stocks T70H-C and Rb4T70H-C.

The method of Searle and Beechey (1974) was used to count sperm from the caput epididymis. Testes were weighted after removal of adherent fat tissue. The capacity of males to produce offspring was tested by matings with two 2-3 months old NMRI (Han) virgin

females (de Boer *et al.*, 1986). Small and large moles and live embryos were classified according to the criteria of Carter *et al.* (1955) and were counted at day 13 after a vaginal plug had been found. A male was declared fertile when at least one conceptus was produced after two successive matings.

Synaptonemal complex (SC) analyses.

Two T70H/+ and two Rb4T70H/Rb4+ males (each two months old), which had been produced in crosses between homozygous parents, were used for SC analysis of the translocation multivalents. Spermatocytes were spread on glass slides by the drying down technique of Peters *et al.* (1997a; chapter 2) and subsequently silver stained (Kodama *et al.*, 1980). Then, the glass slides were coated with falcon plastic and the nuclei were detached from the glass and transferred to the plastic by hydrofluoric acid (Messier *et al.* 1986). About 50 pachytene nuclei per male were analysed at a Jeol 1200 EKII or Philips EM 208 (80 kV) electron microscope. Pachytene was substaged on the basis of the degree of synapsis and the morphology of the sex chromosomes, and the morphology of the attachment plaques (APs) of the autosomes (Tres 1977, Moses 1980, Dietrich and de Boer 1983, Moses *et al.* 1984), as described by Peters *et al.*, (in press; chapter 3). Shortly, pachytene spermatocytes with unsynapsed straight sex chromosome axial elements (sometimes showing the typical little hairpin at their ends) and nuclei with XY synapsis up to 10 % of the length of the Y chromosome in the absence of APs at the autosomal telomeric ends were classified as *early*. Spermatocytes with XY-synapsis varying from 10% to the maximum, both ascending and decending (the difference decided by the morphology of the APs) were classified as *middle* and pachytenes with less than 10 % synapsis of the XY plus pronounced APs were named *late*.

Statistical analyses

Parameters were statistically analysed by the χ^2 test and the Mann-Whitney U test as described by Siegel (1956).

Results.

T70H homozygous segregants are underrepresented in the offspring of crosses between T70H homozygous males and heterozygous females.

Provided that segregation of the translocation and normal chromosomes 1 and 13 at metaphase II in secondary oocytes is not distorted (see fig. 1), approximately equal numbers of fuzzy homozygous and smooth heterozygous T70H segregants should be produced in the

Table 2: Numbers of fuzzy and smooth offspring at weaning in litters of crosses between translocation homozygous males and heterozygous females (summed data of approximately 25 generations over the period 1980-1997).

Stock or Cross	*Cross* male		*female*	*Number of Breeding Pairs*	*Number of phenotypes at weaning* dead (days 0-20)	*fuzzy*	*smooth*	*smooth tri-somic*	*total*	*Percentage of fuzzy offspring*[1] total	*per breeding pair*[2] mean ± sd
T70H-A	T70H/T70H	x	T70H/+	87[3]	10	845	1099	1	1956	43.50[5,8,9]	43.75 ± 11.07
Rb4T70H-B	Rb4T70H/Rb4T70H	x	Rb4T70H/Rb4+	92[4]	53	1425	1647	4	3129	46.39[6,8]	45.69 ± 9.56
T70H/+ hybrids	T70H/T70H	x	T70H/+ hybrid	20[3]	nd	89	168	2	259	34.63[7,9]	nd

sd = standard deviation; nd = not determined
1: relative to normal smooth phenotypes
2: Number of fuzzy and smooth offspring > 15 per breeding pair
3: Mostly 1 mother per breeding pair
4: Mostly 2 mothers per breeding pair (sisters)
5: $\chi^2_1 = 16.39$ $P < 0.005$; compared with 50% fuzzy offspring
6: $\chi^2_1 = 7.88$ $P < 0.005$; compared with 50% fuzzy offspring
7: $\chi^2_1 = 12.35$ $P < 0.0005$; compared with 50% fuzzy offspring
8: $\chi^2_1 = 3.98$ $P < 0.025$; T70H-A stock compared with Rb4T70H-A stock
9: $\chi^2_1 = 6.90$ $P < 0.005$; T70H/+ hybrid compared with T70H-A stock

Table 3: Littersize of crosses between translocation homozygous males and heterozygous females (T70H-A and Rb4T70-A stocks).

Stock or Cross	*% fuzzy mean ± sd*	*Number of Breeding Pairs*	*Number of Offspring*	*Number of Litters*	*Littersize*	*mean ± sd*
T70H-A [1]	43.7 ± 11.1	84	907	256	3.54	3.55 ± 1.13 [3,4]
Rb4T70H-A [2]	45.67 ± 9.58	92	2095	529	3.96	3.98 ± 0.93 [3]
T70H/+ hybrid	34.63	20	195	72	2.71	2.88 ± 0.85 [4]

sd = standard deviation
1: Mostly 1 mother per breeding pair
2: Mostly 2 mothers per breeding pair (sisters)
3: P = 0.0011
4: P = 0.0054

T/T x T/+ crosses of the T70H-A and Rb4T70H-A stocks (figs. 1c, 1d; table 1). Yet, significantly lower numbers of fuzzy offspring were weaned at day 18 after birth in both stocks (relative to 50% fuzzy: $P < 0.005$ in both stocks; table 2). The percentage of fuzzy offspring was significantly lower in the absence of Rb4 (T70H-A versus Rb4T70H-A: $P < 0.025$; table 2). In addition, the mean littersize of the T/T x T/+ crosses was also significantly lower in the absence of Rb4 ($P = 0.0011$; table 3). Only 0.1% and 0.24% of the smooth segregants in the T70H-A and Rb4T70H-A stocks respectively showed the typical skull abnormalities characteristic for the trisomic translocation offspring (table 2). Thus, these trisomics hardly contributed to the excess of smooth offspring. The postnatal death rate of both phenotypes was low in both stocks (table 2). Moreover, similar numbers of smooth and fuzzy offspring died between days 5 and weaning during the last four Rb4T70H-A generations (data not shown). Hence, fewer fuzzy (Rb4)T70H homozygotes than smooth (Rb4)T70H heterozygotes are born in the T/T x T/+ crosses.

Interestingly, T70H homozygous segregants were even more underrepresented in the offspring of crosses between *Mus musculus* T70H homozygous males and T70H/+ female hybrids of *Mus musculus* and *M. musculus molossinus*. These crosses only yielded 34.6% fuzzy offspring which is significantly less than the expected 50% and the percentage fuzzy offspring produced in the T70H-A stock (table 2; $\chi^2_1 = 12.35$, $P < 0.0005$; $\chi^2_1 = 6.90$, $P < 0.005$ respectively). Only 2 out of 170 (1.2%) smooth segregants showed the typical skull malformations characteristic for the translocation trisomics (table 2). The mean littersize of the T70H/+ hybrid females was significantly lower compared with the littersizes of the T70H-A stock ($P = 0.0054$; table 3).

To summarize, a deficit of T70H homozygous segregants is produced in crosses between homozygous males and heterozygous females. The deviation from the expected 1:1 ratio of fuzzy T/T versus smooth T/+ offspring was significantly more severe in the stock

lacking Rb4 (table 2). This deviation was even more pronounced in crosses with T70H/+ hybrid females.

Littersizes of T/T x T/T and T/T x +/+ crosses using translocation homozygotes derived from the T70H-A and Rb4T70H-A stocks.

To analyse whether the deficit of T70H homozygotes either is intrinsic to homozygosity for this translocation or is restricted to T/T x T/+ crosses, we compared the littersizes of T/T x T/T crosses with the ones of T/T x +/+ crosses which have been performed during the same period as the T/T x T/+ crosses (figs. 3c, 3d). During almost all generations homozygous T70H males, derived from the T/T x T/+ crosses (T70H-A stock), produced less T/T offspring in crosses with T70H homozygous females (T70H-C stock) than T/+ offspring in crosses with +/+ females (T70H-B stock; fig. 4b). The distibution of the breeding pairs according to their littersize is shifted to lower littersizes in these T/T x T/T (T70H-C stock) compared with T/T x +/+ crosses (T70H-B stock; $P < 0.00003$; fig 4a; table 4). This finding could be explained by a reduction in ovulation number of the homozygous T70H females. However, this is unlikely because crosses between T70H homozygous females and T(1;13)1Wa homozygous males produced clearly larger litters than contemporary crosses between T70H homozygotes (data not shown). Taken together, these data suggest that the prenatal viability of T70H homozygotes is reduced in comparison to T70H heterozygotes, not only in T/T x T/+ crosses but also in T/T x T/T crosses.

Table 4: Littersize [1] of crosses between T70H homozygotes and between T70H homozygous males and Swiss +/+ females in the absence or presence of Rb4 (T70H-C, -B and Rb4T70-C, -B stocks respectively).

Stock	*Cross*			*Number of Breeding Pairs*	*Number of Offspring*	*Number of Litters*	*Littersize*	
	male		*female*					*mean ± sd*
T70H-B	T70H/T70H	x	+/+	90	2874	275	10.45	10.54 ± 2.43 [2]
T70H-C	T70H/T70H	x	T70H/T70H	84	2084	242	8.61	8.67 ± 1.05 [2]
Rb4T70H-B	Rb4T70H/Rb4T70H	x	Rb4+/Rb4+	97	2393	245	9.77	9.95 ± 2.55
Rb4T70H-C	Rb4T70H/Rb4T70H	x	Rb4T70H/Rb4T70H	124	3238	338	9.58	9.58 ± 1.84

sd = standard deviation
1: Calculated from first three litters per female only.
2: $\chi^2_1 = 4.91$; $P < 0.00003$

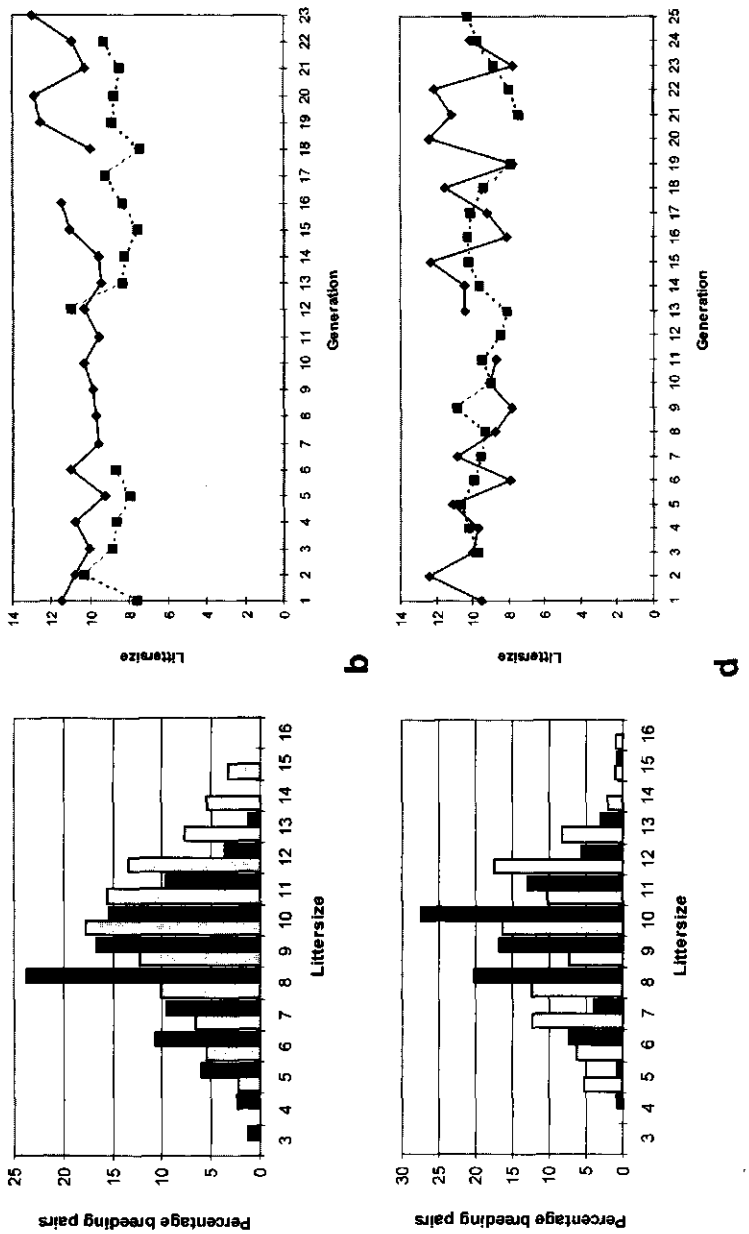


Figure 4: a, c Distribution of breeding pairs according to their littersize (based on the first three litters) in the T/T (m) x T/T (f) and T/T (m) x +/+ (f) crosses (black and grey bars respectively). **b, d** Mean littersize per generation in the T/T (m) x T/T (f) and T/T (m) x +/+ (f) crosses (rectangles and diamonds respectively). Figs. **a, b** represent data of crosses in absence of Rb4. Figs. **c, d** represent data of crosses in presence of Rb4.

In the presence of Rb4, the littersizes of the T/T x T/T crosses were not significantly smaller than the ones of the T/T x +/+ crosses (Rb4T70H-C versus -B stock; table 4; fig. 4c). It is possible that this is partly due to a reduced fertility of the Rb4/Rb4 females in the Rb4T70H-B stock (compare littersizes of RbT70H-B and T70H-B stocks: P = 0.064; figs. 4c, 4a; table 4).

Significantly fewer homozygous offspring was produced in the absence than in the presence of Rb4 in the T/T x T/T crosses (T70H-C versus Rb4T70H-C stock: P = 0.0012; figs. 4a versus 4c). This can not be attributed to a lower degree of fertility of the T70H versus Rb4T70H homozygous males as determination of male fertility estimates of the T70H and Rb4T70H homozygous males revealed the opposite result (table 5). Finally, preliminary data on contemporary crosses of T70H/T70H females with either T70H- or Rb4T70H homozygous males reveal higher littersizes for the latter cross (data not shown). This suggests that the reduction in prenatal viability of the T70H homozygotes is not related to homozygosity for the breakpoints of the T70H reciprocal translocation.

In summary, analogous to the T/T x T/+ crosses in the T70H-A stock, the transmission of the T70H homozygotes is reduced in the T/T x T/T crosses lacking Rb4. Most likely, this is caused by a reduction in prenatal viability of T70H homozygotes.

Table 5: Fertility indices of T70H and Rb4T70H homozygous males generated by homozygous parents.

Stock	*Cross*			*Number of Males*	*Testis Weight (mg) mean ± sd*	*Epididymal Sperm Count mean ± sd*
	male		*female*			
+/+ [1]	+/+	x	+/+	40	-	417 ± 71
T70H-C	T70H/T70H	x	T70H/T70H	36	131.8 ± 17.0	516 ± 119
Rb4T70H-C	Rb4T70H/Rb4T70H	x	Rb4T70H/Rb4T70H	32	98.5 ± 9.6	266 ± 95

sd = standard deviation
1: from de Boer *et al.*, 1986

Homologous and non-homologous chromosome synapsis in the chromosome segments around the T70H breakpoints is more delayed during zygotene and pachytene stage in Rb4T70H/Rb4+ multivalents compared with T70H/+ quadrivalents.

To correlate the genetic data on the transmission distortion in the T/T x T/+ crosses with chromosome behaviour at meiotic prophase, we performed SC analyses of the translocation multivalents in T70H/+ males and Rb4T70H/Rb4+ males. The chromosome segments around

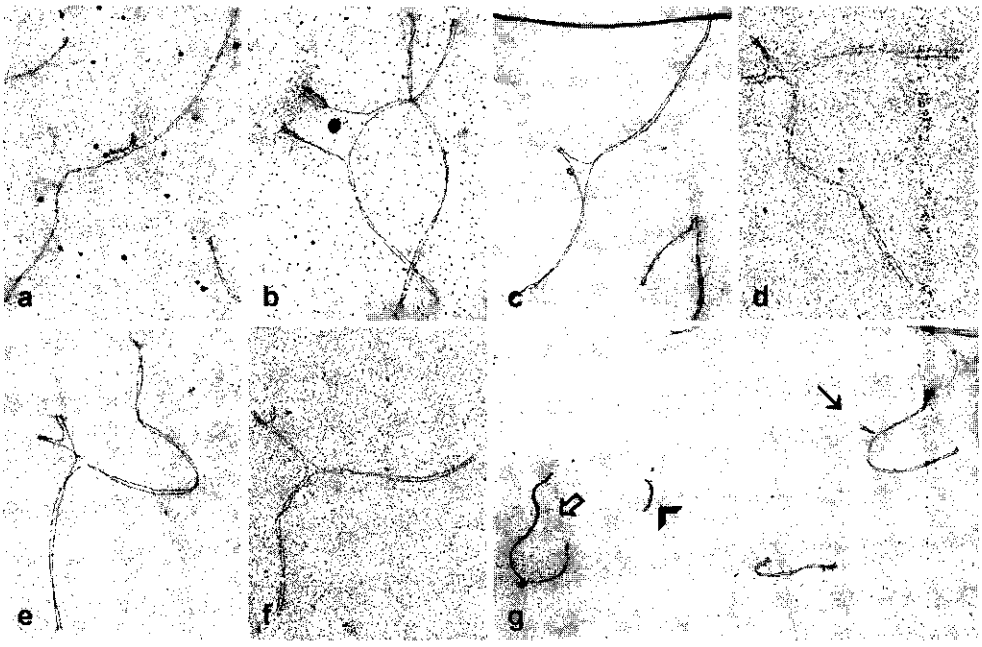
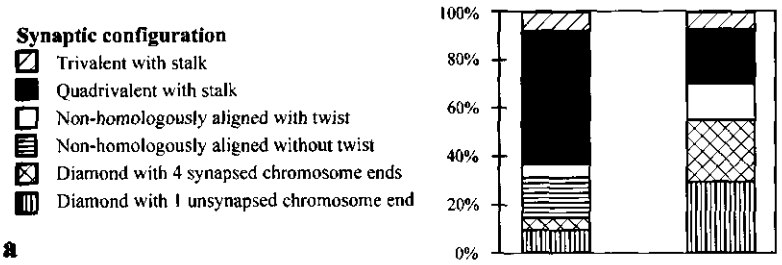


Figure 5: Silver stained SCs of different synaptic multivalent configurations of the segments around the T70H breakpoints. **a:** Complete, presumably, homologous synapsis. **b, c:** Open diamond configurations showing incomplete homologous synapsis. **d, e:** "Alignment" of non-homologous "+" chromosome segments. **f, g :** Stalk configurations showing complete non-homologous synapsis. Figure **g** shows translocation trivalent (closed arrow), $1^{13}H$ univalent (arow head) and sex bivalent (open arrow). Figures **d**, **f** and **g** are from T70H/+ spermatocytes. Figures **a**, **b**, **c** and **e** are from RbT70H/Rb4+ spermatocytes.

the T70H breakpoints display several different synaptic configurations during pachytene stage of both T70H/+ and Rb4T70H/Rb4+ spermatocytes (fig. 5). A symmetrical cross configuration indicating complete homologous synapsis was encountered only once among the 100 analysed nuclei of the Rb4T70H/Rb4+ males (fig. 5a). In (Rb4)T70H/(Rb4)+ multivalents, delayed homologous synapsis results in open diamond like configurations (figs. 5b, 5c), in which the axial elements of one of the short arms are sometimes unsynapsed (fig. 5b). On the basis of the multivalent configurations at diakinesis-metaphase I stage (de Boer, 1976; de Boer *et al.*, 1983), the unsynapsed short arm is most likely the short interstitial segment of chromosome 1.

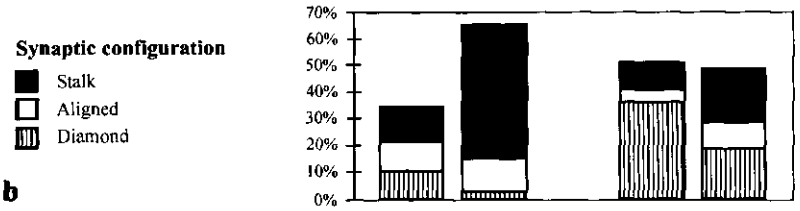
In both karyotypes, segments around the translocation breakpoints were engaged in non-homologous synapsis. Complete non-homologous synapsis led to stalk like multivalent configurations (figs. 5f) from which the small $1^{13}H$ chromosome was sometimes excluded

Karyotype	T70H/+	Rb4BnrT70H/ Rb4Bnr+
n Males	2	2
Age (weeks)	8	8
Testis Weight (mg)	117.8±1.8	70.8±2.8
Epididymal Sperm Count	387.5±1.5	84.5±15.5
n Pachytene nuclei	96	100



a

Karyotype	T70H/+		Rb4BnrT70H/Rb4Bnr+	
Pachytene substage	early/mid	late	early/mid	late
n Pachytene nuclei	33	63	51	49



b

Figure 6: Percentages of different synaptic configurations of the T70H/+ and Rb4T70H/Rb4+ multivalents in all pachytene nuclei (**a**) and nuclei of successive pachytene substages (**b**).

(fig. 5g). The percentages of the stalk configurations increased in successive pachytene substages in both karyotypes (fig. 6b). Moreover, "alignment" of the non-homologous axial elements as shown in figures 5d and 5e suggests that the stalk configurations arose from the open diamond configurations. Rb4T70H/Rb+ males contained more open diamond configurations during the early and mid pachytene substages and less stalk configurations during late pachytene substage compared with the T70H/+ males (fig. 6b). Also the length of the stalk in the fully non-homologously synapsed stalk quadrivalents differed between the two karyotypes. Compared with the Rb4T70H/Rb4+ karyotype, significantly more non-homologously synapsed stalk configurations in the T70H/+ karyotype showed a stalk that was larger than the lateral element of the $1^{13}H$ chromosome (26%, n=23 versus 82%, n=52 respectively; $\chi^2_1 = 20.1$, $P < 0.0005$). Thus, homozygosity for Rb4 clearly impaired the progression of non-homologous synapsis during the pachytene substages.

For Rb4T70H heterozygotes, fewer nuclei were scored in the late pachytene substage (fig. 6b; compared with T70H/+ males, $\chi^2_1 = 4.87$; $P < 0.025$). Moreover, the frequency of multivalents with an unsynapsed short translocation segment was significantly higher in the Rb4T70H/Rb4+ than in the T70H/+ karyotype (fig. 6a; $\chi^2_1 = 8.13$; $P < 0.005$). As this type of synaptic configuration has been correlated with reduced male fertility (reviewed in de Boer and de Jong, 1989), these data suggest the occurrence of cellular loss during progression of the pachytene stage in Rb4T70H/Rb4+ males. Accordingly, testis weights and epididymal sperm counts were reduced in the Rb4T70H/Rb4+ compared with the T70H/+ males (fig. 6a; Nijhoff, 1981; de Boer, 1986).

In a preliminary study on the synapsis of the translocation quadrivalent in pachytene oocytes of fetal T70H/+ females, we found a preponderance of non-homologously synapsed stalk-like configurations, similar to ones in males (data not shown).

Meiotic recombination in the chromosome 1 segment distal of the T70H breakpoint is more suppressed in Rb4T70H/Rb4+ females than T70H/+ females.

To estimate the rate of meiotic recombination for loci which are positioned near the T70H breakpoints we used the microsatellites D1Mit4, D1Mit20 and D1Mit122. All three microsatellites are positioned on chromosome 1 distal to the T70H breakpoint in the region of aberrant synapsis (chapter 4; figs. 5, 6, 9) and are mapped in the following order: T70H - D1Mit4 - D1Mit20 - D1Mit122 (Seldin, 1996; fig. 9). We compared the alleles of these microsatellites of mice which were born in 1992 and had +/+, T70H/T70H and Rb4T70H/Rb4T70H karyotypes respectively.

T/T offspring of both T/T x T/+ crosses (T70H-A and Rb4T70H-A stocks) was homozygous for allele 1 of D1Mit4. As the frequency of this allele was very low in the Swiss random bred stock (fig. 7), this shows that recombination is clearly suppressed for the interval T70H-D1Mit4 during female (and earlier male) T/+ meiosis. Likewise, no exchange of D1Mit20 alleles was found in the Rb4T70H-A stock. In the absence of Rb4 however, several of the T70H homozygous segregants of the T70H-A stock harboured Swiss random bred "new" alleles for D1Mit20 (fig. 7). For D1Mit122, Rb4T70H homozygotes contained allele 1 which was only found at a very low frequency in the Swiss random bred stock and which was absent in T70H homozygotes (fig. 7).

Thus meiotic recombinational exchange is reduced in the chromosome 1 segment distal of the T70H breakpoint in a distance dependent manner. This effect is more pronounced in the Rb4T70H-A stock than in the T70H-A stock.

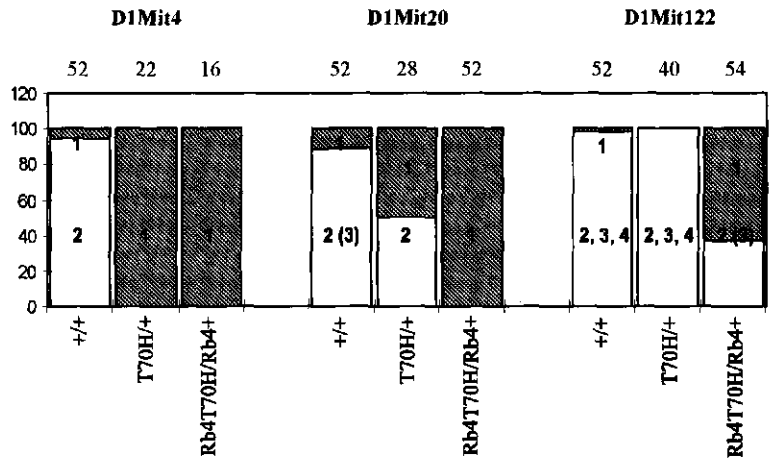


Figure 7: Percentages of "new" (light bars) and "original" (dark bars) alleles of three microsatellites positioned distal to the T70H breakpoint in the 1992 generations of the random bred Swiss (+/+), T70H-A and Rb4T70H-A stocks. The code number of the individual alleles belonging to a specific microsatellite are depicted within the bars.

Littersize in the Rb4T70H-B stock appears to be correlated with the introgression of new D1Mit122 alleles from the Swiss stock.

In an attempt to correlate the variation in mean littersizes of the T/T x T/T cross over successive generations (fig. 4d) with meiotic recombination occurring in the translocated segment of chromosome 1, we performed microsatellite analysis of the parents of these crosses. Pedigree analysis revealed a specific combination of alleles of D1Mit4, D1Mit20, D1Mit121 and D1Mit122 (haplotype A) which was present in all analysed Rb4T70H homozygous offspring of the Rb4T70H-A stock of the generations 18, 19 and 20. As the microsatellites D1Mit4, D1Mit20 and D1Mit122 of haplotype A contained an allele which either was not detected or was only found at a very low frequency in the wildtype Swiss random bred genome at that time ("old" alleles in fig. 7), we consider this haplotype to be excluded from meiotic recombination for at least many generations.

An increase in the mean littersize was observed during the last four successive generations of the Rb4T70H-C stock (fig. 4d; top of fig. 8). At generations 22 and 23, almost all T/T parents which were derived from the Rb4T70H-A stock (fig. 8) carried a new Swiss allele for D1Mit122 on one of their 11-13^{1}H chromosomes. These mice were heterozygous with the original haplotype A and the new haplotype F (table 6). Repeated intercrossing of Rb4T70H homozygotes which were homozygous for haplotype A did not result in an increase in littersize during generations 22 and 23 (fig. 8).

Table 6: Microsatellite alleles of D1Mit4, -20, -121 and -122 belonging to haplotypes A, C and F.

Haplotype	*Microsatellite Alleles* [1]			
	D1Mit4	*D1Mit20*	*D1Mit121*	*D1Mit122*
A	1	1	1	1
C [2]	1	1	2	3
F [2]	1	1	1	2

1: Relative position of microsatellites towards the T70H breakpoint is as follows: T70H-D1Mit4-D1Mit20-D1Mit121-D1Mit122 (Seldin, 1996; this chapter)
2: Haplotypes C and F are recombinational products from the ancestral haplotype A and the wild type Swiss genome

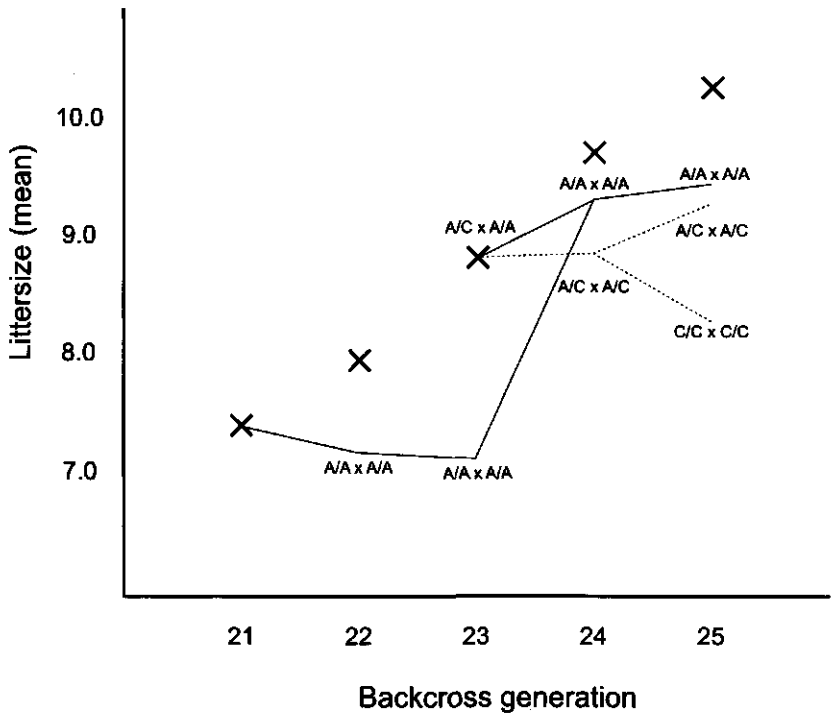


Figure 8: Littersizes of crosses between Rb4T70H homozygotes with different microsatellite haplotypes. Crosses represent the inflow of Rb4T70H translocation chromosomes from the Rb4T70H-A stock into the Rb4T70H-C stock. Solid lines indicate crosses between Rb4T70H homozygotes which are derived from the RbT70H-C stock and which are homozygous for haplotype A (A/A x A/A crosses). Dashed lines designate crosses between Rb4T70H homozygotes which are heterozygous for haplotypes A and C or homozygous for haplotype C as indicated.

At generation 23, one parent of the T/T x T/T crosses of the Rb4T70H-B stock (fig. 8), contained a new haplotype C on one of its 11-13[1]H translocation chromosomes. Haplotype C contains new Swiss alleles for D1Mit121 and D1Mit122 (table 6) which most likely have been recombined into the original haplotype A during the previous Rb4T70H/Rb4+ meiosis. Crosses between Rb4T70H homozygotes which were heterozygous with haplotypes A and C had large littersizes at generation 24 (fig. 8). Subsequent intercrosses between haplotype C homozygotes had slightly smaller litters (generation 25; fig. 8).

Interestingly, crosses between haplotype A homozygotes which were derived from parents carrying the haplotypes A/C (male) and A/A(female) (characterized by a large littersize at generation 23; see fig. 8) and from parents with haplotypes A/A and A/A (characterized by a small littersizes at generation 23; see fig. 8) had large littersizes at generation 24. At generation 25, littersizes of subsequent intercrosses between these haplotype A homozygotes remained high (fig. 8).

Thus, these data suggest that the littersize of crosses between Rb4T70H homozygotes is correlated with the microsatellite "haplotype". Moreover, the haplotype C "littersize increasing effect" is transferable to haplotype A by one meiotic passage through a heterozygous A/C male.

Discussion

(Non)-homologous chromosome synapsis and meiotic recombination in segments near the T70H breakpoints during meiosis of T70H/+ and Rb4T70H/Rb4+ females.

Classical linkage analysis of the T70H translocation breakpoint and the phenotypic markers fuzzy and leaden, revealed "signs of some crossover suppression" for chromosome 1 in T70H heterozygotes (Searle *et al.*, 1971). In the present study we confirm this notion. In both T70H/+ and Rb4T70H/Rb4+ females, meiotic recombination is clearly suppressed in the chromosome 1 interval between the T70H breakpoint and the microsatellite D1Mit4 (fig. 7). D1Mit4 is located distal of and close to the breakpoint (chapter 4; Seldin, 1996; fig. 9a). Analysis of two more distally positioned microsatellites D1Mit20 and D1Mit122 (fig. 9a) revealed that in comparison with the situation in T70H/+ females, the addition of homozygosity for Rb4 reduced the recombination rate in the translocated chromosome 1 segment near the T70H breakpoint even more (Rb4T70H/Rb4+ female meiosis, fig. 7).

This suppression of recombination correlates with the synaptic behaviour of the translocation multivalents in T70H/+ and Rb4T70H/Rb4+ spermatocytes and T70H/+ oocytes (males: figs. 5, 6). Homologous synapsis of the regions close around the T70H breakpoints was clearly impaired as a homologously synapsed configuration was found only once (n=196;

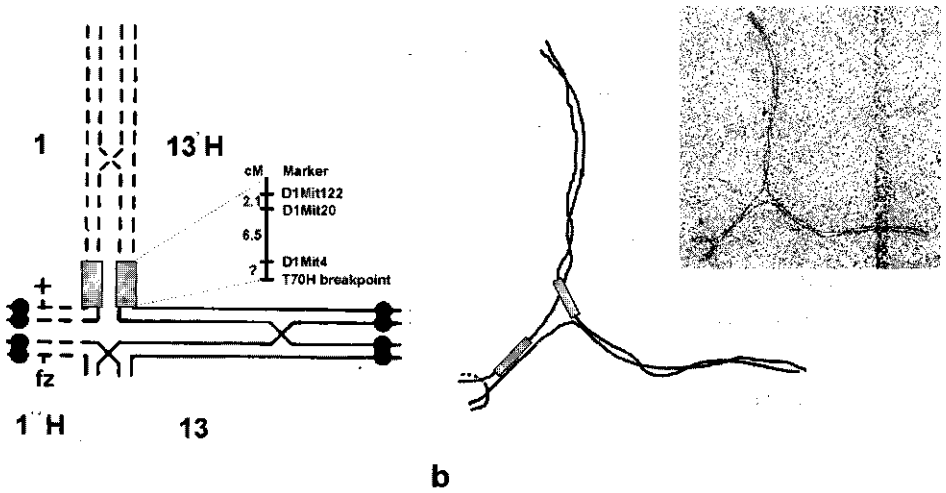


Figure 9: Schematic representation of homologous (**a**) and non-homologous (**b**) synapsis within T70H/+ quadrivalents. The localization of the microsatellites D1Mit4, D1Mit20 and D1Mit122 is given in **a**. The genetic distances are from Seldin (1996). The grey bars in **a** and **b** represent the translocated chromosome 1 region near the T70H breakpoint. The T70H/+ quadrivalent in **b** is identical to fig. **5f**.

fig. 5a). Moreover, as indicated by the short interstitial chromosome 1 segment, homologous synapsis of segments which are located little away from the T70H breakpoints was more delayed during the zygotene stage and successive pachytene substages of spermatocytes in the presence of Rb4 (fig. 6). Thus, if synapsis in (Rb4)T70H/(Rb4)+ females is comparable to the situation in the analysed (Rb4)T70H/(Rb4)+ males and assuming that the degree of synapsis represents the pairing behaviour of meiotic chromosomes, the data on meiotic recombination and homologous synapsis suggest that homology search interactions between the homologous sequences on non-sister chromatids are more disturbed in the presence of Rb4 resulting in a lower initiation rate of meiotic recombination.

In T70H/+ males, we found high percentages of cells in which the segments around the translocation breakpoints were non-homologously synapsed (figs. 5, 6). These configurations were also observed at the early and middle pachytene stages which might suggest that non-homologous synapsis also occurs at the zygotene stage. Depending on when meiotic recombination is initiated, non-homologous synapsis could correlate with a reduction in meiotic recombination. Non-homologous synapsis at the (late) pachytene stage, however, does not seem to prevent the occurrence of crossing-over, at least not all. In this respect, it would be

interesting to analyse the position of the mismatch repair enzym Mlh1, which has been implicated in mammalian meiotic reciprocal recombination (Baker *et al.*, 1996; Edelmann *et al.*, 1996), on the segments near the T70H breakpoints of the different synaptic configurations of (Rb4)T70H male and female heterozygotes.

Deficit of T70H homozygotes: segregation distortion or prenatal lethality ?

The major object of this study was to test the following hypothesis: does the absence of crossing-over in segments near the T70H translocation breakpoints during many successive backcross generations of translocation heterozygotes lead to the accumulation of detrimental and/or lethal mutations. Analysis of the survival rates of the T/T versus contemporary T/+ progeny of crosses between T/T males and T/+ females revealed a significant deficit of fuzzy T/T offspring which was more severe in the T/T x T/+ stock lacking Rb4 (table 2).

Two different mechanisms can be envisaged to explain the deficit of (Rb4)T70H homozygotes in the T/T x T/+ crosses: a) segregation distortion of dyads containing the $13^{1}H$; 13 chromatids at the second meiotic division, b) increased prenatal lethality of T70H homozygotes. According to chromosome segregation analysis in secondary oocytes of T70H/+ females (Wauben-Penris and Prins, 1983), about equal numbers of fuzzy T/T and smooth T/+ offspring should be produced in the crosses between T/T males and T/+ females if chromosome segregation at the second meiotic division is not distorted. Otherwise, the decrease of the percentage fuzzy offspring could be caused by preferential segregation of the $13^{1}H$ chromosome into the polar body at the second meiotic division of (Rb4)T70H/(Rb4)+ females. Yet, littersizes of intercrosses between T70H homozygotes were smaller compared with the ones of crosses between T70H/T70H females and +/+ males. As the difference in littersizes is most likely not due to a reduced ovulation rate of T70H homozygous females, this indicates that the deficit of T70H homozygotes is not restricted to T/T x T/+ crosses only. Therefore, we do not favour segregation distortion at the 2^{nd} meiotic division as the cause of the deficit of T70H homozygotes in the T/T x T/+ crosses although we can not exclude it. Hence, we attribute the major shortage of the fuzzy (Rb4)T70H homozygous offspring in the T70H-A, Rb4T70H-A and T70H-C stocks to a reduction of the prenatal survival of fuzzy T/T segregants.

Acquisition of lethal mutations in non-homologously synapsed translocation multivalents by disturbance of interchromosomal meiotic recombination.

As indicated by the higher littersizes of crosses between T70H homozygous females and Rb4T70H homozygous males than of crosses between T70H homozygotes, the increased lethality of T70H homozygotes is not due to homozygosity for the T70H breakpoints (see

second section of the results). What then is the cause of the prenatal lethality of (Rb4)T70H homozygotes?

Combining the data of the two T/T x T/+ backcross stocks regarding the prenatal death rate of the T/T segregants with the degree of homologous and non-homologous chromosome synapsis and the rate of meiotic recombination in the regions near the translocation breakpoints in T70H/+ and Rb4T70H//Rb4+ karyotypes, two correlations arise. The prenatal death rate is positively correlated not only with the degree and the extent of non-homologous synapsis but also with the level of meiotic recombination. Moreover, we did not observe an increase in the transmission distortion over successive backcross generations in both stocks (data not shown). This suggests that accumulation of recessive mutations, of somatic and /or germ line origin, in segments near the T70H breakpoints due to a diminished level of meiotic recombination over many successive generations is most likely not the cause of the increased lethality of the (Rb4)T70H homozygotes.

An acquisition of mutations especially during meiosis and subsequent accumulation of recessive lethal mutations of genetic nature during successive transmissions through T/+ females, however, could explain the shortage of the fuzzy T70H homozygotes in the T70H-A and Rb4T70H-A stocks. We propose that the processing and resolution of interchromosomal meiotic recombination intermediates in one or more segments surrounding the T70H breakpoints is disturbed by non-homologous chromosome synapsis in a high percentage of recombination events leading to mutation of the synapting partners. Thus, on the one hand, unsuccessful meiotic recombination leads to a mutational load on the segments near the T70H breakpoints of the translocation and possibly normal wildtype genomes. On the other hand, successful meiotic recombination introduces new undamaged copies of these segments from the normal "+" genome into the translocation genome. As the recombination frequency and the degree and extent of non-homologous synapsis in segments near the T70H breakpoints is higher in absence of Rb4, the mutation rate in these regions is also higher and the percentage fuzzy offspring is lower in the T70H-A compared with the Rb4T70H-A stock.

Additional support for this model can be gained from the following observations. In T70H/+ males, the proximal chiasma of two on the translocated chromosome 1 segment (1_t) of T70H/+ male quadrivalents is closely positioned near the breakpoint in 40% of the meiocytes (n = 260 nuclei; de Boer, 1979). In T70H/+ females, 67% of 215 diakinesis-metaphase I oocytes have 2 chiasmata in the 1_t segment (de Boer *et al.*, 1983). A large portion of these chiasmata is presumably also positioned near the T70H breakpoint. In backcrosses between hybrid T70H/+ females (*"Swiss"Mus musculus/Mus musculus molossinus* genomes) and T70H/T70H (Swiss) males, only 35% of the offspring had a fuzzy phenotype (table 2). In T70H/+ diakinesis-metaphase I spermatocytes of the same hybrid genotype the frequency of two chiasmata in the 1_t segment was significantly increased compared with T70H/+ males on

a Swiss background (de Boer and Nijhoff, 1981). Thus, the major deficit of fuzzy offspring in the "hybrid" crosses might be related to an increased recombination frequency near the T70H breakpoint. In addition, the significantly lower littersizes in the T70H/+ hybrid females in comparison with T70H/+ Swiss females having a similar percentage fuzzy offspring (P = 0.02; data not shown) suggests that the non-translocation and translocation chromosomes gained dominant lethal mutations too during meiosis of the T70H/+ hybrid females. However, differences in ovulation rates can not be ruled out. It is tempting to speculate that the increased mutation rate in these hybrid females is related to the sequence divergence between the synapting chromosomes influencing the inititation rate of meiotic recombination and/or the processing of recombination intermediates.

Accumulation of recessive lethal mutations on the (Rb4)T70H translocation chromosomes.
Accumulation of recessive lethal mutations could explain the littersize reduction as observed in the crosses between T70H homozygotes (T70H-C stock) compared with the crosses between T70H homozygous males and +/+ females (T70H-B stock; table 4; figs. 4a, 4b). Littersizes of intercrosses between the Rb4T70H homozygotes were significantly higher than the littersizes of the T70H homozygotes (table 4, figs, 4a, 4c). This is in agreement with the proposed higher mutation rate in the absence than in the presence of Rb4 in T70H heterozygous females. The preliminary data on crosses between T70H and Rb4T70H homozygotes (see above) indicate that these translocation chromosomes can complement one anothers' mutations suggesting the existence of mutations in more than one locus.

The introduction of a new "+" segment into the 13^{1}H translocation chromosome (as indicated by D1Mit121 and D1Mit122 microsatellite alleles) resulted in an increase in littersize (fig. 8) suggesting complementation of the recessive lethal mutations on the "original" translocation chromosome by undamaged loci on the newly introgressed segment of the other translocation chromosome. As the "littersize increasing effect" of the 11-13^{1}H chromosome with haplotype C is transferable to the 11-13^{1}H chromosome with haplotype A (without changing the alleles of the microsatellites within haplotype A) by one meiotic passage through a T/T male which is heterozygous with haploypes A and C male, deleterious mutations on the original 11-13^{1}H chromosome with haplotype A have to be positioned distal of D1Mit122.

Acquisition of epigenetic mutations in relation to non-homologous chromosome synapsis.
Instead of interfering with meiotic recombination, non-homologous synapsis itself could be mutagenic. In this model, non-homologous synapsis at meiosis would lead to an irreversible epigenetic modification of DNA or chromatin in the segments near the T70H breakpoint. In the subsequent generations, these modifications act like recessive epimutations. Similar to the

previous model, meiotic recombination introduces new unaffected copies of these segments into the translocation genome. Thus, the difference in distortion of the percentage of T/T segregants in the T70H-A and Rb4T70H-A stocks (table 2) is, in this model, the outcome of the balance between the epigenetic mutation rate of non-homologous synapsis and the reciprocal exchange rate of meiotic recombination around the translocation breakpoints.

In analogy with one of the explanations for position effect variegation observed in *Drosophila melanogaster*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pomble*, Cattanach's translocation in the mouse (Cattanach, 1974) and other mouse and human X-autosome translocations (see review by Hendrich and Willard, 1995), inactivation of sequences on the T70H translocation chromosomes might be caused by heterochromatinization. With respect to the model for non-homologous synapsis, the epigenetic modification must be heritable, at least to a large extent.

Implications of the presumed mutational activity of non-homologous synapsis in relation to meiotic recombination.

As non-homologous synapsis has been observed in many reciprocal translocation as well as other types of chromosome aberrations (Ashley *et al.*, 1981; Ashley and Cacheiro, 1990; Borodin *et al.*, 1990; Moses and Poorman, 1981; Poorman *et al.*, 1981; Winking *et al.*, 1993), these systems could be used to test the validation of the proposed mutation models. To be able to discern between the proposed genetic or epigenetic mutation mechanism, systems in which chromosome segments undergo non-homologous synapsis but are not engaged in meiotic recombination should be informative. Differences in the time period of non-homologous synapsis, namely either during the zygotene and early pachytene stages (G-synapsis: Ashley, 1988) or during the progression of pachytene stage (synaptic adjustment: Poorman *et al.*, 1981; Moses *et al.*, 1981), have to be taken into account in these analyses.

Acknowledgments.

The assistance of Bert Weijers for breeding of numerous mice is gratefully acknowledged. We thank Maud M. Giele for technical assistance in the microsatellite typings. We acknowledge Ing. Richard P.M.A. Crooijmans and drs Martin A.M Groenen and Jan J. van der Poel for making available PCR facilities and technical support.

Chapter 6

Non-homologous chromosome synapsis in heteromorphic bivalents and exclusion from meiotic recombination does not impair survival of progeny.

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Abstract

In this study we analyzed the consequences of generations long suppression of crossing over in two chromosomal segments, positioned between translocation breakpoints on chromosomes 1 and 13 (Δ1 and Δ13). To this purpose, mice double heterozygous for two near-identical reciprocal translocations, T(1;13)70H and T(1;13)1Wa, were used. Survival of heterozygous and homozygous carriers for these segments was taken as the phenotype of the study.

We observed no differences in the pre- and postnatal survival rates of the T70H/T1Wa double heterozygous progeny in which the Δ1 and Δ13 segments of the T1Wa translocation chromosomes had either no, a one-generation or a multi-generation history of non-homologous synapsis in heteromorphic bivalents during previous meioses. In addition, expected numbers of homozygous carriers for Δ1 and Δ13 on T1Wa translocation chromosomes were found after 20-22 successive transmissions via double heterozygous females. Thus, exclusion of these segments from meiotic crossing over within non-homologously synapsed heteromorphic bivalents during 20 to 25 successive generations does not result in an accumulation of recessive lethal mutations or an increased susceptibility for gaining dominant lethal mutations.

Yet, haplotype analysis revealed a high rate of intergenerational instability for the tetranucleotide microsatellite D1Mit122 which is positioned in the Δ1 segment. The mutation frequency was higher for chromosomes derived from the parental double heterozygous genotype which suggests the mutations to be of meiotic origin. On the basis of the identity of the mutations and the observation of ectopic homologous contacts of the Δ1 segments during zygotene, we speculate that these mutations are the result of ectopic homologous gene conversion events, most likely occurring in the absence of a synaptonemal complex.

Introduction.

During meiosis, heterozygosity for reciprocal translocations can lead to partial asynapsis or non-homologous synapsis around translocation breakpoints (de Boer and de Jong, 1989). Accordingly, meiotic crossing over is often suppressed or absent in the vicinity of translocation breakpoints (Beechey and Evans, 1996). A low level of meiotic recombination over many generations has been associated with a reduction in fitness via Muller's ratchet (Muller, 1964). In this study we have exploited the exclusion from crossing over of two segments positioned between the breakpoints of two reciprocal translocations on mouse chromosome 1 and 13 in following the consequences for pre- and postnatal survival of heterozygous and homozygous carriers after 20 to 25 successive generations. The system by

which crossover suppression around translocation breakpoints can be extended to the segments between two adjacent translocation breakpoints works as follows.

The T(1;13)70H and T(1;13)1Wa reciprocal translocations resemble one another regarding the position of their breakpoints (fig. 1a). The breakpoints of the T70H reciprocal translocation are located in the R bands A4 and D1 of chromosomes 1 and 13, respectively (fig. 1a; de Boer and Gijsen, 1974). The T1Wa breakpoints are positioned either in the R bands 1C1.2 and 13D2.2 or in the R bands 1C2 and 13D1 (chapter 4). Both translocations are characterized by having a long and short marker chromosome. The region between the T70H and T1Wa breakpoints on chromosome 1 (Δ1) is about 10 cM and is positioned on the long T70H chromosome (13^{1}H) and the small T1Wa translocation chromosome (1^{13}Wa)(fig. 1). The position of the T1Wa breakpoint relative to the T70H breakpoint on chromosome 13 is unknown. Therefore, the segment between these breakpoints (Δ13) is located either on the long T1Wa (13^{1}Wa) and the short T70H (1^{13}H) translocation chromosomes or on the 13^{1}H and 1^{13}Wa translocation chromosomes (for the breakpoint orders: T70H - T1Wa - distal chr. 13 telomere and T1Wa - T70H - distal chr. 13 telomere respectively). The first possibility is represented in figs. 1 and 2. The Δ13 segment is much smaller than the Δ1 segment (fig. 1).

In mice, heterozygous for these two translocations, the two pairs of largely homologous translocation chromosomes (fig. 1b) form two differently sized heteromorphic bivalents during first meiotic prophase of both sexes (fig. 1c; Wauben-Penris *et al.*., 1983, de Boer *et al.*, 1986, Peters *et al.*, 1997b, chapter 3). During the zygotene and pachytene stages of meiotic prophase, the Δ1and Δ13 segments are incorporated into complete synaptonemal complexes (SCs) by non-homologous synapsis of the heteromorphic bivalents, irrespective from their chromosomal position (fig. 1d; Peters *et al.*, 1997b, chapter 3). During the zygotene and early pachytene stages, the axial/lateral elements of the two ectopically positioned homologous Δ1 segments were situated close towards one another in a minority of dried down spermatocytes and oocytes (Peters *et al.*, 1997b, chapter 3). Yet, at the later stages of meiotic prophase and at diakinesis in both sexes, quadrivalent configurations have never been encountered (Wauben-Penris et a., 1983, de Boer *et al.*, 1986, Peters *et al.*, 1997b, chapter 3). Thus, in T70H/T1Wa males and females, the segments between the breakpoints of the two translocations (Δ1 and Δ13) are excluded from meiotic crossing over. The occurrence of meiotic gene conversion can not be excluded, however.

Since 1979, the T1Wa translocation chromosomes have been transmitted for 25 generations in successive backcrosses between double heterozygous T70H/T1Wa females and T70H homozygous males which also were homozygous for the Rb(11.13)4Bnr Robertsonian translocation (Rb4) (Mat(ernal)+Rb stock; table 1; fig. 2b). Each new generation, the Rb4T70H homozygous fathers were obtained from a separate Rb4T70H stock (chapter 5).

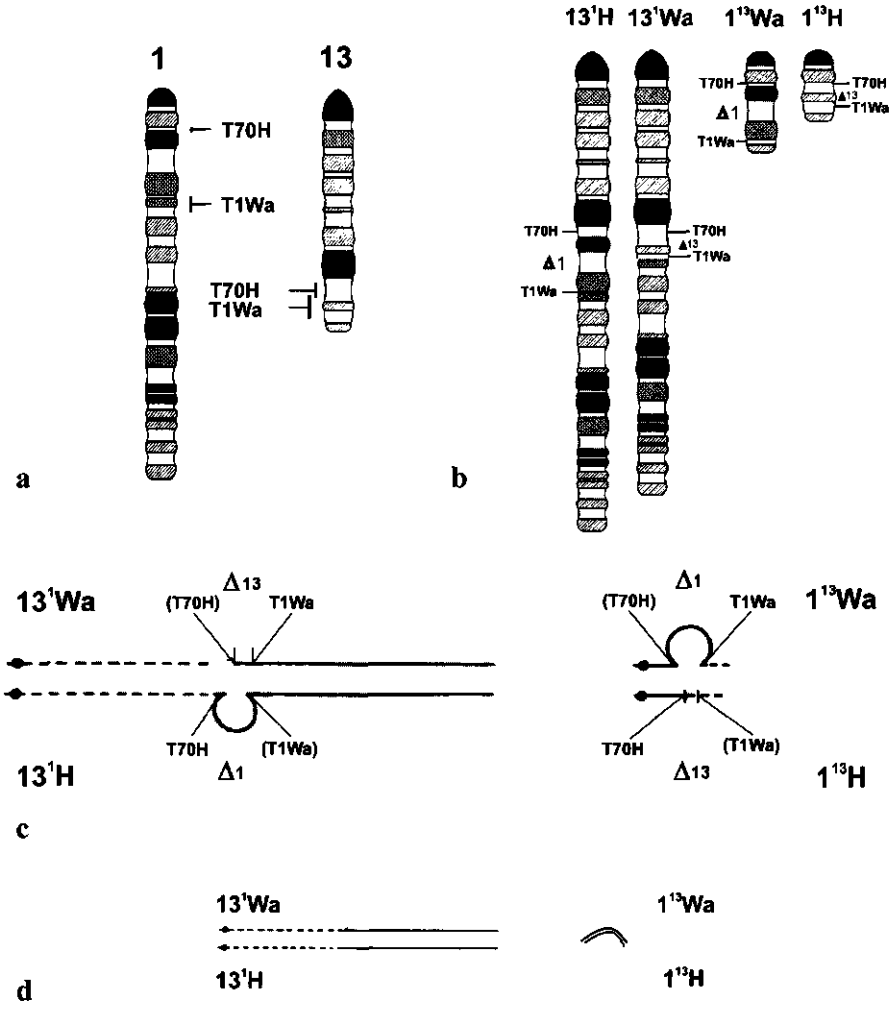

Figure 1: a Position of the T(1;13)70H and T(1;13)1Wa translocation breakpoints along the G-banded mitotic chromosomes 1 and 13. The breakpoints of T70H are located in the R bands 1A4 and 13D1 (de Boer and Gijsen, 1974). The T1Wa breakpoints are in the R bands 1C1.2 and 13D2.2 or in 1C2 and 13D1 (chapter 4). **b** T(1;13)70H and T(1;13)1Wa translocation chromosomes in which we arbitrarily positioned the T1Wa breakpoint distal of the T70H breakpoint on chromosome 13. The regions between the T70H and T1Wa breakpoints on chromosomes 1 and 13 are referred to as Δ1 and Δ13 respectively. **c** Schematic representation of homologous synapsis in the large and small heteromorphic bivalents of the T70H/T1Wa genotype in which the Δ1 and Δ13 segments between the T70H and T1Wa breakpoints are unsynapsed. The Δ1 segment is located on the large 13^{1}H and the small 1^{13}Wa translocation chromosomes. In fig. c, the position of the chromosome 13 T70H and T1Wa breakpoints is as in fig. b. The Δ13 segment is then localized on the large 13^{1}Wa and the small 1^{13}H translocation chromosomes. **d** Non-homologous synapsis of the Δ1 and Δ13 segments results in fully synapsed SC configurations.

Hence, the existence of this double heterozygous translocation breeding line enabled us to assess the question whether non-homologous synapsis in heteromorphic bivalents accompagnied by exclusion from crossing over of the Δ1 and Δ13 segments on the T1Wa translocation chromosomes during many successive backcross generations, leads to an increase in the vulnerability of these chromosomes for gaining dominant lethal mutations during non-homologous synapsis and/or to an accumulation of recessive lethal mutations.

To assay for dominant lethal mutations, we compared the postnatal survival of the double heterozygous progeny in which the T1Wa translocation chromosomes had either no, a one-generation or a multi-generation history of synapsis in heteromorphic bivalents during previous meioses. In order to find indication for recessive lethal mutations, we performed intercrosses between double heterozygotes from the 20th to 22nd backcross generations of the Mat+Rb stock and determined the survival rate of the offspring that were homozygous for the Rb4T1Wa translocation chromosomes relative to that of double heterozygous littermates. These assays revealed no indications for increased lethality of heterozygous or homozygous carriers of the Rb4T1Wa translocation chromosomes. In summary, our results suggest that non-homologous synapsis in heteromorphic bivalents in the absence of reciprocal recombination is not severely mutagenic.

Yet, karyotyping of the offspring produced in the backcrosses and intercross by the use of the microsatellites D1Mit4, D1Mit20, D1Mit121 and D1Mit122, located in the Δ1 segment, revealed a high rate of intergenerational instability for the D1Mit122 marker. The mutation frequency was significantly higher for chromosomes derived from the parental double heterozygous genotype. The mutations are thus likely of meiotic origin. On the basis of the identity of the mutations and the existence of chromatin contact between the ectopically positioned Δ1 segments (Peters et al., 1997b, chapter 3), we speculate that these mutations might be the results of ectopic homologous gene conversion events, most likely occurring in the absence of a SC.

Material and Methods.

Reciprocal and Robertsonian translocation stocks

Over 18 years the T(1;13)70H, T(1;13)1Wa and Rb(11.13)4Bnr translocation stocks have been maintained by crossing homozygous translocation (T/T) males with heterozygous translocation (T/+) females according to a family rotation scheme as described in chapter 5. The Rb(11.13)4Bnr-T(1;13)70H (abbreviation: Rb4T70H) stock has been maintained by crossing homozygous Rb4T70H males with Rb4T70H/Rb4+ females. From these crosses, male T/T offspring is selected by peripheral blood lymphocyte karyotyping (de Boer *et al.*,

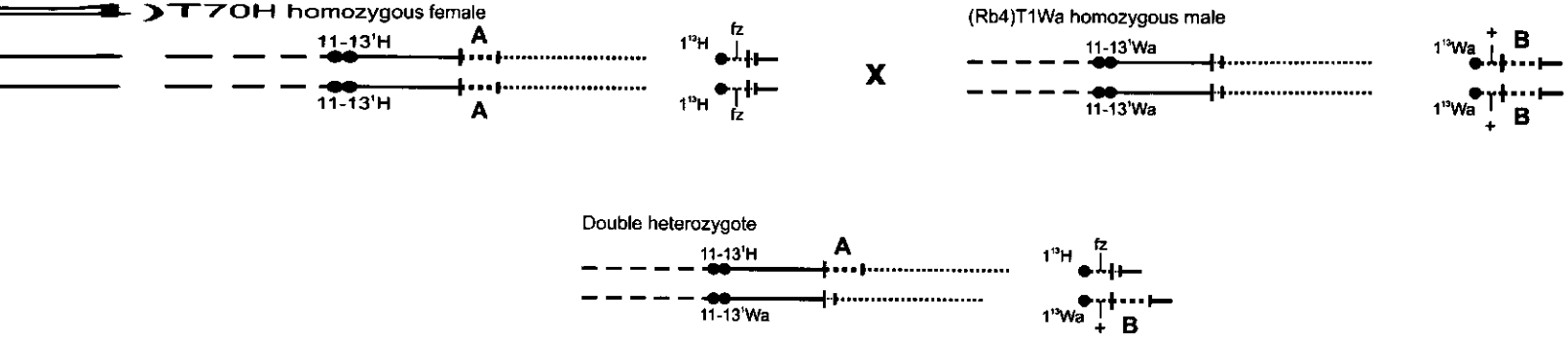

Figure 2: a Breeding of Rb4F1 double heterozygotes by homozygous Rb4T70H females and homozygous Rb4T1Wa males. The Rb4T70H homozygotes have a fuzzy hair coat as result of the recessive fuzzy allele (fz) located on the proximal chr. 1 end of the 1^{13}H chromosome. The 1^{13}Wa chromosome carries the wildtype allele. The Δ1 segments are situated on the large 13^{1}H and small 1^{13}Wa translocation chromosomes containing microsatellite haplotypes A and B respectively. This figure presents the situation in which the T1Wa breakpoint is distal of the T70H breakpoint on chromosome 13 (see fig. 1b). The Δ13 segments are then localized on the large 13^{1}Wa and small 1^{13}H translocation chromosomes (between two vertical bars representing the T70H and T1Wa breakpoint positions). The segments originating from chromosomes 1 and 11 are indicated by dotted and dashed lines respectively. Chromosome 13 segments are represented by a black line.

b Backcrosses between (Rb4)T70H/(Rb4)T1Wa double heterozygotes and (Rb4)T70H homozygotes produce four types of offspring. Figure **b** displays the situation in which chromosomes 11 are fused to chromosomes 13 due to the Robertsonian translocation Rb(11.13)4Bnr (Rb4). Karyotyping is performed by haplotyping with microsatellites positioned in the Δ1 segment as indicated (see also table 3) or by chromosomal karyotyping in combination with assessment of the phenotype (table 2). In the Mat+Rb and Pat+Rb stocks, most Rb4T70H homozygous fathers were homozygous with haplotype A. In the RbB1-C and RbB25-C crosses, Rb4T70H/Rb4T70H fathers, homozygous with haplotype C were used. (Rb4) double heterozygous parents originate from the Rb4F1 cross or from one of the successive backcross generations (table 1). (Rb4)T70H homozygous parents are produced by (Rb4)T70H homozygotes which originate from the "outcross" stocks (chapter 5).

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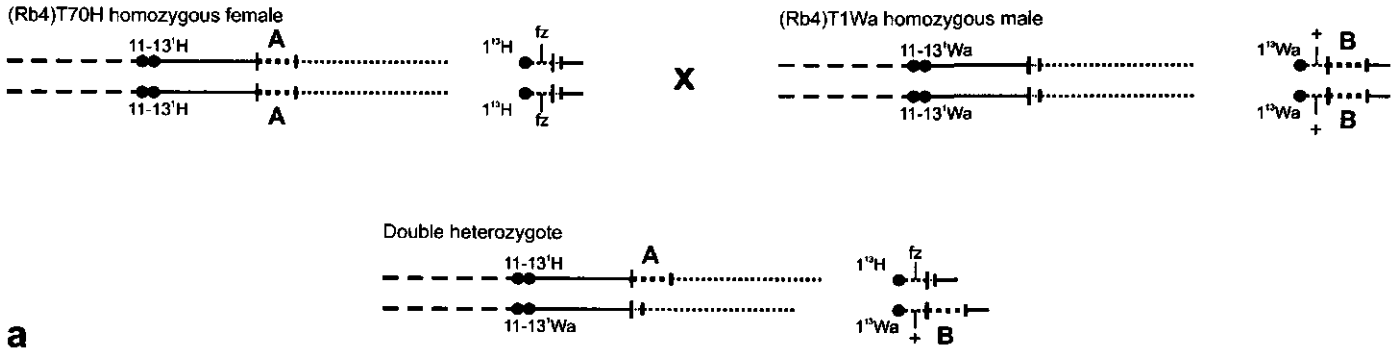

Figure 2: **a** Breeding of Rb4F1 double heterozygotes by homozygous Rb4T70H females and homozygous Rb4T1Wa males. The Rb4T70H homozygotes have a fuzzy hair coat as result of the recessive fuzzy allele (fz) located on the proximal chr. 1 end of the 1^{13}H chromosome. The 1^{13}Wa chromosome carries the wildtype allele. The Δ1 segments are situated on the large 13^{1}H and small 1^{13}Wa translocation chromosomes containing microsatellite haplotypes A and B respectively. This figure presents the situation in which the T1Wa breakpoint is distal of the T70H breakpoint on chromosome 13 (see fig. 1b). The Δ13 segments are then localized on the large 13^{1}Wa and small 1^{13}H translocation chromosomes (between two vertical bars representing the T70H and T1Wa breakpoint positions). The segments originating from chromosomes 1 and 11 are indicated by dotted and dashed lines respectively. Chromosome 13 segments are represented by a black line.

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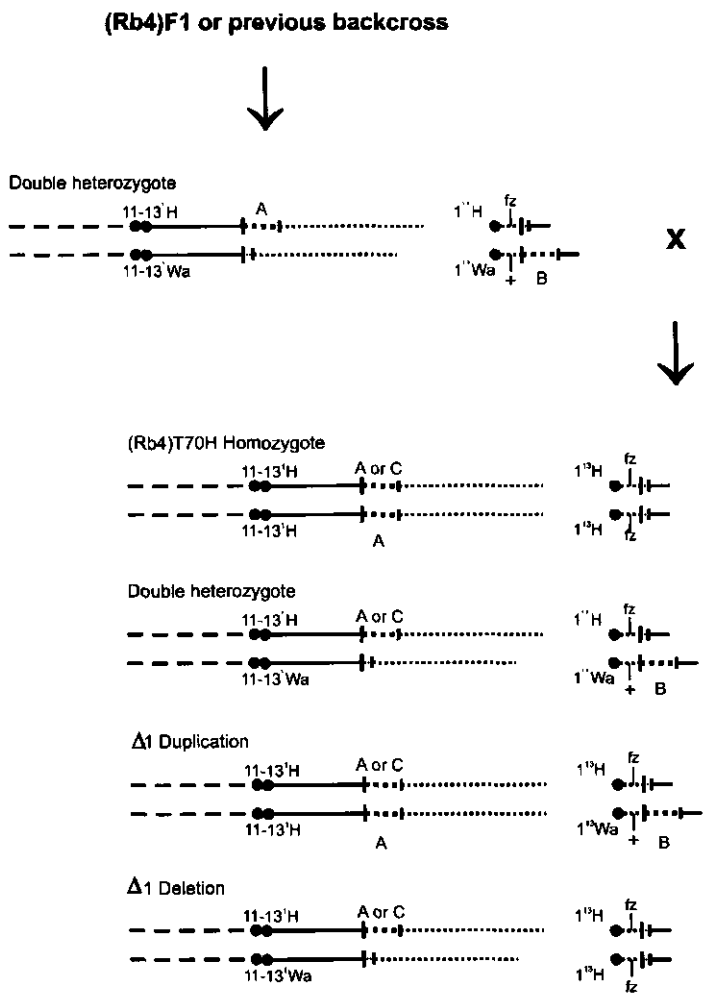

Phenotype	Haplotype in crosses: *RbB1-A* *RbB23-A*	*RbB1-C* *RbB25-C*
Fuzzy	A/A	C/A
Smooth	A/B	C/B
Smooth runted	A/A/B	C/A/B
Embryonic dead	A	C

1977) or an embryonic lethality test (Searle *et al.*, 1971). All types of T/+ females are generated by crossing T/T males with Swiss random bred +/+ (Cpb:SE(S)) females. Rb4T70H/Rb4+ females are obtained from crosses between Rb4T70H homozygous males and Rb4/Rb4 females.

In 1996, we generated mice homozygous for both Rb(11.13)4Bnr and T(1;13)1Wa (abbreviation: Rb4T1Wa) by the use of T1Wa and Rb4 homozygotes as founder animals. Rb4T1Wa homozygous males, used in the RbF1 cross (see below), were obtained from crosses between Rb4T1Wa/Rb4+ mice and from crosses between Rb4T1Wa homozygotes.

Description of different backcross stocks.

We determined the pre- and postnatal viability and death rates of the different genotypes produced in one RbF1 cross (see below) and several backcrosses which are shown in table 1. In most crosses, parents were also homozygous for the Robertsonian translocation Rb(11.13)4Bnr. The Rb4T1Wa translocation chromosomes have been transmitted for 25 generations in backcrosses between double heterozygous females and Rb4T70H homozygous males (Mat+Rb stock, table 1). During the first 19 generations of the Mat+Rb stock the Rb4T1Wa genome was transmitted by successive backcrosses of Rb4T70H/Rb4T1Wa females with homozygous Rb4T70H males (fig. 2b). From backcross generation 19 onwards (RbB19), the (Rb4)T1Wa genome has been transmitted in three different ways (table 1): maternally (Mat+Rb) and paternally (Pat+Rb) in the presence of Rb4 and maternally (Mat-Rb) in the absence of Rb4. The Mat-Rb stock was derived from the Mat+Rb stock by removal of Rb4 in two successive backcrosses with T70H homozygous males (start at the RbB18 generation).

Meiotic chromosome segregation in the (Rb4)T70H/(Rb4)T1Wa double heterozygous karyotype

Crosses between female Rb4T70H and male Rb4T1Wa homozygotes produced the "RbF1" double heterozygous offspring (fig. 2a). Backcrosses between (Rb4)T70H/(Rb4)T1Wa double heterozygotes and (Rb4)T70H homozygotes produce four types of offspring (fig. 2b) as the result of random assortment of the four different translocation chromosomes from the two heteromophic bivalents present at metaphase I (Wauben-Penris *et al.*, 1983). For simplicity, fig. 2 presents the situation in which the T1Wa breakpoint is distal of the T70H breakpoint on chromosome 13 and the Δ13 segment is localized on the large 13^{1}Wa and the small 1^{13}H translocation chromosomes (fig. 2; see introduction). Segregants with the parental genotypes are viable and can be distinguished from each other by their phenotype. The (Rb4)T70H homozygotes have a fuzzy hair coat as result of the recessive fuzzy allele (fz/fz, Dickie and Woolley, 1950) in the proximal chromosome 1 region of the 1^{13}H chromosome (figs. 2, 5; Searle *et al.*, 1971). The wildtype allele (+) on the 1^{13}Wa chromosome is closely linked to the

Table 1: Bird's eye view of names and characteristics of the F1 crosses and the backcross stocks.

Name of Cross or Stock	*Backcross Generation of Cross*	*Year of Breeding*	*Parental and Offspring Karyotypes*		
			Mother	*Father*	*Offspring*
RbF1	-	1995	Rb4T70H/Rb4T70H	Rb4T1Wa/Rb4T1Wa	Rb4T70H/Rb4T1Wa
RbB1	RbB1	1996	Rb4T70H/Rb4T1Wa	Rb4T70H/Rb4T70H	4 types[1]
Backcross stocks:					
Mat+Rb[3]	RbB1-RbB26	1979-1996	Rb4T70H/Rb4T1Wa	Rb4T70H/Rb4T70H	4 types[1]
Pat+Rb[4]	RbB20-RbB24	1992-1996	Rb4T70H/Rb4T70H	Rb4T70H/Rb4T1Wa	4 types[1]
Mat-Rb[5]	B20-B27	1992-1996	T70H/T1Wa	T70H/T70H	4 types[1]
Intercross	RbB20 - RbB22	1992-1993	Rb4T70H/Rb4T1Wa	Rb4T70H/Rb4T1Wa	9 types[2]

1: Due to random assortment of the 4 translocation chromosomes of the double heterozygous parent, 4 types of offspring are produced: see figure 2b
2: Due to random assortment of the 4 translocation chromosomes of both double heterozygous parents, 9 types of offspring are produced: see figure 7
3: Maternal transmission of T1Wa translocation chromosomes in the presence of Rb4Bnr
4: Paternal transmission of T1Wa translocation chromosomes in the presence of Rb4Bnr
5: Maternal transmission of T1Wa translocation chromosomes in the absence of Rb4Bnr

segment between the T70H and T1Wa breakpoints and causes a smooth hair phenotype (figs. 2, 5). Duplication mice, which are trisomic for the Δ1 segment and monosomic (or trisomic in case the T70H breakpoint is distal from the T1Wa breakpoint) for the small Δ13 segment, have a low viability and have a runted smooth-haired phenotype (figs. 2, 4). Deletion mice, which are monosomic for the Δ1 segment and trisomic (or monosomic) for the small Δ13 segment die prenatally (fig. 2; de Boer *et al.*, 1986; Peters *et al.*, 1997b, chapter 3).

Description of the Mat+Rb stock.

In 1979, the Mat+Rb stock was founded by crossing T1Wa/+ males with homozygous Rb4T70H females (fig. 3). Subsequently, double heterozygous Rb4T70H/+T1Wa females (checked by analysis of peripheral blood lymphocyte chromosomes (de Boer *et al.*, 1977)) were mated with Rb4T70H/Rb4T70H males to produce the first backcross (1980-RbB1) generation (fig. 3). On the basis of genotype analysis, smooth female offspring, homozygous for Rb4 and with the differently sized 1^{13}Wa and 1^{13}H chromosomes (fig. 3) was selected to produce the second backcross generation. Likewise, during the subsequent 17 generations of the Mat+Rb stock, smooth offspring used for successive backcrosses was only checked for the presence of the small 1^{13}Wa chromosome by chromosome karyotyping in order to select against crossovers between the fz locus and the T70H breakpoint in the proximal part of the small heteromorphic bivalent (figs. 2, 3).

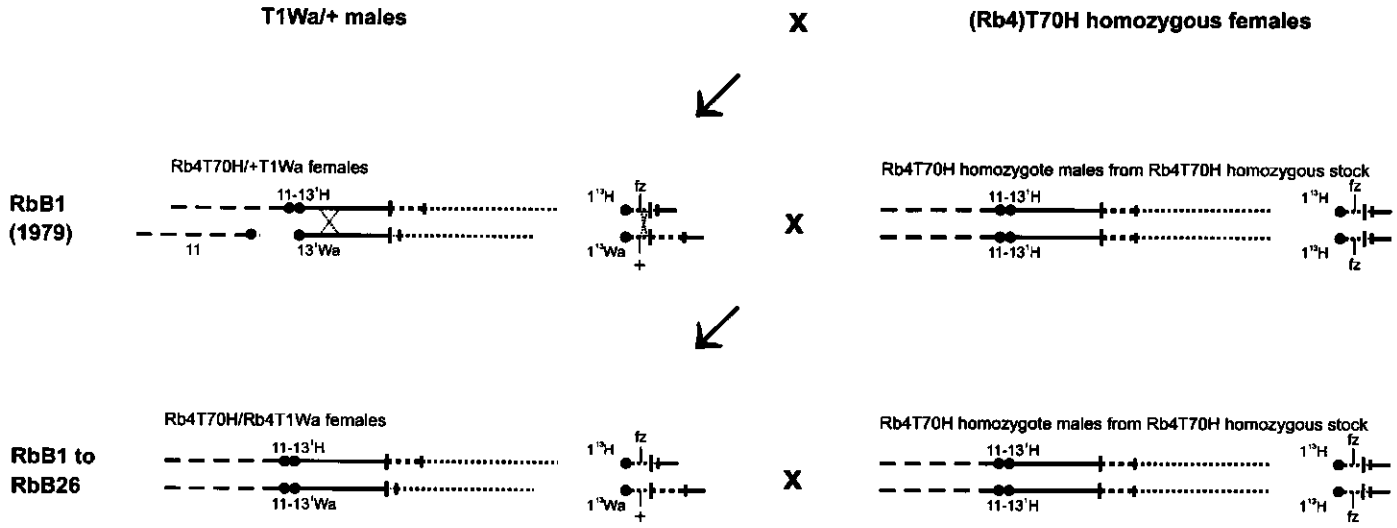

Figure 3: Chromosomal history of the Mat+Rb stock as described in the tekst. The offspring of the RbB1-RbB19 generations of the Mat+Rb stock is most likely only produced by double heterozygous females though no cytological selection was performed for the presence of the 11.13^{1}Wa chromosome during these generations. Breeding females were only checked for the presence of the 1^{13}Wa chromosome in order to select against crossovers in the region between the fuzzy locus and the T70H breakpoint (dotted cross in small heteromorphic bivalent). Chromosome 11 was attached to the 13^{1}Wa chromosome by a crossover in the chr. 13 segment of the 13^{1} bivalent during meiosis of the RbF1 female.

Table 2: Genotyping by a combination of methods.

Backcross Offspring[1]	Karyotype[2]	Phenotype	G-Band Analysis[3]	Normal karyotype analysis		Postnatal Viability[6]	Bodyweight at Days 28 and 42[6]
				13^{1} chromosomes[4]	1^{13} chromosomes[5]		
(Rb4)T70H Homozygotes	$13^{1}H$; $13^{1}H$; $1^{13}H$; $1^{13}H$	fuzzy	+	large, large	small, small	normal	normal
Double Heterozygotes (Rb4)T70H/(Rb4)T1Wa	$13^{1}H$; $13^{1}Wa$; $1^{13}H$; $1^{13}Wa$	smooth	+	large, small	small, large	normal	normal
Duplication Mice	$13^{1}H$; $13^{1}H$; $1^{13}H$; $1^{13}Wa$	smooth runted	+	large, large	small, large	reduced	± 60% reduced

1: See figure 1a
2: For clarity, chromosomes 11 which are fused to the 13^{1} translocation chromosomes in the Rb containing karyotypes are not depicted.
3: The G-band karyotype of the $13^{1}H$ and the $1^{13}Wa$ chromosomes show two large G-bands (1A5 and 1C1) in the middle region of both chromosomes. These bands are absent from the $13^{1}Wa$ and the $1^{13}H$ chromosomes. (see fig. 1b of chapter 4). The "+" indicates that the karyotype can be determined by G-banding.
4: Mitotic length of the $13^{1}Wa$ is 87.6% of the $13^{1}H$ chromosome (see comment #3).
5: Mitotic length of the $1^{13}H$ is 50% of the $1^{13}Wa$ chromosome (see comment #3).
6: Unpublished observations

Genotyping of backcross and intercross progeny.

Determination of the pre- and postnatal viability and death rates of the different genotypes produced in the successive backcross generations of the Mat+Rb (RbB20-RbB26), Pat+Rb (RbB20-RbB24) and Mat-Rb (B20-B27) stocks was based on phenotype analysis. Because the recombination frequency between the fuzzy locus and the T70H breakpoint is about 5% only (data not shown), we estimated the percentages of Rb4T70H homozygotes and Rb4T70H/Rb4T1Wa double heterozygotes on the basis of their fuzzy and smooth hair coat at adulthood and on the basis of their curled or smooth whiskers at day 5 after birth (table 2). Variation in body weight among smooth offspring was visible at day 5 after birth. Postnatal death rate of each genotype was calculated from the difference in numbers of mice with a specific phenotype at days 5 and 20. Mice used for backcrossing were karyotyped by analysis of either the length of the different translocation chromosomes in chromosome preparations of peripheral blood lymphocytes (de Boer *et al.*, 1977) or of their G-band karyograms (table 2).

Offspring, produced in the RbB1, RbB23, RbB25 backcrosses and the intercross between RbB20-RbB22 double heterozygotes of the Mat+Rb stock was genotyped at 20 to 30 days of age by segregation analysis of 4 (or 3 for the intercross) polymorphic microsatellites as described in chapter 4 (see figs. 2, 8). In an earlier study we determined the order of these microsatellites in the Δ1 segment as follows: T70H - D1Mit4 - D1Mit20 - D1Mit121 - D1Mit122 - T1Wa (chapter 5). Previously we indirectly showed that all four microsatellites of haplotype A had retained alleles of the chromosome 1 in which the T70H translocation was produced (table 3; chapter 5). Haplotype C is a recombinant of the ancestral haplotype A and the Swiss random bred background stock (chapter 5) as it contains "Swiss" alleles for the D1Mit121 and D1Mit122 microsatellites (table 3). The small 1^{13}Wa chromosome of all double heterozygous parents of the RbB20-RbB26 generations of the Mat+Rb and Pat+Rb stocks carried haplotype B (table 3; fig. 2b). Most of these parents had a large Rb4-T70H chromosome (11.13^{1}H) with haplotype A. Similarly, most Rb4T70H/Rb4T70H parents were homozygous for haplotype A. As the Δ1 segmenten are almost completely excluded from crossing over in the double heterozygous karyotype (see below) and meiotic recombination is suppressed distal of the T70H breakpoint upto D1Mit122 in the outcross stock of Rb4T70H (see chapter 5 for haplotype A), most likely all double heterozygous genotypes were also heterozygous with haplotypes A and B in the RbB1 to RbB19 generations of the Mat+Rb stock. Some parents of the RbB20-RbB22 generations carried haplotype F on one of their 11.13^{1}H chromosomes (table 3). In the RbB1-C and RbB25-C backcrosses, double heterozygous A/B females were mated with Rb4T70H/Rb4T70H males homozygous for haplotype C to be able to indisputably determine the genotype of the offspring (table 3).

Table 3a: Microsatellite alleles of D1Mit4, -20, -121 and -122 belonging to haplotypes A, B, C and F.

Translocation Genome	*Haplotype*	*Microsatellite Alleles*[1]			
		D1Mit4	*D1Mit20*	*D1Mit121*	*D1Mit122*
Rb4T70H	A	1	1	1	1
Rb4T1Wa	B	2	2	3	2
Rb4T70H	C[2]	1	1	2	3
Rb4T70H	F[2]	1	1	1	2

1: The number code of a microsatellite allele is inversely related to the length of the PCR product.
2: Haplotypes C and F are recombinational products of the ancestral haplotype A and the wild type Swiss genome; see chapter 5

Table 3b: Repeat sequence of microsatellites D1Mit4, -20, -121 and -122[1]

Microsatellite	*Position on genetic map of chr.1 (cM)*	*Sequence of repeat*
D1Mit4	9.2	-$(TG)_{19}$-AGAN-$(AG)_5$-NG-$(AG)_7$-
D1Mit20	17	-$(TG)_8$-NGTGTA-$(TG)_{10}$-TA-$(TG)_2$-
D1Mit121	18.1	-$(TG)_{21}$-
D1Mit122	19.8	-$(TATC)_{14}$-

1: From: Mouse genome database of Whitehead/MIT centre for genome research (USA)

Littersizes

The littersize data of the different crosses and stocks refer to the number of live plus dead pubs from the first three pregnancies of a single female. Some "breeding pairs" of the Mat+Rb stock consisted of two double heterozygous females and one male. Accordingly, the littersize data of these stocks refer to the first 6 pregnancies, 3 pregnancies per female.

Measurement of body weight.

Body weights of all types of offspring produced in the RbF1 and RbB1 crosses as well as in the backcrosses of the Mat+Rb, Pat+Rb and Mat-Rb stocks from generation 20 onwards were measured at days 28 and 42 after birth to facilitate phenotypic analysis of the progeny.

Statistical analyses

Parameters were analysed by the χ^2 test or the Mann-Whitney U test as described by Siegel (1956). The difference in mutation frequency of the microsatellite D1Mit122 between the two groups of different genotypic origin of mutation was tested using the following test for small

chances. Because the numbers of mutations were low, while the sample sizes were not small, the parameters scored have been assumed to follow a poisson-distribution: with k1 and k2 the numbers of mutations of the two samples, with sizes N1 and N2 respectively, the expression k1/N (where N = k1 + k2) follows a binomial distribution with P = N1 / (N1 + N2), provided that the two small fractions are equal (H_0 hypothesis).

Results

The T1Wa translocation chromosomes have been transmitted by double heterozygous Rb4T70H/Rb4T1Wa females for 25 generations in the Mat+Rb stock..

During the first 19 backcross generations of the Mat+Rb stock, the identity of the large translocation chromosomes of the breeding females was not systematically determined. Therefore, no distinction was made between the double heterozygous or duplication genotype of the females used for backcrossing (see fig. 2). G-band karyotype analysis at the RbB15 and RbB19 generations and genotype analysis by microsatellite haplotyping (see below) which have continuously been performed since RbB19, revealed the presence of the large 11.13[1]Wa chromosome in all different "1[13]Wa pedigrees" of the Mat+Rb stock indicating the presence of the Rb4T1Wa translocation in the balanced form from the onset of this stock on (see figs. 3). This also indicates that at least the large majority of the offspring in the RbB1 to RbB18 generations was produced by double heterozygous and not by duplication females.

Table 4: Littersizes[1] of crosses between double heterozygotes and (Rb4)T70H homozygotes.

Stock	*Cross*			*Generation*	*Number of Breeding Pairs*	*Number of Offspring*	*Number of Litters*	*Littersize mean ± sd*
	Father		*Mother*					
Mat+Rb	RbH/RbH	x	RbH/RbWa	RbB_2-RbB_{26}	193	3017	546	5.56 ± 1.72
Mat+Rb	RbH/RbH	x	RbH/RbWa	RbB_2-RbB_{12}	66	1060	187	5.71 ± 1.52
Mat+Rb	RbH/RbH	x	RbH/RbWa	RbB_{13}-RbB_{19}	49	643	127	5.14 ± 2.07
Mat+Rb	RbH/RbH	x	RbH/RbWa	RbB_{20}-RbB_{26}	78	1314	232	5.71 ± 1.62
Pat+Rb	RbH/RbWa	x	RbH/RbH	RbB_{20}-RbB_{24}	27	429	77	5.55 ± 1.36
Mat-Rb	H/H	x	H/Wa	B_{20}-B_{27}	46	795	140	5.69 ± 1.60

H/H = T70H/T70H; H/Wa = T70H/T1Wa; RbH/RbH = Rb4BnrT70H/Rb4BnrT70H; RbH/RbWa = Rb4BnrT70H/Rb4BnrT1Wa

1: First three litters only were used for calculation

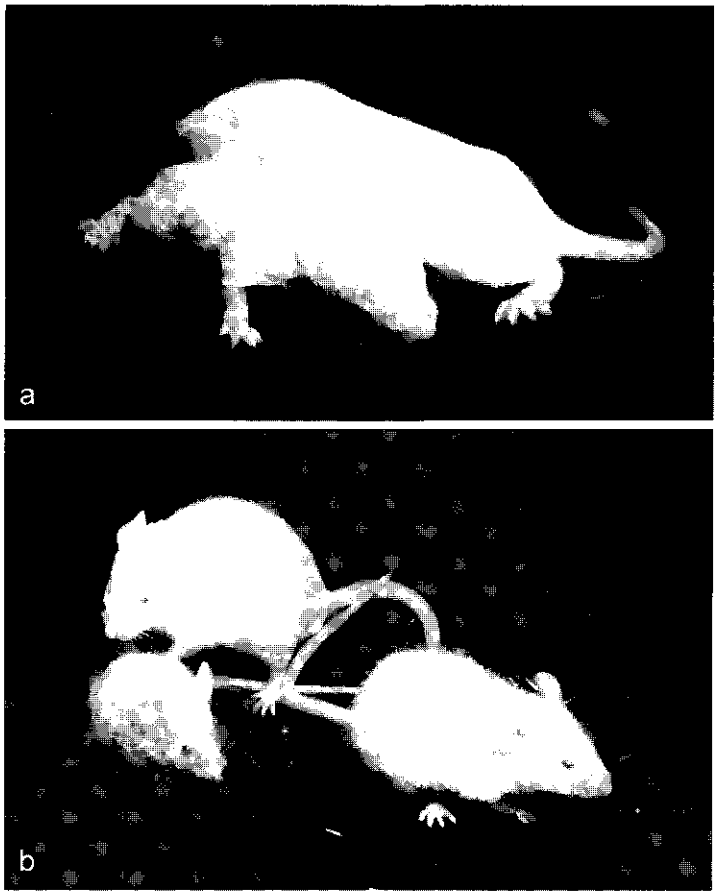

Figure 4: a Eight days old smooth double heterozygous (normal body size) and duplication (small body size) littermates. **b** Seven weeks old fuzzy Rb4T70H homozygous, smooth double heterozygous and smooth runted duplication littermates.

Development of duplication mice with a smooth runted phenotype from generation 20 onwards in the different backcross stocks.

During the first 19 backcross generations, the percentage of smooth offspring which was alive at weaning fluctuated around 50% (data not shown; see also table 6). From RbB20 onwards, smooth mice were born which were smaller (body weight is about 60% of the weight of balanced littermates at day 42; data not shown) and had an unhealthy appearance (tables 5, 6; fig. 4). This class of runted progeny had the duplication genotype as judged from G-band karyotyping (table 2), length measurements of the translocation chromosomes (table 2) or haplotype analysis (see below). In both the Pat+Rb and Mat-Rb stocks (see table 1), which are derived from the Mat+Rb stock, duplication mice also had a runted phenotype.

Postnatal death rate of progeny and fertility of double heterozygous mothers is variable over successive generations in the Mat+Rb stock.

Phenotype analysis at day 5 revealed a small excess of smooth double heterozygous offspring over fuzzy (Rb4)T70H homozygous progeny in all three backcross stocks from backcross generation 20 onwards (table 5). Compared with the situation at day 5, the ratio between smooth and fuzzy offspring was reduced in all three stocks at weaning (same backcross generations; table 6). The percentage of offspring that died before weaning was significantly lower in the first 12 backcross generations than in the last 7 generations of the Mat+Rb stock (table 6). As shown in table 7, the unbalanced duplication genotype contributes only minorly to the postnatal death rate between day 5 and weaning. The increased postnatal death rate can have different explanations of which two opposite extremes are given: if the 10% preweaning death during the first 12 generations and the 8.3% death between days 0-5 of the last 7 generations of the Mat+Rb stock are attributable to loss of duplication segregants, the postnatal death rates of the balanced segregants must have increased in the Mat+Rb stock. Alternatively, if the 10% dead mice of the first 12 generations is due to loss of balanced segregants only (no duplication mice born) and the 8.3% loss between days 0-5 of the last 7 generations of the Mat+Rb stock is caused by loss of duplication segregants, fertility of the double heterozygous females must have decreased in the Mat+Rb as the expected littersize would be 11.3 for the first 12 generations and 9.65 for the last 7 generations (table 5).

The high death rate of the double heterozygotes is intrinsic to the genotype

The death rates between day 5 and weaning of the different phenotypes is presented in table 8. In all three stocks significantly more smooth Rb4T70H/Rb4T1Wa than Rb4T70H homozygous mice died during days 5 to 20 (table 8). Analysis of the death rates at different backcross generations of the Mat+Rb stock confirmed this observation (fig. 5). The higher death rate of the double heterozygous versus the Rb4T70H homozygous segregants in the backcross stock could have two reasons:

(*i*) it is intrinsic to the double heterozygous genotype or

(*ii*) repeated transmission of the T1Wa translocation chromosomes through heteromorphic bivalents leads to an increase in the vulnerability of these chromosomes for gaining dominant lethal mutations during non-homologous synapsis within a heteromorphic bivalent.

To distinguish between these two possibilities, we compared the postnatal survival of the double heterozygous progeny in which the T1Wa translocation chromosomes had either no, a one-generation or a multi-generation history of non-homologous synapsis in heteromorphic bivalents during previous meioses.

Table 5: Offspring analysis at day 5 after birth according to phenotypic appearance (Mat+Rb, Pat+Rb and Mat-Rb backcross stocks).

						Offspring analysis at day 5 in %					
	Cross				*Littersize*	*total (n)*	*fuzzy (Rb4T70H/ Rb4T70H)*	*smooth (Rb4T70H/ Rb4T1Wa)*	*smooth runted (duplication)*	*death (between days 0-5)*	*ratio smooth: fuzzy*	*Expected Littersize*[1]
Stock	*Father*		*Mother*	*Generation*	*mean ± sd*							
Mat+Rb	RbH/RbH	x	RbH/RbWa	RbB20-RbB26	5.71 ± 1.62	1989	40.3	45.0	6.5	8.2	1.12	9.73
Pat+Rb	RbH/RbWa	x	RbH/RbH	RbB20-RbB24	5.55 ± 1.36	729	40.6	42.5	10.7	6.2	1.05	9.23
Mat-Rb	H/H	x	H/Wa	RbB20-RbB27	5.69 ± 1.60	1314	42.9	44.3	8.1	4.7	1.03	9.93

H/H = T70H/T70H; H/Wa = T70H/T1Wa; RbH/RbH = Rb4BnrT70H/Rb4BnrT70H; RbH/RbWa = Rb4BnrT70H/Rb4BnrT1Wa
1: Calculation of the expected littersize is based on the follwing assumption: the fuzzy and normal smooth segregants have only a low rate of embryonic and postnatal early death. Hence, the total number of these two segregants alive at day 5 represent about 50% of the litter.

Table 6: Offspring analysis at weaning according to phenotypic appearance (Mat+Rb, Pat+Rb and Mat-Rb backcross stocks).

		Offspring analysis at weaning (in %)					χ^2_1		
		total (n)	*fuzzy (Rb4T70H/ Rb4T70H)*	*smooth (Rb4T70H/ Rb4T1Wa)*	*smooth runted (duplication)*	*death (between days 0-20)*	*(versus B_2--B_{12} generation)*		*ratio smooth: fuzzy*
Stock	*Generation*								
Mat+Rb	RbB2-RbB12	2046	44.9	45.1	0	10.0			1.004
Mat+Rb	RbB20-RbB26	1989	37.4	40.1	5.5	17.0	41.52	P<0.0005	1.07
Pat+Rb	RbB20-RbB24	729	38.7	36.6	8.4	16.3	20.10	P<0.0005	0.95
Mat-Rb	RbB20-RbB27	1314	40.3	38.9	7.6	13.2	7.98	P<0.0005	0.97

Table 7: Distribution of postnatal death over different phenotypes in the Mat+Rb, Pat+Rb and Mat-Rb backcross stocks.

		% death between day 0 and weaning	*% death*	*% death between day 5 and weaning according to phenotype*		
Stock	*Generation*	*(N mice born)*	*between days 0-5*	*fuzzy*	*smooth*	*smooth runted*
Mat+Rb	RbB2-RbB12	10 (2046)	nd	nd	nd	nd
Mat+Rb	RbB20-RbB26	17.0 (1989)	8.3	2.9	4.9	1.0
Pat+Rb	RbB20-RbB24	16.3 (729)	6.2	1.9	5.9	2.3
Mat-Rb	RbB20-RbB27	13.2 (1314)	4.7	2.6	5.4	0.5

nd = not determined

Table 8: Postnatal death rates (between day 5 and weaning) of different phenotypes in progeny of the Mat+Rb, Pat+Rb and Mat-Rb backcross stocks.

Stock	*Generation*	*Death Rate (%) between Day 5 and Weaning per Phenotype (N at day 5)*		
		Fuzzy (Rb4T70H/Rb4T70H)	*Smooth (Rb4T70H/Rb4T1Wa)*	*Smooth Runted (Duplication)*
Mat+Rb	RbB20-RbB26	7.1 (801) [1]	10.9 (894) [1]	15.4 (130)
Pat+Rb	RbB20-RbB24	4.7 (296) [2]	13.9 (310) [2]	21.8 (78)
Mat-Rb	B20-B27	6.2 (564) [3]	12.2 (582) [3]	5.7 (106)

1: $\chi^2_1 = 6.69$, $P < 0.005$ 2: $\chi^2_1 = 13.79$, $P < 0.0005$ 3: $\chi^2_1 = 11.55$, $P < 0.0005$

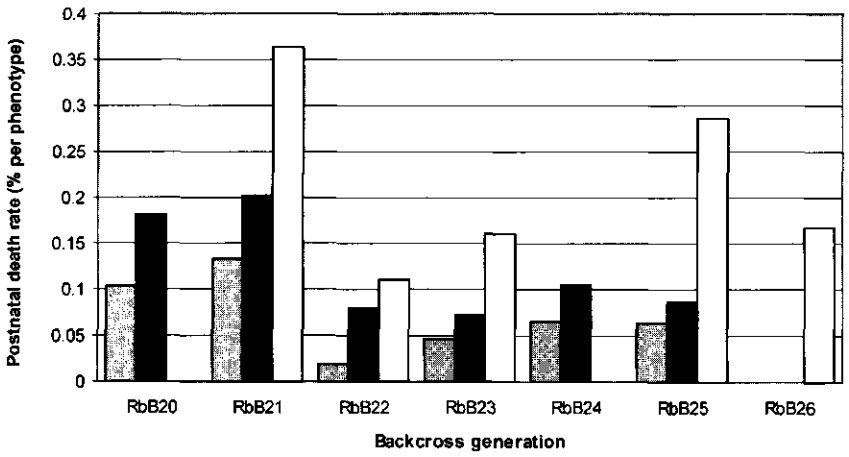

Figure 5: Postnatal death rates (between day 5 and weaning) of the different progeny phenotypes of successive backcross generations of the Mat+Rb stock (in % per phenotype). Fuzzy Rb4T70H homozygotes are represented by grey bars; smooth double heterozygotes and smooth runted duplication mice are represented by black and white bars respectively.

Double heterozygotes (RbF1) that had no history of synapsis in heteromorphic bivalents were produced in crosses between Rb4T70H homozygous females and Rb4T1Wa homozygous males (table 1). Double heterozygotes (RbB1) carrying Rb4T1Wa chromosomes which had a one-generation history of non-homologous synapsis in heteromorphic bivalents were produced in crosses between RbF1 females and Rb4T70H homozygous males (table 1). Finally, double heterozygotes (RbB23 and RbB25) carrying T1Wa chromosomes which had a multi-generation history of non-homologous synapsis in heteromorphic bivalents were produced in crosses between Rb4T70H homozygous males and double heterozygous females of the Mat+Rb stock (RbB22 and RbB24 generations).

All crosses were set up to allow karyotyping of the progeny by haplotype analysis as shown in fig. 2b. The parents of the RbF1 cross and consequently the double heterozygous mothers of both RbB1-A and RbB1-C crosses (see below) carried haplotypes A and B on their large 11-13^{1}H and small 1^{13}Wa translocation chromosomes respectively (figs. 2a, 2b; table 3). Similarly, double heterozygous mothers from the Mat+Rb stock were also heterozygous for haplotypes A and B (fig. 2b; table 3). Backcrosses utilized two types of Rb4T70H homozygous males:

(a) RbB1-A and RbB23-A backcrosses were performed with Rb4T70H/Rb4T70H males which were homozygous for haplotype A (fig. 2b; table 3). In crosses (a), not all fuzzy and smooth offspring was genotyped. The genotype of most smooth runted mice was determined by haplotyping to confirm the duplication genotype.

(b) RbB1-C and RbB25-C backcrosses were performed with Rb4T70H/Rb4T70H males, homozygous for haplotype C (fig. 2b; table 3). All offspring of crosses (b) were genotyped by haplotype analysis shortly after weaning (age of mice: 20-30 days).

Comparison of the littersizes of the RbB1-A x RbB23-A and RbB1-C x RbB25-C crosses revealed no significant differences (table 9). Thus, repeated transmission of the T1Wa translocation chromosomes via heteromorphic bivalents has no deleterious effect on prenatal survival of the offspring in comparison with one such transmission.

The average littersize of the haplotype C breeding pairs was slightly larger compared with the haplotype A breeding pairs (not significant; table 9). The littersizes of all four backcrosses were slightly larger compared with the littersize of all breeding pairs of the RbB20-RbB26 generations in the Mat+Rb stock (tables 4, 9) despite the fact hat the RbB23-A cross had the same haplotype A as most breeding pairs of the Mat+Rb stock.

Finally, considering that the littersize of the double heterozygous females is about 40% reduced due to prenatal death of the duplication and deletion genotypes (see fig. 2), double heterozygous females have a larger expected littersize compared with the observed littersizes of the Rb4T70H homozygous females used in the RbF1 and control crosses (table 9).

Table 9: Littersizes[a] of RbF1-A, RbB1-A, RbB23-A, RbB1-C and RbB25-C backcrosses.

Stock	*Cross*			*N*	*N*	*N*	*Littersize*
	Father		*Mother*	*Females*	*Progeny*	*Litters*	*mean ± sd*
Haplotype A:							
RbF1-A	RbWa/RbWa	x	RbH/RbH	11	225	25	9.17 ± 1.54
RbB1-A	RbH/RbH	x	RbH/RbWa	11	210	31	6.68 ± 1.00
RbB23-A	RbH/RbH	x	RbH/RbWa	11	192	30	6.64 ± 1.04
Control-A	RbH/RbH	x	RbH/RbH	8	129	18	7.12 ± 1.71
Haplotype C:							
RbB1-C	RbH/RbH	x	RbH/RbWa	10	199	27	7.30 ± 1.71
RbB25-C	RbH/RbH	x	RbH/RbWa	17	313	46	7.04 ± 1.01
Control-C	RbH/RbH	x	RbH/RbH	5	99	12	8.02 ± 1.91

RbH/RbH = Rb4BnrT70H/Rb4BnrT70H; RbH/RbWa = Rb4BnrT70H/Rb4BnrT1Wa;
RbWa/RbWa = Rb4BnrT1Wa/Rb4BnrT1Wa
a: Only first three litters of breeding pairs were used for calculation.

Phenotype analysis at day 5 (with subsequent confirmation of the genotype by haplotype analysis) revealed no significant differences in the percentages of Rb4T70H homozygous, double heterozygous and duplication segregants between either groups with different backcross history or groups with different haplotypes (table 10). However, the percentage of pubs dying early (between days 0-5) was significantly higher in both haplotype C crosses compared with haplotype A crosses which correlates with the slightly larger littersize of the haplotype C breeding pairs. Most likely, these early dying pubs have the duplication genotype because the expected littersize is similar for the groups with different haplotypes under this assumption (table 10). Interestingly, the expected littersizes of the haplotype A groups (RbB1-A and RbB23-A) were larger than the expected littersize of the RbB20-RbB26 generations of the Mat+Rb stock (tables 5, 10). This results from a larger littersize (about 6.6 versus 5.7; tables 10 versus 3) and a smaller postnatal preweaning death rate (about 10.6% versus 17%; $\chi^2_1 = 15.3$, $P < 0.0005$, tables 11 versus 6) for the RbB1-A and RbB23-A haplotype A groups versus the Mat+Rb stock.

Comparison of the postnatal death rates of the Rb4T70H/Rb4T1Wa mice produced in either the RbF1 crosses or any of the backcrosses revealed no significant differences (table 11). Similarly, no significant differences were found for the postnatal survival of the Rb4T70H homozygotes of the different crosses. Only a small trend is visible: less Rb4T70H/Rb4T70H mice heterozygous for haplotypes A and C (produced in haplotype C crosses) die compared with Rb4T70H homozygotes with haplotypes A/A (produced in

Table 10: Offspring analysis at day 5 after birth according to phenotypic appearance in type-A crosses and by haplotyping[1] in type-C crosses.

						Offspring analysis at day 5 (in %)					
Cross Code	*Cross Father*		*Mother*	*Littersize mean ± sd*	*total*	*fuzzy Rb4T70H/ Rb4T70H*	*smooth Rb4T70H/ Rb4T1Wa*	*smooth runted duplication*	*death (between days 0-5)*	*ratio smooth: fuzzy*	*Expected Littersize*
Haplotype A:											
RbF1-A	RbWa/RbWa	x	RbH/RbH	9.17 ± 1.54	252	-	99.6	-	0.4		
RbB1-A	RbH/RbH	x	RbH/RbWa	6.68 ± 1.0	374	39.8	46.8	9.6	3.8 [1]	1.18	11.57
RbB23-A	RbH/RbH	x	RbH/RbWa	6.64 ± 1.04	289	44.3	42.5	8.7	4.5 [1]	0.96	11.53
Control	RbH/RbH	x	RbH/RbH	7.12 ± 1.71	129	100	-	-	-		
Haplotype C:											
RbB1-C	RbH/RbH	x	RbH/RbWa	7.30 ± 1.71	318	39.6	39.0	7.6	13.8 [1]	0.98	11.48
RbB25-C	RbH/RbH	x	RbH/RbWa	7.04 ± 1.01	409	36.4	44.2	9.0	9.8 [1]	1.21	11.43
Control	RbH/RbH	x	RbH/RbH	8.02	99	97			3		

RbH/RbH = Rb4BnrT70H/Rb4BnrT70H; RbH/RbWa = Rb4BnrT70H/Rb4BnrT1Wa; RbWa/RbWa = Rb4BnrT1Wa/Rb4BnrT1Wa
1: Haplotyping of progeny was performed at days 20-30. Karyotype of mice died between day 5 and haplotyping was determined on basis of phenotype. Totals at day 5 is sum of both groups of mice.
2: $\chi^2_1 = 25.41$ (RbB_1-A + RbB_{23}-A versus RbB_1-C + RbB_{25}-C)

Table 11: Postnatal death rates between day 5 and weaning of different phenotypes in progeny of RbF1, RbB1, RbB23 and RbB25 crosses.

					Death rate between day 5 and weaning per phenotype (total mice per phenotype)		
Cross Code	*Cross Father*		*Mother*	*% death durng days 0-20 (of total mice born)*	*fuzzy (Rb4T70H/ Rb4T70H)*	*smooth (Rb4T70H/ Rb4T1Wa)*	*smooth runted (duplication)*
Haplotype A:							
RbF1-A	RbWa/RbWa	x	RbH/RbH	5.95 (252)	-	5.6 (251)	-
RbB1-A	RbH/RbH	x	RbH/RbWa	10.2 (374)	4.0 (149) [1]	6.9 (175) [1]	16.7 (39)
RbB23-A	RbH/RbH	x	RbH/RbWa	11.1 (289)	4.7 (128) [1]	7.3 (123) [1]	16.0 (25)
Control	RbH/RbH	x	RbH/RbH	10.9 (129)	10.9 (129)	-	-
Haplotype C:							
RbB1-C	RbH/RbH	x	RbH/RbWa	19.5 (318)	2.4 (126) [2]	9.7 (124)[2]	12.5 (24)
RbB25-C	RbH/RbH	x	RbH/RbWa	15.4 (409)	2.0 (149) [2]	10.9 (183)[2]	0.0 (37)
Control	RbH/RbH	x	RbH/RbH	7.1 (99)	4.2 (97)	-	-

RbH/RbH = Rb4BnrT70H/Rb4BnrT70H; RbH/RbWa = Rb4BnrT70H/Rb4BnrT1Wa; RbWa/RbWa = Rb4BnrT1Wa/Rb4BnrT1Wa

1: $\chi^2_1 = 1.48$ (fuzzy RbB_1-A + RbB_{23}-A versus smooth RbB_1-A + RbB_{23}-A)

2: $\chi^2_1 = 14.8$ (fuzzy RbB_1-C + RbB_{23}-C versus smooth RbB_1-C + RbB_{23}-C)

haplotype A crosses; table 11). In addition, in all backcrosses more double heterozygous segregants died between day 5 and weaning than Rb4T70H homozygous segregants.

In conclusion, we observed no significant differences between the postnatal death rates of the double heterozygous genotypes produced either by homozygous parents or by double heterozygous females and Rb4T70H homozygous males. Yet, the postnatal death rate of the double heterozygous segregants is higher than that of Rb4T70H homozygous segregants. Thus, the synaptic history of the T1Wa translocation chromosome has no effect on the postnatal survival of the Rb4T70H/Rb4T1Wa genotype. The relatively high postnatal death rate is therefore an intrinsic characteristic of this genotype.

Assessment of accumulation of recessive lethal mutations.

The second goal of this study was to investigate whether exclusion from crossing over of the Δ1 and Δ13 segments on the T1Wa translocation chromosomes during the successive transmissions through heteromorphic bivalents results in an accumulation of recessive lethal mutations. To address this question we estimated the survival rate of Rb4T1Wa homozygotes in comparison to Rb4T70H/Rb4T1Wa littermates which were obtained from intercrosses between double heterozygotes from the 20th to 22nd backcross generation of the Mat+Rb stock.

In these intercrosses, 9 different genotypes are produced as a result of random assortment of the T70H and T1Wa translocation chromosomes from the heteromorphic bivalents of the double heterozygous parents (fig. 6; table 12).

	$13^{1}H, 1^{13}H$	$13^{1}Wa, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$	$13^{1}Wa, 1^{13}H$
$13^{1}H, 1^{13}H$	$13^{1}H, 1^{13}H$	$13^{1}Wa, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$
	$13^{1}H, 1^{13}H$	$13^{1}H, 1^{13}H$	$13^{1}H, 1^{13}Wa$	$13^{1}Wa, 1^{13}H$
$13^{1}Wa, 1^{13}Wa$	$13^{1}H, 1^{13}H$	$13^{1}Wa, 1^{13}Wa$	$13^{1}Wa, 1^{13}Wa$	$13^{1}Wa, 1^{13}Wa$
	$13^{1}Wa, 1^{13}Wa$	$13^{1}Wa, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$	$13^{1}Wa, 1^{13}H$
$13^{1}H, 1^{13}Wa$	$13^{1}H, 1^{13}H$	$13^{1}Wa, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$	$13^{1}Wa, 1^{13}H$
	$13^{1}H, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$
$13^{1}Wa, 1^{13}H$	$13^{1}H, 1^{13}H$	$13^{1}Wa, 1^{13}Wa$	$13^{1}H, 1^{13}Wa$	$13^{1}Wa, 1^{13}H$
	$13^{1}Wa, 1^{13}H$	$13^{1}Wa, 1^{13}H$	$13^{1}Wa, 1^{13}H$	$13^{1}Wa, 1^{13}H$

Figure 6: Random assortment of the translocation chromosomes from two double heterozygous parents (left column and top row) results in nine different offspring genotypes. Balanced karyotypes are depicted in bold. For clarity, the chromosomes 11 are omitted.

Table 12: Expected frequencies of offspring, generated in intercrosses between double heterozygotes.

Karyotype (fig. 7)	*Phenotype*	*Expected Frequency*	*Haplotype (fig. 8)*	*Number of Individual Segments*			*Name*	*Fitness*
				Δ1	*Δ13*[a]	*Δ13*[b]		
$13^{1}H, 1^{13}H, 13^{1}H, 1^{13}H$ [c]	fz	6.25	AA	2	2	2	Rb4T70H Homozygote	
$13^{1}H, 1^{13}H, 13^{1}Wa, 1^{13}Wa$	sm	25 [c]	AB	2	2	2	Double Heterozygote	
$13^{1}Wa, 1^{13}Wa, 13^{1}Wa, 1^{13}Wa$	sm	6.25	BB	2	2	2	Rb4T1Wa Homozygote	
$13^{1}H, 1^{13}H, 13^{1}H, 1^{13}Wa$	sm	12.5	AAB	3	1	3	Duplication	reduced
$13^{1}Wa, 1^{13}Wa, 13^{1}H, 1^{13}Wa$	sm	12.5	ABB	3	1	3	Duplication	dead [d]
$13^{1}H, 1^{13}H, 13^{1}Wa, 1^{13}H$ [c]	fz	12.5	A	1	3	1	Deletion	dead
$13^{1}Wa, 1^{13}Wa, 13^{1}Wa, 1^{13}H$	sm	12.5	B	1	3	1	Deletion	dead
$13^{1}H, 1^{13}Wa, 13^{1}H, 1^{13}Wa$	sm	6.25	AABB	4	0	4	-	dead
$13^{1}Wa, 1^{13}H, 13^{1}Wa, 1^{13}H$	fz	6.25	-	0	4	0	-	dead

a: If the T1Wa breakpoint is positioned distal of the T70H breakpoint on chromosome 1.
b: If the T70H breakpoint is positioned distal of the T1Wa breakpoint on chromosome 1.
c: 50% are complementation types.
d: In contrast to $13^{1}H, 1^{13}H, 13^{1}H, 1^{13}Wa$ duplication mice, $13^{1}Wa, 1^{13}Wa, 13^{1}H, 1^{13}Wa$ duplication mice are embryonic lethals suggesting a position effect

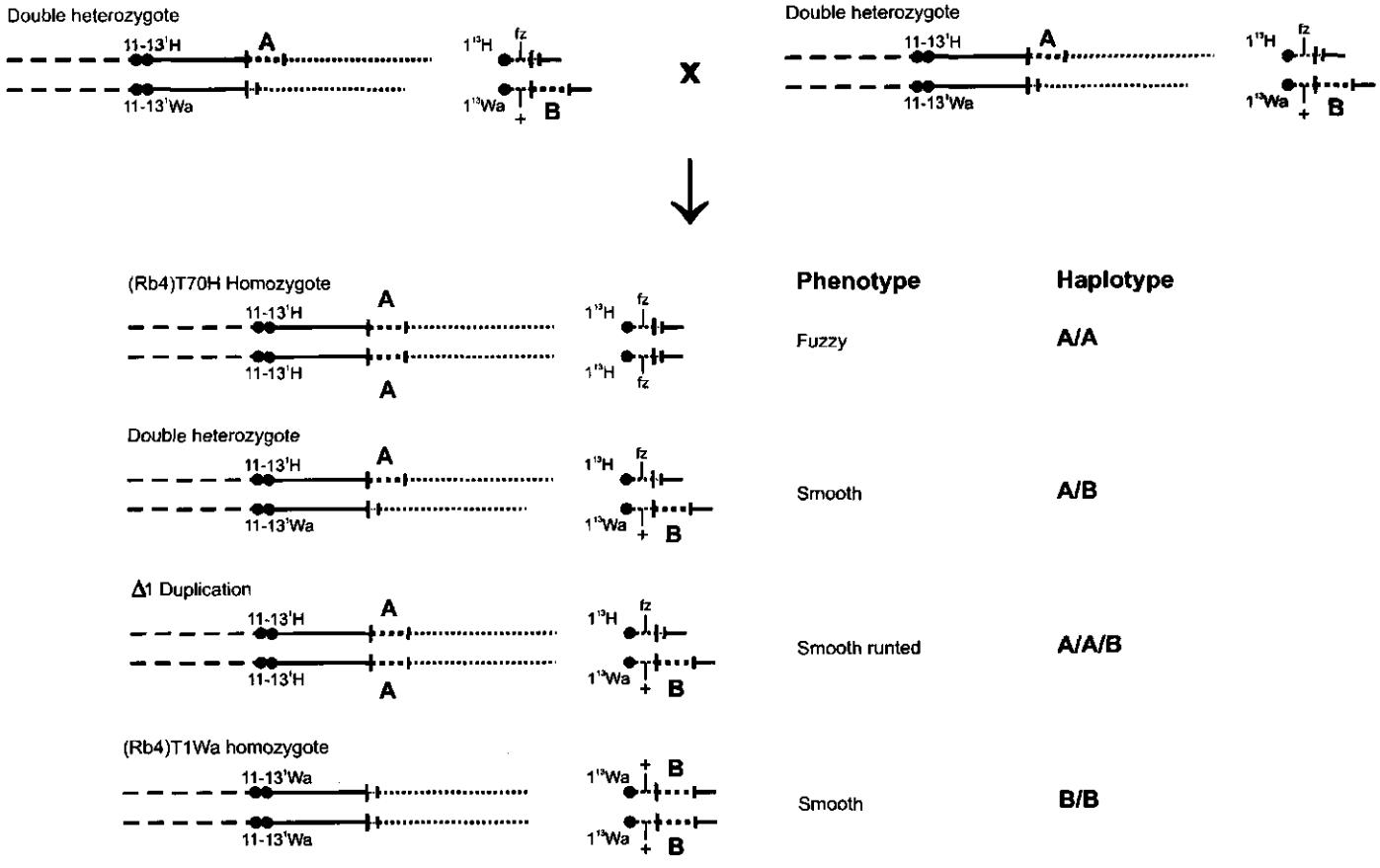

Figure 7: Haplotype analysis of viable progeny produced in intercrosses between double heterozygous parents. Rb4T1Wa homozygous offspring is easily identified due to homozygosity for haplotype B.

Table 13: Observed frequencies of offspring, generated in intercrosses between double heterozygotes.

At Day 5 after Birth		*After Weaning (Days 20-30 after Birth)*				
Phenotype	*Number of mice*	*Karyotype*	*N Mice karyotyped*	*N Mice died before karyotyping*	*Observed Numbers of Offspring*	*Expected[1] Numbers of Offspring*
Fuzzy	22	Rb4T70H/Rb4T70H	18	1	19	22.75
Smooth	105	Rb4T70H/Rb4T1Wa	82	9	91	91
		Rb4T1Wa/Rb4T1Wa	18		18	22.75
Smooth runted	8	Duplication	6	1	7	45.5
Dead	31	Unkaryotyped dead offspring:				
		Fuzzy	1	↑		
		Smooth	9	} → ┘ interpretation		
		Smooth runted	1			

1: See table 12 for expected frequencies of progeny.

First of all, we scored the phenotype of the progeny at day 5 after birth. Subsequently, progeny of these crosses was genotyped by analysis of the microsatellites D1Mit4, D1Mit20 and D1Mit122 (chapter 4; table 6). All Rb4T70H/Rb4T1Wa parents were heterozygous for haplotypes A and B (table 3) and could therefore be used for a haplotype progeny analysis as shown in fig. 7. As a control for the haplotypings, the identity of the small marker chromosomes (1^{13}H and 1^{13}Wa) was determined in chromosome preparations of peripheral blood lymphocytes of the majority of the offspring produced (de Boer *et al.*, 1977).

As shown in table 13, the phenotype of 135 of the 166 pubs born was determined at day 5 after birth. Subsequently, 11 mice died between day 5 after birth and genotyping. Yet, on the basis of their phenotype and the known estimate of the postnatal death rate of the double heterozygous genotypes (table 8), we included these mice into the final score (table 13). The observed littersize was 4.35 ± 1.64 (23 litters produced by 8 breeding pairs). If we assume that most of the mice that die between days 0-5 have an unbalanced genotype, the (expected) littersize of 9.54 is comparable with the one of the Mat+Rb stock (9.73; table 5). If the Rb4T1Wa homozygotes would have a normal viability, a 4:1 ratio is expected between the numbers of double heterozygous and the Rb4T1Wa homozygous littermates produced in intercrosses between double heterozygous parents (see table 12). As we found exactly this (table 13), we conclude that exclusion from crossing over of the Δ1 and Δ13 segments on the T1Wa translocation chromosomes during the successive transmissions through heteromorphic bivalents does not results in an accumulation of recessive lethal mutations.

Increased instability of D1Mit122 in the double heterozygous genotype

Fundamental to karyotyping with microsatellites is the stable intergenerational transmission of the repeat number of a microsatellite. However, among the offspring produced in several different backcrosses (table 10, other unpublished backcrosses) and the intercross, we observed changes in the repeat number of the tetranucleotide simple sequence repeat D1Mit122 (table 14, fig. 8). All mutations have been confirmed by use of a second different DNA isolation sample. In contrast, we observed no change in repeat number of any of the other three dinucleotide repeats D1Mit4, D1Mit20 and D1Mit121 (number of informative typings: 978, 831 and 767 respectively). Due to the use of different alleles of D1Mit122 for marking the different parental translocation chromosomes (see figs. 2b, 8) we could determine the origin of 8 out of 9 D1Mit122 mutations with respect to the karyotype of the parent (table 14).

The mutation frequency of D1Mit122 was siginificantly higher for translocation chromosomes transmitted by the double heterozygous parent (table 14) than by Rb4T70H homozygous parent suggesting the mutations to be of meiotic origin ($P < 0.025$; test for small chances). Four out of 7 mutations derived from double heterozygotes were of maternal origin

Table 14: Mutation frequency and spectrum of mutations of the tetra-nucleotide microsatellite D1Mit122.

Parental Origin of Mutation	*Number of Mutations*	*Number of Analyzed Alleles*	*Mutation Frequency*	*Interpretation[1]* *Spontaneous Mutation* *N (freq.)*	*Ectopic Gene Conversion* *N (freq.)*
Rb4T70H/Rb4T1Wa	7	1226	5.7×10^{-3}	3 (2.4×10^{-3}) H: +1 Wa: -1 Wa: -1	4 (3.3×10^{-3}) H: -1 Wa: +1 H: -1 Wa: +1
Rb4T70H/Rb4T70H	1	1026	0.97×10^{-3}	1 (0.97×10^{-3}) H: +1	
Rb4T70H/Rb4T1Wa or Rb4T70H/Rb4T70H	1			H: -1	

1: Number, (frequency) and identity of mutations
H: + 1 indicates an increase in repeatsize by one repeat unit of the microsatellite positioned on the large T70H translocation chromosome.

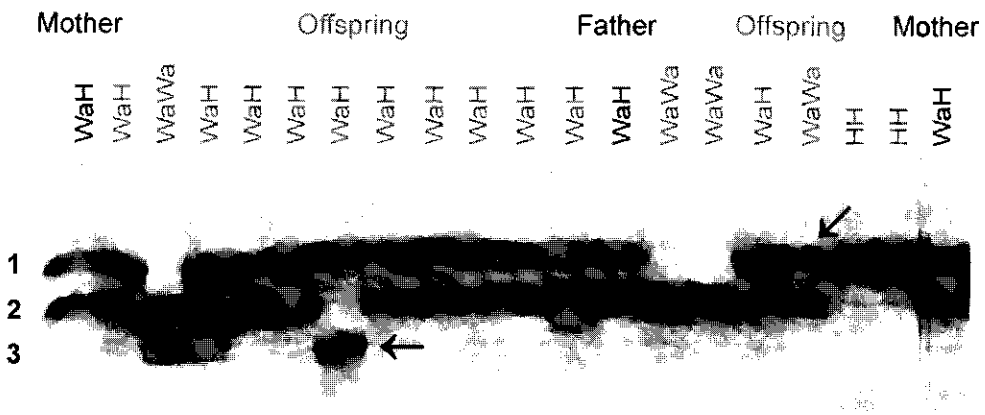

Figure 8: Segregation of alleles 1 and 2 of D1Mit122 belonging to haplotypes A and B respectively in progeny of intercrosses between double heterozygous parents (WaH). Allele 3 is a mutation of the allele 2 of haplotype B. Rb4T70H homozygotes (HH) are homozygous for allele 1 belonging to haplotype A. One Rb4T1Wa homozygote (WaWa) carries alleles 1 and 2 instead of the expected alleles 2/2 belonging to haplotypes B/B.

(n = 1026 meioses). The sex origin of the other 3 mutations is unknown as they were found in progeny of the intercrosses between double heterozygotes (n = 200 meioses).

Mutation analysis shows that the change in the number of repeat units is restricted to one. Moreover, D1Mit122 markers on both the 11-13^{1}H and 1^{13}Wa chromosomes show gain and loss of a repeat unit (table 14, fig. 8). Three out of 7 mutations showed a new allele which did not exist in the parental chromosomes of the double heterozygous genotype. In 2 other mutations, the repeat unit number of the D1Mit122 marker on the 1^{13}Wa chromosomes was changed into the repeat number characteristic for the 11-13^{1}H chromosome of the double heterozygous parent. The reciprocal phenomenon was observed for two other mutations.

Discussion

Consequences of non-homologous synapsis of the T1Wa translocation chromosomes in meiotic heteromorphic bivalents for viability of its carrier.

In this paper, we analyzed the question whether repeated transmission of the T1Wa translocation chromosomes through heteromorphic bivalents with most likely a non-homologously synapsed SC configuration leads to an increased vulnerability of these chromosomes for gaining dominant and recessive lethal mutations during such meiotic passages. Chromosome analysis of fertilized oocytes (arrested at the first cleavage) obtained from Rb4T70H/Rb4T1Wa females revealed equal percentages of pronuclei with either the 1^{13}H or 1^{13}Wa chromosome (51.3 % and 48.7% respectively of 111 numerically balanced pronuclei; de Boer and van der Hoeven, 1991). In agreement with these observations, we found almost equal percentages of fuzzy Rb4T70H homozygotes and smooth double heterozygotes at day 5 after birth in progeny of backcrosses of the double heterozygous translocation breeding lines (from RbB20 onwards; tables 1, 5), though the percentage fuzzy segregants at day 5 was slightly lower in most backcross generations (table 5). For the subsequent period till weaning, the death rate of the double heterozygous segregants was higher than that of Rb4T70H homozygous segregants in all backcross generations of the Mat+Rb stock (fig. 5; table 8). The relatively high postnatal death rate is an intrinsic characteristic of the double heterozygous genotype as it was independent from the synaptic history of the T1Wa translocation chromosomes. The T1Wa translocation chromosomes present in the RbF1 and RbB1 crosses (tables 9 to 11) had been transmitted for many successive generations in the T1Wa stock via meiotic quadrivalent and homomorphic bivalent configurations of T1Wa heterozygous females and homozygous males respectively. As the expected littersizes in the RbF1, RbB1, RbB23 and RbB25 crosses were high (table 10), repeated transmission of the T1Wa chromosomes via either meiotic heteromorphic bivalent or

quadrivalent and homomorphic bivalent configurations has no major deleterious effects on the stability of these chromosomes as measured in this lethality assay.

Genotype analysis of progeny produced in intercrosses of double heterozygotes revealed the expected frequency of Rb4T1Wa homozygotes, indicating that exclusion from crossing over of the Δ1 and Δ13 segments on the T1Wa translocation chromosomes during the successive transmissions through heteromorphic bivalents does also not result in an accumulation of recessive lethal mutations. Similarly, repeated alternated transmission of T1Wa chromosomes via meiotic quadrivalent and homomorphic bivalent configurations (normal T1Wa stock; see Mat. and Meth. section) presumably does not lead to accumulation of recessive mutations (in regions around the T1Wa breakpoint) as (Rb4)T1Wa homozygotes were generated in the expected frequency in intercrosses of Rb4T1Wa/Rb4+ heterozygotes (n = 69; $\chi^2_2 = 1.75$) and in crosses between T1Wa homozygous males and heterozygous females (n = 64; $\chi^2_1 = 0$)(data not shown).

Mat+Rb stock: a dynamic breeding line

Yet, the double heterozygous translocation Mat+Rb stock is a dynamic breeding line considering (a) the increased postnatal survival of the duplication offspring from RbB20 onwards and

(b) the positive effects of haplotype C (on the $11.13^{1}H$ chromosome) on the postnatal survival rate of the duplication segregants (increased littersizes of the RbB1-C and RbB25-C crosses, table 9) and Rb4T70H segregants (tables 10, 11).

Two sources of genetic variation come to mind considering these results:

(*i*) random genetic change (genetic drift) in the Swiss random bred stock used as a genetic background

(*ii*) genetic variation that is confined to the breakpoint areas of the translocation chromosomes derived from the Rb4T70H/Rb4T70H backcross parent, the nature of which has been approached by Δ1 haplotype analysis (see chapter 5 for a discussion of the meiotic history of this segment in the Rb4T70H stock).

As haplotype C is a recombinant of the $11.13^{1}H$ chromosome with the ancestral haplotype A and chromosome 1 of the Swiss random bred background stock (chapter 5; table 3), the haplotype C effects mentioned in (b) hint at a role of genetic variation in regions around the T70H breakpoint for the fitness of the duplication and possibly Rb4T70H homozygous segregants produced in the different backcross stocks.

Intergenerational instability of the D1Mit122 microsatellite: examples of increased spontaneous mutations and/or ectopic homologous gene conversion events.

Genotype offspring analysis by haplotyping revealed intergenerational instability of the tetranucleotide microsatellite D1Mit122 whereas the dinucleotide loci D1Mit4, D1Mit20 and D1Mit121 were stable. These findings are in agreement with the reported almost four times higher mutation frequency of 12 tetranucleotide repeats versus 15 dinucleotide repeats positioned on human chromosome 19 (2.1×10^{-3} versus 5.6×10^{-4}; Weber and Wong, 1993). On the other hand, on the basis of a large scale statistical analysis of distributions of allele sizes of different microsatellite loci, grouped according to repeat motif types, Chakraborty *et al.* (1997) estimated that dinucleotide repeats have an 1-2 times higher mutation rate than tetranucleotide repeats. These authors attributed the results of Weber and Wong (1993) to non-random sampling of loci (see also Zahn and Kwaitkowski, 1995), and in other cases to linkage with a disease gene (see Hastbacka *et al.*, 1992).

Interestingly, the mutation rate was significantly higher for alleles with a double heterozygous parental origin, suggesting these mutations to be generated during meiosis. In the offspring from intercrosses we found 3 mutations (n = 200 meiotic events). In backcrosses of double heterozygous females (table 10 and similar backcrosses not discussed in this chapter) only 4 mutations in 1026 meiotic passages were observed. The reason for this difference is unknown although it might be related to parental sex differences.

The higher mutation frequency in double heterozygotes versus Rb4T70H homozygotes could be related to non-homologous synapsis of the Δ1 segments within heteromorphic bivalents suggesting this configuration to be mutagenic. Accordingly, the three mutations with an allele which are different from any of the parental ones, can be classified as spontaneous mutations (table 14). On the other hand, in 4 out of 7 mutations the repeat size specific for either the 1^{13}Wa or 11.13^{1}H chromosome was changed into the repeat size characteristic for the other parental chromosome of the double heterozygous parent. Thus these latter 4 or even maybe all 7 mutations could be interpreted as the product of successful (4) or unsuccessful (3) ectopic homologous meiotic gene conversion events occurring between D1Mit122 markers present on the 1^{13}Wa and $11\text{-}13^{1}$H chromosomes. Interestingly, as D1Mit122 is located in the seventh intron of the St2 gene, also referred to as T1 (Klemenz *et al.*, 1989, Tominaga *et al.*, 1991), chromatin accessibility could be involved in the proposed occurrence of ectopic gene conversions. Yet, although the axial elements of the ectopic homologous Δ1 regions of the 1^{13}Wa and $(11.)13^{1}$H translocation chromosomes were pointing towards one another in about 20% of spread zygotene spermatocytes, suggesting chromatin contact, an SC configuration was never observed between these Δ1 segments (Peters *et al.*, 1997b, chapter 3). In an *in situ* hybridization experiment of spermatocyte nuclei of double heterozygous males, we observed fusion of the four chromatid signals belonging to the 40 kb genomic probe of the St2 gene

positioned in the two ectopic homologous Δ1 segments (see chapter 8). We interpret this finding as indicating the meiotic homology search between these ectopic St2/T1 sequences (containing D1Mit122) to be successful. However, for validation of the the hypothesized occurrence of ectopic gene conversion, sequencing of the D1Mit122 flanking DNA sequences would be elucidative.

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Chapter 7

The capacity of chromosomes to synapse non-homologously during meiosis is heritable over successive generations, most likely through an epigenetic mechanism.

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Abstract

For many reciprocal translocations in the mouse it has been shown that in the heterozygous condition the degree of male fertility is related to the type of translocation. Here we demonstrate that male fertility of mice, heterozygous for two nearly identical reciprocal translocations T(1;13)70H and T(1;13)1Wa, is additionally modified by the pairing or synaptic history in previous meioses of the translocation chromosomes involved. In the T70H/T1Wa karyotype, the four different translocation chromosomes always produce one large and one small heteromorphic bivalent during female and male meiosis. Each heteromorphic bivalent contains one copy of a segment of chromosome 1 of about 10 cM (Δ1 segment), which is positioned between the T70H and T1Wa translocation breakpoints. These insertion-like Δ1 segments are able to synapse non-homologously during meiotic prophase of double heterozygous males and females. In males, the percentage of spermatocytes showing a fully non-homologously synapsed small heteromophic bivalent is about linearly correlated with fertility-related characteristics like testis weight and epididymal sperm count. Male fertility of double heterozygous offspring was determined (a) after transmission of both reciprocal translocation genomes through homozygous parents (F_1) and (b) after single (B_1) or multi-generation (B_x; $x > 20$) transmission of the T1Wa translocation chromosomes through double heterozygotes. The T70H genome of the backcross double heterozygotes originated from T70H homozygotes. Male fertility was significantly reduced in the B_1 and even more in the B_x T70H/T1Wa offspring which correlated with a decrease in the capacity of the small heteromorphic bivalent to synapse non-homologously. Introduction of the Robertsonian translocation Rb(11.13)4Bnr in the B_x T70H/T1Wa double heterozygous karyotype restored male fertility by stimulating non-homologous synapsis of the small heteromorphic bivalent. Multi-generational transmission of the T1Wa translocation chromosomes via Rb4T70H/Rb4T1Wa females favoured non-homologous chromosome synapsis in the small heteromorphic bivalent and fertility of the Rb4T70H/Rb4T1Wa double heterozygous male offspring. Thus, the structural organisation of meiotically synapsed chromosomes is heritable through the germline likely by an epigenetic mechanism. The proposed non-homologous synapsis imprint is able to influence the reorganisation of chromatin during the subsequent meiotic prophase. Rb4 functions as a modifier of the imprint possibly by increasing the duration of meiotic prophase.

In addition, the effect of the presence of a specific allele for D1Mit122 in the Δ1 segment for male fertility could be interpreted as indicating a QTL for that trait in the region distal from D1Mit122 on mouse chromosome 1.

Introduction

In many mammalian species, heterozygosity for structural chromosomal aberrations correlates with impairment of gametogenesis (de Boer, 1986; de Boer and de Jong, 1989). For example, extensive variation in male fertility exists in a stock of mice heterozygous for two semi-identical reciprocal translocations, T(1;13)70H and T(1;13)1Wa (de Boer *et al.*, 1986; Peters *et al.*, 1997b, chapter 3). The breakpoints of the T(1;13)70H (T70H) and T(1;13)1Wa (T1Wa) translocations are positioned about 10 cM apart from each other on the proximal part of chromosome 1 and are cytologically indistinguishable at the distal end of chromosome 13 (fig. 1a). The karyogram of the double heterozygotes is therefore characterized by the presence of two small and two large semi-identical translocation chromosomes (fig. 1b). During meiotic prophase and diakinesis these chromosomes produce two differently sized heteromorphic bivalents in both sexes. A quadrivalent has never been encountered (Wauben-Penris *et al.*, 1983; de Boer *et al.*, 1986; Peters *et al.*, 1997b, chapter 3). At pachytene stage, the small heteromorphic bivalent displays several synaptonemal complex (SC) configurations. Either synapsis is absent or restricted to homologous segments or the bivalents form a fully non-homologously synapsed horseshoe like configuration (fig. 1c). We previously showed that the degree of male fertility within this double heterozygous karyotype was positively correlated with the percentage of spermatocytes with a fully synapsed small heteromorphic bivalent leading to a "symmetrical" SC (Peters *et al.*, 1997b, chapter 3).

Fertility of the T70H/T1Wa males which were generated in crosses between T70H and T1Wa homozygotes (F1 crosses) varied in a family dependent manner and over subsequent generations of the parental (outbred) stocks. All variation was attributed to the random bred nature of the Swiss background stock (Peters *et al.*, 1997b, chapter 3). Preliminary data on fertility of double heterozygous males which were produced in backcrosses between double heterozygous females and T70H homozygous males, however, suggested that male fertility is not only determined by the chromosomal and genetic constitution of the carrier but also by the pairing or synaptic history in previous meioses of the small translocation chromosomes involved. To analyze this finding more thrroughly, we determined fertility-related characteristics like testis weights, sperm counts and the capacity to propagate of the male T70H/T1Wa offspring that was obtained from several F1 and further backcrosses (table 1). Further, we analyzed the synaptic behaviour of the small heteromorphic bivalent in relation to the synaptic history of the T1Wa genome.

Since its production in 1977 (de Boer *et al.*, 1977), the T1Wa translocation has been maintained by the use of T1Wa/+ males or females. Since 1980, the T1Wa translocation chromosomes have also been transmitted for 25 generations in successive backcrosses between Rb4T70H/Rb4T1Wa females and Rb4T70H homozygous males which additionally

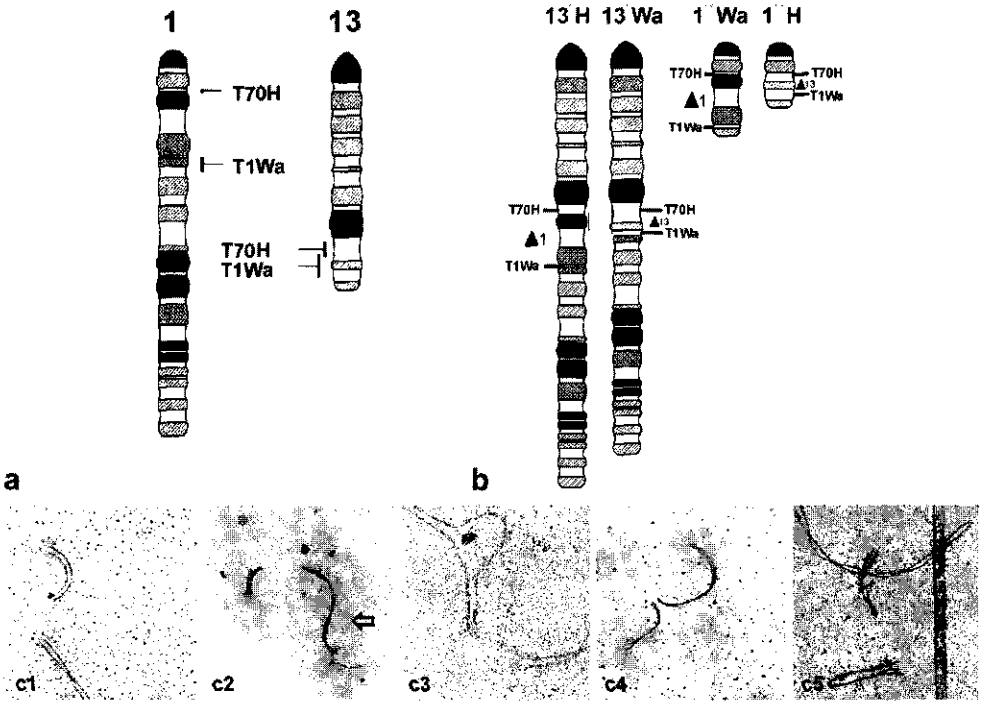


Figure 1: a Position of the T(1;13)70H and T(1;13)1Wa translocation breakpoints along the G-banded mitotic chromosomes 1 and 13. The breakpoints of T70H are located in the R bands 1A4 and 13D1 (de Boer and Gijsen, 1974). The T1Wa breakpoints are in the R bands 1C1.2 and 13D2.2 or in 1C2 and 13D1 (chapter 4). **b** T(1;13)70H and T(1;13)1Wa translocation chromosomes in which we positioned the T1Wa breakpoint distal of the T70H breakpoint on chromosome 13. **c** Silver stained synaptonemal complexes of the different synaptic configurations of the small heteromorphic bivalent: complete non homologous synapsis in a symmetrical (1) or asymmetrical (2) horse-shoe configuration. The latter shows many pseudo-axes and is located near the XY chromosomes (arrow). Restriction of synapsis to two or one homologous chromosome end(s) in loop (3) or fork (4) configurations. (5) Univalents of the 1^{13}Wa (with hairpin, bottom) and 1^{13}H (with pseudo-axis) chromosomes.

were homozygous for the Robertsonian translocation Rb(11.13)4Bnr (Mat+Rb stock; table 1; chapter 6). In an earlier study we observed high sperm counts and a nearly exclusive presence of fully synapsed small heteromorphic bivalents in three Rb4T70H/Rb4T1Wa males produced at the fifth backcross generation of this stock. This suggests that Rb4 might directly or indirectly influence the synaptic behaviour of the small 1^{13}Wa and 1^{13}H translocation chromosomes. On the other hand, this high rate of fully synapsed small bivalents could have been due to successive backcrossing via the double heterozygous females also carrying Rb4. To be able to discern between these two explanations we determined the fertility-related

Table 1: Bird's eye view of names and characteristics of the F1 crosses and the backcross stocks.

Name of Cross or Stock	*Backcross Generation of Cross*	*Year of Breeding*	*Parental and Offspring Karyotypes*		
			Mother	*Father*	*Offspring*
F1	-	1991 + 1996	T70H/T70H	T1Wa/T1Wa	T70H/T1Wa
F1	B1	1991 + 1996	T70H/T1Wa	T70H/T70H	4 types [1]
F1	B1	1991 + 1996	T70H/T70H	T70H/T1Wa	4 types [1]
RbF1	-	1995	Rb4T70H/Rb4T70H	Rb4T1Wa/Rb4T1Wa	Rb4T70H/Rb4T1Wa
RbB1	RbB1	1996	Rb4T70H/Rb4T1Wa	Rb4T70H/Rb4T70H	4 types [1]
Backcross stocks:					
Mat+Rb	RbB1-RbB19	1979-1992	Rb4T70H/Rb4T1Wa	Rb4T70H/Rb4T70H	4 types [1]
Mat+Rb	RbB20-RbB25	1992-1996	Rb4T70H/Rb4T1Wa	Rb4T70H/Rb4T70H	4 types [1]
Pat+Rb	RbB20-RbB24	1992-1996	Rb4T70H/Rb4T70H	Rb4T70H/Rb4T1Wa	4 types [1]
Mat-Rb	B20-B27	1992-1996	T70H/T1Wa	T70H/T70H	4 types [1]

1: Due to random assortment of the four translocation chromosomes of the double heterozygous karyotype, four types of gametes are produced: see figure 2b

characteristics of the double heterozygous male offspring containing T1Wa chromosomes which had been meiotically transmitted for many generations either via a quadrivalent configuration (T1Wa/T1Wa x T1Wa/+ crosses) or via a heteromorphic bivalent configuration (Rb4T70H/Rb4T1Wa x Rb4T70H/Rb4T70H crosses of the Mat+Rb stock). The synaptic behaviour of the small heteromorphic bivalent in relation to the synaptic history of the T1Wa genome was also analyzed in some of these Rb4 containing double heterozygotes.

The variation in fertility and in non-homologous synapsis within the small heteromorphic bivalent of the double heterozygous males produced in the different F1, B1 and multi-generation backcross generations could not be explained by the introgression of alleles of potential genetic modifiers into regions near the translocation breakpoints of any of the different translocation chromosomes. Hence, in analogy with the inheritance of allelic blueprints for methylation patterns as described in humans and mice (Silva and White, 1988; Sasaki *et al.*, 1991) and the cumulative epigenetic inheritance of the TKZ751 transgene (Allen *et al.*, 1990), we present a model in which the T1Wa translocation chromosomes acquire an epigenetic modification through non-homologous synapsis during meiotic prophase to explain the transmission of the capacity of non-homologous synapsis over successive generations and the consequences for male fertility. We further show that Rb4 improves fertility by promoting non-homologous synapsis in the small heteromorphic bivalent.

Materials and Methods

Animals of reciprocal and Robertsonian translocation stocks

Over 18 years the T(1;13)70H, T(1;13)1Wa and Rb(11.13)4Bnr translocation stocks have been maintained by crossing homozygous translocation (T/T) males with heterozygous translocation (T/+) females according to a family rotation scheme (chapter 5). The Rb(11.13)4Bnr-T(1;13)70H (abbreviation: Rb4T70H) stock has been maintained by crossing homozygous Rb4T70H males with Rb4T70H/Rb4+ females (chapter 5). From these crosses, male T/T offspring was selected by peripheral blood lymphocyte karyotyping (de Boer *et al.*, 1977) or an embryonic lethality test (Searle *et al.*, 1971). All types of T/+ females are generated by crossing T/T males with Swiss random bred +/+ (Cpb:SE(S)) females. Rb4T70H/Rb4+ females are obtained from crosses between Rb4T70H homozygous males and Rb4/Rb4 females. In this way, genetic variation is maintained at the level of the Swiss random bred stock except for the regions around the breakpoints where crossing-over is reduced or absent (see chapters 4, 5 and 6; Cattanach *et al.*, 1972; Philips *et al.*, 1980; Davisson and Akeson, 1993). Recently, mice homozygous for both Rb(11.13)4Bnr and T(1;13)1Wa (abbreviation: Rb4T1Wa) were produced by using T1Wa and Rb4 homozygotes as founder animals (chapter 6). Rb4T1Wa homozygous males, used in the RbF1 cross, were obtained from intercrosses between Rb4T1Wa/Rb4+ mice and from crosses between Rb4T1Wa homozygotes.

Meiotic chromosome segregation in the (Rb4)T70H/(Rb4)T1Wa double heterozygous karyotype

The breakpoints of the T(1;13)70H reciprocal translocation are located in the R bands A4 and D1 on chromosomes 1 and 13, respectively (fig. 1a; de Boer and Gijsen, 1974). The breakpoints of the T(1;13)1Wa reciprocal translocation are positioned in the R bands 1C1.2 and 13D2.2 or in 1C2 and 13D1 (chapter 4). The regions between the T70H and T1Wa breakpoints on chromosomes 1 and 13 are called Δ1 and Δ13, respectively.

We analyzed male fertility-related characteristics like testis weights and epididymal sperm counts of (Rb4)T70H/(Rb4)T1Wa double heterozygous and (Rb4)T70H homozygous offspring produced in several F1- and backcrosses (B_x; table 1; fig. 2). In a number of these crosses mice were also homozygous for the Robertsonian translocation Rb(11.13)4Bnr (RbF1 and RbB_x crosses; table 1; fig. 2). In this paper the abbreviation (Rb4)F1 refers to both the F1 and Rb4F1 crosses. (Rb4)F1 double heterozygous offspring was produced in crosses between female (Rb4)T70H and male (Rb4)T1Wa homozygotes (fig. 2a). Following random assortment of the four different translocation chromosomes from the two heteromophic bivalents present at metaphase I (Wauben-Penris *et al.*, 1983), backcrosses between

(Rb4)T70H/(Rb4)T1Wa double heterozygotes and (Rb4)T70H homozygotes produce four types of offspring (fig. 2b). For simplicity, figure 2 presents the situation in which the T1Wa breakpoint is in the chromosome 13D2.2 band. Accordingly, the T1Wa breakpoint is distal of the T70H breakpoint on chromosome 13 and the Δ13 segment is localized on the large 13^{1}Wa and the small 1^{13}H translocation chromosomes (fig. 2). Segregants with the parental karyotypes are viable and can be distinguished from each other by their phenotype. The (Rb4)T70H homozygotes have a fuzzy hair coat as result of the recessive fuzzy allele (fz, Dickie and Woolley, 1950) in the proximal chromosome 1 region of the 1^{13}H chromosome (fig. 2; Searle *et al.*, 1971). The wildtype allele (+) on the 1^{13}Wa chromosome is closely linked to the segment between the T70H and T1Wa breakpoints and causes a smooth hair phenotype (fig. 2). Duplication mice, which are trisomic for the Δ1 segment and monosomic (or trisomic in case the T70H breakpoint is distal from the T1Wa breakpoint) for the small Δ13 segment, have a low viability and have a runted smooth-haired phenotype (fig. 2; chapter 6). Deletion mice, which are monosomic for the Δ1 segment and trisomic (or monosomic) for the small Δ13 segment die prenatally (fig. 2; table 8; de Boer *et al.*, 1986; Peters *et al.*, 1997b, chapter 3).

The Rb4T1Wa translocation chromosomes have been transmitted for 25 generations in backcrosses between double heterozygous females and Rb4T70H homozygous males (Mat+Rb stock, table 1). During the first 19 generations of the Mat+Rb stock (table 1), the Rb4T1Wa genome was transmitted by successive backcrosses of Rb4T70H/Rb4T1Wa females with homozygous Rb4T70H males (fig. 3; chapter 6). From backcross generation 19 onwards, the (Rb4)T1Wa genome has been transmitted in three different ways (table 1): maternally (Mat+Rb) and paternally (Pat+Rb) in the presence of Rb4 and maternally (Mat-Rb) in the absence of Rb4. The Mat-Rb stock was derived from the Mat+Rb stock by removal of Rb4 in two successive backcrosses with T70H homozygous males (start at the RbB18 generation).

Genotyping of backcross offspring

Offspring produced in both first backcrosses of 1995/1996 (B1 and RbB1) and in the 25th backcross of the Mat+Rb stock (RbB25; fig 3) was karyotyped by segregation analysis of 4 polymorphic microsatellites as described in chapters 4 and 6. In an earlier study we determined the order of these microsatellites in the Δ1 segment as follows: T70H - D1Mit4 - D1Mit20 - D1Mit121 - D1Mit122 - T1Wa (chapter 5).

Irrespective of the cross analyzed, all 1^{13}Wa chromosomes harboured the same combination of alleles of the 4 microsatellites, haplotype B, which is specific for the T1Wa genome (tables 2,3; see chapter 6). Haplotypes A and C (tables 2, 3) were used as markers for the 11-13^{1}H chromosome of the Rb4T70H genome in the RbF1, RbB1 and RbB25 (Mat+Rb)

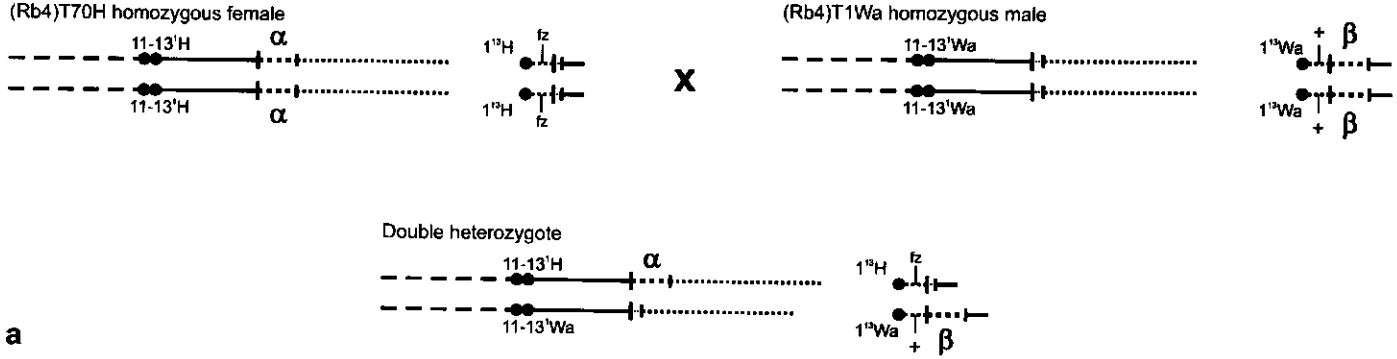
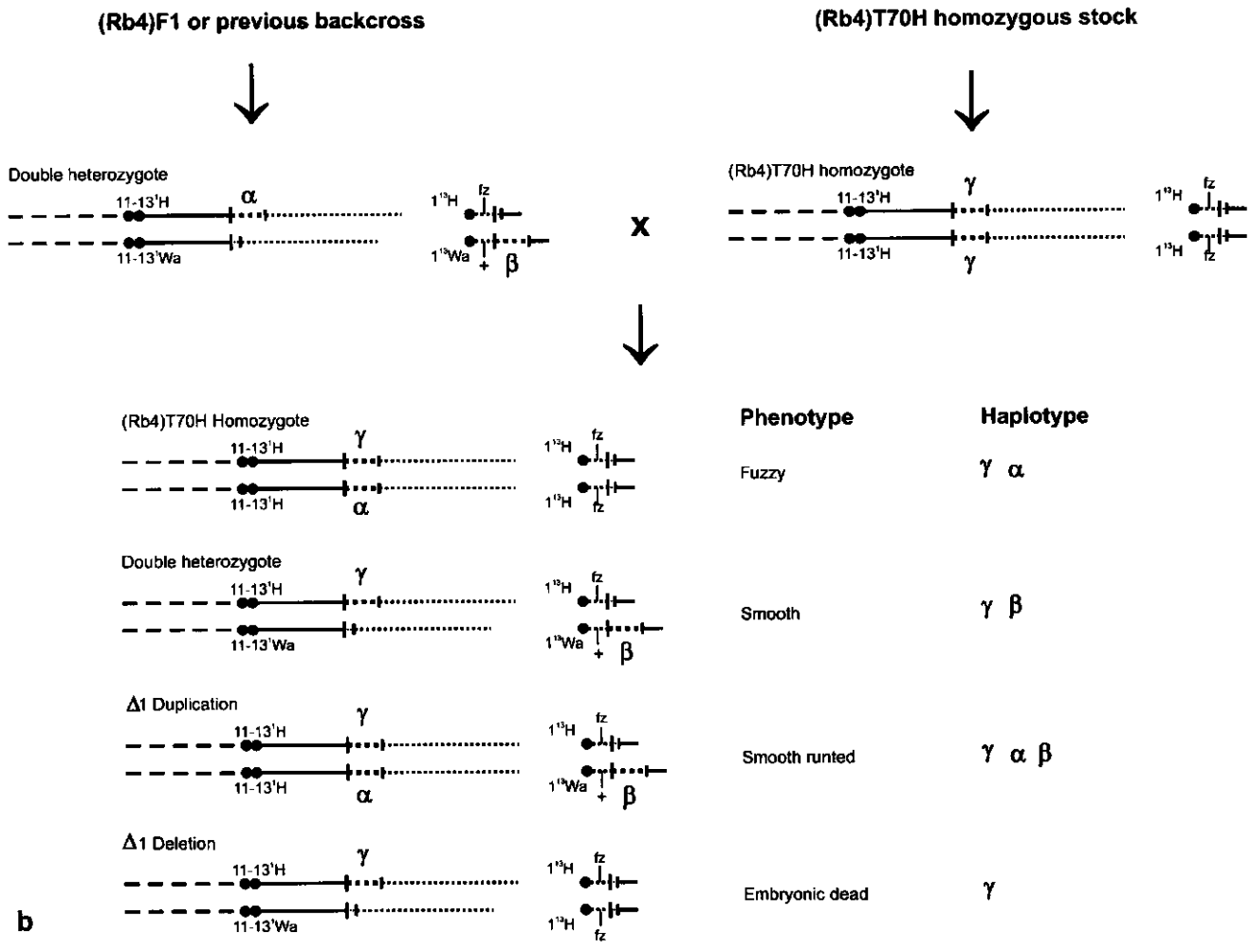


Figure 2: a Breeding of (Rb4)F1 double heterozygotes by homozygous (Rb4)T70H females and homozygous (Rb4)T1Wa males. Within a specific cross, both parents are homozygotes for Rb4 (depicted by (Rb4)) or not. The (Rb4)T70H homozygotes have a fuzzy hair coat as result of the recessive fuzzy locus (fz) located on the proximal chr. 1 end of the 1^{13}H chromosome. The 1^{13}Wa chromosome carries the wildtype allele. For simplicity, this figure presents the situation in which the T1Wa breakpoint is distal of the T70H breakpoint on chromosome 13 (see fig. 1b). The small region between the two breakpoints on chromosome 13 (Δ13 segment) is then localized on the large 13^{1}Wa and the small 1^{13}H translocation chromosomes. The region between the breakpoints on chromosome 1 (Δ1 segment) is localized on the large 13^{1}H and the small 1^{13}Wa translocation chromosomes.

b Backcrosses between (Rb4) double heterozygotes and (Rb4) homozygotes produce four types of offspring. Karyotyping is performed by haplotyping (α,β,γ) as shown in tables 2 and 3 or by chromosomal karyotyping in combination with assessment of the phenotype (table 4). (Rb4) double heterozygous parents originate from the (Rb4)F1 cross or from one of the successive backcross generations (table 1). (Rb4)T70H homozygous parents are produced by (Rb4)T70H homozygotes which originate from the "outcross" stocks (chapter 5).


(Rb4)F1 or previous backcross
(Rb4)T70H homozygous stock
Double heterozygote
11-13'H
α
11-13'Wa
fz
β
X
(Rb4)T70H homozygote
γ
(Rb4)T70H Homozygote
Double heterozygote
Δ1 Duplication
Δ1 Deletion
Phenotype
Haplotype
Fuzzy
γ α
Smooth
γ β
Smooth runted
γ α β
Embryonic dead
γ

b

Table 2: Karyotyping by microsatellite haplotype analysis in B1, RbB1, RbB20-RbB24 and RbB25 backcrosses.

Karyotype	*Phenotype*	*Haplotype Code from Figure 2*	*Backcross Generations*				
			F1 (1996)	*B1 (1996)*	*RbF1 (1996)*	*RbB1 + RbB19-RbB24*[1]	*RbB1 + RbB25*
F1 Parents							
(Rb4)T70H/(Rb4)T70H	fuzzy	α/α	D/D		A/A		
(Rb4)T1Wa/(Rb4)T1Wa	smooth	β/β	B/B		B/B		
F1 Offspring:							
(Rb4)T70H/(Rb4)T1Wa	smooth	α/β	D/B		A/B		
Backcross Parents:							
(Rb4)T70H/(Rb4)T70H	fuzzy	γ/γ		E/E		A/A	C/C
(Rb4)T70H/(Rb4)T1Wa	smooth	α/β		D/B		A/B	A/B
Backcross Offspring:							
(Rb4)T70H/(Rb4)T70H	fuzzy	γ/α		E/D		A/A	C/A
(Rb4)T70H/(Rb4)T1Wa	smooth	γ/β		E/B		A/B	C/B
Duplication	smooth runted	γ/α/β		E/D/B		A/A/B	C/A/B
Deletion	embryonic dead	γ		E		A	C

1: Some Rb4T70H/Rb4T70H parents of the RbB20 to RbB22 generations were heterozygous for haplotypes A and F. Accordingly, some offspring of these parents had haplotype C instead of haplotype A on their 11-13[1]H chromosomes.

Table 3: Microsatellite alleles of D1Mit4, -20, -121 and -122 belonging to haplotypes A to F.

Translocation	*Haplotype Code*	*Haplotype*	*Microsatellite Alleles*[1]			
Genome	*from Figure 2*		*D1Mit4*	*D1Mit20*	*D1Mit121*	*D1Mit122*
Rb4T70H	α & γ	A	1	1	1	1
(Rb4)T1Wa	β	B	2	2	3	2
Rb4T70H	γ	C[2]	1	1	2	3
T70H	α	D[2]	1	x[3]	x[3]	4
T70H	γ	E[2]	1	2	2	3
Rb4T70H	α & γ	F[2]	1	1	1	2

1: The number code of a microsatellite allele is inversely related to the length of the PCR product.
2: Haplotypes C to F are recombinational products of the ancestral haplotype A and the wild type Swiss genome
3: x = allele 1 or 2

crosses (table 1; fig 3; chapter 6). Most Rb4T70H homozygous parents of the RbB20-RbB24 generations from the Mat+Rb and Pat+Rb stocks were homozygous for haplotype A. In the RbB20-RbB22 generations however, some of these parents were heterozygous for haplotypes A and F (tables 2, 3). Haplotypes D and E (tables 2, 3) were used as markers for the T70H genomes in the F1 and B1 crosses, respectively (table 1; fig. 2; chapter 6). Each microsatellite in haplotype A contains an allele which either has not been detected or has been found only at a very low frequency in the wildtype Swiss random bred genome since 1992 (table 3, chapter 5). Haplotypes C to F are therefore most probably products of recombination between the ancestral T70H haplotype A and the Swiss random bred stock (chapter 5). Haplotypes C and E both originated from a single maybe different wild type chromosome 1 (tables 2, 3). Haplotype D probably originated from more than one wild type chromosome 1 (tables 2, 3).

Offspring which was produced in the B1 of 1991 (table 5), at successive backcross generation of the Mat+Rb, Pat+Rb, Mat-Rb stocks and in crosses presented in table 9 was karyotyped by several different methods. Some generations were karyotyped by analysis of G-banded chromosome preparations made from peripheral blood lymphocytes (table 4; de Boer *et al.*, 1977). If G-banding was not performed, the difference in length between the two small 1^{13} chromosomes was used to discern between the karyotypes of the fuzzy potentially (Rb4)T70H homozygous and smooth potentially double heterozygous segregants (table 4; fig 1b in chapter 4). The mitotic length of the two large 13^{1} chromosomes at the prometaphase I stage was measured (after magnification using a microscope drawing tube) to distinguish between the smooth double heterozygous (Rb4)T70H/(Rb4)T1Wa and smooth runted duplication ((11-)13^{1}H; (11-)13^{1}H; 1^{13}Wa; 1^{13}H) karyotypes (the mitotic length of the 13^{1} Wa chromosome is 87.6 % of the 13^{1}H chromosome; fig 2; chapter 6). Likewise, the growth rate,

as determined on the basis of the body weights at days 28 and 42 after birth (see below; chapter 6), and the post natal viability of the animal (chapter 6) were also taken into account to distinguish between the two possible karyotypes of smooth offspring (table 4).

Table 4: Genotyping by a combination of methods (RbB5, RbB11, RbB20 to RbB24 of the three backcross stocks).

Backcross Offspring[1]	*Karyotype*[2]	*Pheno-type*	*G-Band Ana-lysis*[3]	*Normal karyotype analysis* 13^1 *chr.*[5]	1^{13} *chr.*[4]	*Postnatal Viability*[6]	*Bodyweight at Days 28 and 42*[6]
(Rb4)T70H	13^1H; 1^{13}H;	fuzzy	+	large	small	normal	normal
Homozygotes	13^1H; 1^{13}H			large	small		
Double	13^1H; 1^{13}H;	smooth	+	large	small	normal	normal
Heterozygotes	13^1Wa; 1^{13}Wa			small	large		
Duplication	13^1H; 1^{13}H;	smooth	+	large	small	reduced	± 60% reduced
Mice	13^1H; 1^{13}Wa	runted		large	large		

1: See figure 3B
2: For clarity, chromosomes 11 which are fused to the 13^1 translocation chromosomes in the Rb containing karyotypes
are not given.
3: The G-band karyotype of the 13^1H and the 1^{13}Wa chromosomes show two large G-bands (1A5 and 1C1) in the middle region of both chromosomes. These bands are absent from the 13^1Wa and the 1^{13}H chromosomes (see fig. 1b of chapter 4). The "+" indicates that the karyotype can be determined by G-banding.
4: Mitotic length of the 1^{13}H is 50% of the 1^{13}Wa chromosome (see comment #3).
5: Mitotic length of the 13^1Wa is 87.6% of the 13^1H chromosome (see comment #3).
6: unpublished observations.

Fertility estimates.

The method of Searle and Beechey (1974) was used to count sperm from the caput epididymis. Testis were weighted after removal of adherent fat tissue. The capacity of males to produce offspring was tested by mating them to two 2-3 months old NMRI (Han) virgin females (de Boer *et al.*, 1986). Small and large moles and live embryos were classified according to the criteria of Carter *et al.* (1955) and were counted at day 13 after a vaginal plug had been found. A male was declared fertile when at least one conceptus was produced after two successive matings.

Body weights

Homozygosity for Rb4 was correlated with reduced body weight in the RbF1 and RbB1 crosses (table 1; chapter 6). Body weight also depended on littersize which differed between the RbF1 and RbB1 generations (chapter 6). In addition, body weights decreased over time in both double heterozygous and homozygous segregants of the Mat+Rb and Pat+Rb stocks from RbB20 onwards (chapter 6). In order to compare the testis weights and sperm counts from mice of F1 and different backcross generations and with or without the Rb4 translocation, we normalised the testis weights (T_x) of males in almost all groups presented in tables 5 to 9 for their body weights (B_x), measured at day 42 after birth, using the mean body weight of the "fertile family" 1996 F1 T70H/T1Wa males ($B_{F1\text{-}fertile}$; table 5) as a standard. For example, the normalized testis weight of a male in group "x" is determined as follows: $(T_x \times B_{F1\text{-}fertile})/ B_x$. For the RbB5 to RbB19 segregants in the Mat+Rb stock, we equalled the unrecorded body weights to the body weights of the RbB20 segregants. Testis weight and sperm count of the 1991 F1 and B1 crosses were not normalised.

Synaptonemal complex analyses.

Spermatocytes from three double heterozygous males of the fifth backcross generation (1985) of the Mat+Rb stock were spread according to the following drying down method. One testis was minced with a pair of curved forceps in Hanks Balanced Salt solution. After centrifugation for 5 min. at 800 rpm, cells were resuspended in 1 ml 0.1 M sucrose and kept on ice for 5 min. Subsequently, cells were fixed for 5 min by addition of 2 ml of a 4% paraformaldehyde in 1.5% sucrose solution (pH 8.5). After centrifugation for 5 min at 500 rpm and removal of the supernatant, 4 ml of fixative was added. Cells were kept on ice for an additional 30 min. Subsequently, cells were spread by adding successively one volume of cells, and two volumes of fixative to glass or falcon plastic-coated slides. Slides were dried over night at room temperature. Nuclei were silverstained using the Ag-gelatin method of Howell and Black (1980). 150 pachytene nuclei (50 per male) were analysed by light microscopy. An additional 50 spermatocytes were scored by electron microscopy.

Spermatocytes and oocytes from double heterozygous and/or homozygous segregants of the RbF1 (1996) and all other backcross generations (1992-1996) were spread on falcon plastic coated slides using the drying down technique of Peters *et al.* (1997; chapter 2) and analysed in a Jeol 1200 EKII or Philips EM 208 (80 kV) electron microscope. Pachytene substaging of spermatocytes was based on the degree of synapsis and the morphology of the sex chromosomes (Tres 1977, Moses 1980, Dietrich and de Boer 1983, Moses *et al.* 1984) as described by Peters *et al.* (1997b, chapter 3). Female meiocytes were obtained from fetuses at days 16 and 18 of gestation (vaginal plug formation defined as day 0 of development). Fetuses were karyotyped on the basis of Giemsa stained normal chromosome preparations from fetal

Table 5: Testis weights and epididymal sperm counts of double heterozygous and T70H homozygous offspring produced in F1, B1 and T70H homozygous control crosses performed in 1991 and 1996.

Cross Code	*Cross*			*Trans-mission of T1Wa*	*Fertility Family Code*	*Number of Families*	*T70H/T1Wa*			*T70H/T70H*		
	Father	*x*	*Mother*				*Number of Mice*	*Testis Weight mean ± sd*[a]	*Sperm Count mean ± sd*	*Number of Mice*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*
1996-F1	Wa/Wa	x	H/H		Sterile	3	10	45.5 ± 7.3 [1]	1.5 ± 3.0 [2]			
1996-F1	Wa/Wa	x	H/H		Fertile	4	20	80.3 ± 29.5 [1,3,5]	108.0 ± 101.6 [2,4,6]			
1996- B1	H/H	x	H/Wa	Maternal	Sterile	5	9	42.0 ± 6.2 [7]	0 ± 0	6	115.7 ± 10.3	488.2 ± 105.6 [8,9]
1996- B1	H/H	x	H/Wa	Maternal	Fertile	6	10[b]	55.4 ± 17.0 [3,7]	24.1 ± 60.8 [4]	6	133.9 ± 23.3	666.3 ± 138.3 [8]
1996- B1	H/Wa	x	H/H	Paternal	Fertile	3	13[c]	57.8 ± 16.1 [5,7]	21.2 ± 45.3 [6]	14	131.3 ± 11.7	726.8 ± 131.7 [9]
1996- control	H/H	x	H/H			8				29	127.3 ± 16.4	498.1 ± 115.3
1991-F1	Wa/Wa	x	H/H			4	43	45.7 ± 22.9	43.9 ± 115.8			
1991- B1	H/H	x	H/Wa	Maternal		9	22	46.1 ± 18.1	12.5 ± 32.8	22	115.3 ± 16.0	414.1 ± 102.6 [10]
1991- B1	H/H	x	H/Wa	Paternal		1	9	45.5 ± 11.9	12.9 ± 22.5	11	116.9 ± 14.4	522.7 ± 148.1 [10,11]
1991- control	H/H	x	H/H			5				18	104.6 ± 8.0	380.3 ± 106.0 [11]

H/H = T70H/T70H; H/Wa = T70H/T1Wa; Wa/Wa = T1Wa/T1Wa

a: sd = standard deviation

b: Fertility data of one male not included in mean ± sd: testis weight: 117 mg; sperm count: 300

c: Fertility data of one male not included in mean ± sd: testis weight: 142 mg; sperm count: 660

Mann-Whitney U tests:

1: $P < 0.001$ 2: $P < 0.001$ 3: $P < 0.025$ 4: $P < 0.01$ 5: $P < 0.025$ 6: $P < 0.01$ 7 (B1-sterile versus B1-fertile pooled): $P < 0.0027$

8: $P < 0.047$ 9: $P < 0.01$ 10: $P < 0.0179$ 11: $P < 0.025$

liver cells (Meredith, 1966). The female meiotic prophase was substaged according to the criteria of Speed (1982) and Dietrich and Mulder (1983).

Statistical analyses

Parameters were analysed by the Fischer exact probability test, the χ^2 test or the Mann-Whitney U test as described by Siegel (1956).

Results

Fertility of T70H/T1Wa males is reduced after one passage of the T1Wa genome through meiosis of a double heterozygous parent.

To analyse the influence of one meiotic passage of the 1^{13}Wa translocation chromosome through a heteromorphic bivalent configuration on fertility of T70H/T1Wa males, we determined testis weights, sperm counts and the capacity to sire of the double heterozygous offspring from the F1 and the first backcrosses performed in 1996 and 1991. T70H/T70H male segregants were used as controls. Offspring of the 1996 B1 crosses was karyotyped by haplotype analysis of 4 polymorphic microsatellite markers (see table 2 and Mat. & Meth.). The progeny of the 1991 B1 cross was karyotyped by G-banding (table 5).

Fertility of T70H/T1Wa males obtained in 1996 from intercrosses (F1) between T70H and T1Wa homozygotes varied in a family dependent manner (Peters *et al.*, 1997b, chapter 3). Here we show that three F1 families produced sterile sons only (total of 10 sons tested) whereas four other F1 families produced fertile sons as well (15 out of 20 tested sons were fertile). Mean testis weight and epididymal sperm count were both significantly lower in the "sterile" versus the "fertile" F1 families (table 5; {1, 2} $P < 0.001$ for both parameters). Mean testis weight and sperm count of the T70H/T1Wa B1 sons produced by T70H/T1Wa females (fig. 2b) from the "sterile" F1 families equalled those of the "sterile family" F1 males (table 5). Maternal and paternal transmission of the T1Wa translocation chromosomes from the "fertile" F1 families resulted in a significant decrease of mean testis weight {3 ,5} and sperm count {4, 6} compared with F1 males from the "fertile" families (table 5; {3, 5} $P< 0.025$ and {4, 6} $P< 0.01$). Among both the maternally and paternally derived B1 double heterozygous sons from the "fertile" families, we found one male with a high testis weight and sperm count (see table 5).

Fertility of T70H/T1Wa males obtained from intercrosses between T70H and T1Wa homozygotes performed in 1991 varied in a family dependent manner as well (Peters *et al.*, 1997b, chapter 3). Compared with offspring from intercrosses performed in 1996, fewer males were fertile: out of 134 males tested (of 6 families), 8 were fertile (of 3 families). Mean testis

Table 6: Testis weight and epididymal sperm count of offspring produced in RbF1, RbB1 and Rb4T70H homozygous control crosses of 1996.

Cross	*Cross*			*Family*	*Number*	*Rb4T70H/Rb4T1Wa*				*Rb4T70H/Rb4T70H*			
Code	*Father*		*Mother*	*Lineage Code*	*of Families*	*Number of Mice*	*Haplo-type*	*Testis Weight mean ± sd*[a]	*Sperm Count mean ± sd*	*Number of Mice*	*Haplo-type*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*
RbF1[b]	RbWa/RbWa	x	RbH/RbH	1	2	12	A/B	81.9 ± 24.2	97.2 ± 78.3				
RbF1[b]	RbWa/RbWa	x	RbH/RbH	2	5	22	A/B	68.9 ± 15.9[1]	41.5 ± 44.8[2]				
RbB1	RbH/RbH	x	RbH/RbWa	1	4	9	A/B	75.1 ± 14.1	89.1 ± 70.3	3	A/A	104.1 ± 17.8[3]	300.2 ± 5.72[4]
RbB1	RbH/RbH	x	RbH/RbWa	2	7	18	C/B	86.3 ± 18.4[1]	125.6 ± 124.9[2]	16	C/A	128.6 ± 13.9[3]	430.7 ± 116.8[4,5]
Controls	RbH/Rb0H	x	RbH/RbH		1					2	A/A	130.8 ± 15.1	324.5 ± 113.6
										4	C/A	138.0 ± 3.7	379.6 ± 109.1
Controls	RbH/RbH	x	RbH/RbH		6					19	A/A	118.9 ± 13.9	303.0 ± 137.7[5]

RbH/RbH = Rb4BnrT70H/Rb4BnrT70H; RbH/RbWa = Rb4BnrT70H/Rb4BnrT1Wa; RbWa/RbWa = Rb4BnrT1Wa/Rb4BnrT1Wa

a: sd = standard deviation

b: RbF1 data are presented in two groups of families (1 and 2) of which double heterozygous female offspring was mated either with haplotype A/A (1) or haplotype C/C (2) Rb4T70H homozygous males.

Mann-Whitney U tests: 1: $P < 0.0007$ 2: $P < 0.0011$ 3: $P < 0.025$ 4: $P < 0.025$ 5: $P < 0.01$

weight and sperm count of 43 1991 F1 males were similar to the F1 males of the 1996 "sterile" families (table 5). Mean sperm count of double heterozygous 1991 B1 offspring produced by F1 females was slightly lower than sperm counts of offspring from the 1991 F1 males. One fertile F1 male which produced a high number of implants in the fertility test (testis weight and sperm count unknown) was backcrossed to T70H homozygous females. As in 1996, the double heterozygous B1 segregants had a low mean testis weight and sperm count (table 5). Considering the high fertility of the F1 father, the fertility estimates of these B1 males are probably reduced in comparison with the father.

In summary, we observed that one passage of the 1^{13}Wa chromosomes via F1 double heterozygous males or females of the fertile families correlates with a decrease in male fertility of the B1 double heterozygous male segregants carrying the "same" 1^{13}Wa chromosomes. After transmission of the 1^{13}Wa chromosome via females of the sterile families we did not observed a change in male fertility of the B1 double heterozygotes as all B1 males were also sterile.

In the T70H/T70H segregants of both B1 generations we observed an opposite effect. Sperm counts of the B1 T70H/T70H segregants obtained through maternal or paternal transmission from the "fertile" 1996 F1 families and through transmission from the fertile 1991 F1 father were significantly higher than sperm counts of the contemporary T70H homozygous controls (table 5; 1991: {11} $P < 0.025$). In addition, sperm counts of these B1 T70H homozygous males were significantly higher than the sperm counts of the maternally derived segregants from the sterile 1996 F1 families (table 5; mat: {8} $P < 0.047$; {9} pat: $P < 0.01$) and maternally derived segregants from the 1991 F1 females (table 5; {10} $P < 0.0179$).

Fertility of double heterozygous males is not reduced after one passage of the T1Wa genome through meiosis of a double heterozygous female carrying the Rb4 translocation.

Sperm counts of three Rb4T70H/Rb4T1Wa males which were produced in the fifth backcross generation of the Mat+Rb stock (1985; table 1) were high in comparison with the sperm counts of contemporary T70H/T1Wa males lacking Rb4 (de Boer *et al.*, 1986) or of the F1 and B1 T70H/T1Wa males from 1991 and 1996. Therefore, we wanted to investigate the effect of homozygosity for the Robertsonian translocation Rb4 on male fertility of the double heterozygous karyotype.

First we determined the fertility-related characteristics of the progeny obtained from intercrosses between Rb4T70H and Rb4T1Wa homozygotes (RbF1; table 1). Fertility of the Rb4T70H/Rb4T1Wa males was less family dependent than fertility of the 1996 F1 T70H/T1Wa males. Six out of seven RbF1 families generated fertile offspring. Yet, both the mean testis weight and sperm count of the RbF1 males were not significantly different from the mean testis weight and sperm count calculated for all 1996 F1 males (data of the "sterile"

Table 7: Testis weight and epididymal sperm count of double heterozygous and homozygous offspring produced at different generations of the Mat+Rb and Pat+Rb stocks. RbF1 and RbB1 crosses are given for comparison.

Cross	*Backcross*	*Rb4T70H/Rb4T1Wa*				*Rb4T70H/Rb4T70H*			
Code	*Generation*	*Number of Mice*	*Haplo-type*	*Testis Weight mean ± sd*[a]	*Sperm Count mean ± sd*	*Number of mice*	*Haplo-type*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*
RbF1[b]		34	A/B	73.6 ± 20.5	58.9 ± 63.3				
RbB1[c]		9	A/B	75.1 ± 14.1	89.1 ± 70.3	3	A/A	104.1 ± 17.8	300.2 ± 5.72
RbB1		18	C/B	86.3 ± 18.4[1]	125.6 ± 124.9	16	C/A	128.6 ± 13.9	430.7 ± 116.8
Mat+Rb[c]	B5	3	(A/B)[1]	-	202.4 ± 22.9				
Mat+Rb	B15	20	(A/B)[1]	91.5 ± 18.9	273.7 ± 136.5	21	(A/A)[1]	92.9 ± 12.2	262.2 ± 139.6
Mat+Rb	B20	14	(A/B)[2]	78.3 ± 11.5	121.2 ± 92.7	14	(A/A)[2]	87.7 ± 10.8	230.5 ± 72.0
Mat+Rb	B24	13	A/B	72.3 ± 20.7	71.2 ± 96.4				
Mat+Rb	B25	22	C/B	86.6 ± 16.3	172.7 ± 143.1	21	C/A	130.9 ± 12.9	540.6 ± 149.4
Pat+Rb[c]	B20	14	(A/B)[2]	73.8 ± 12.0	158.5 ± 83.4	19	(A/A)[2]	91.8 ± 8.1	289.9 ± 57.4
Pat+Rb	B22	6	(A/B)[2]	92.6 ± 12.0	243.6 ± 99.3				
Pat+Rb	B23	8	A/B	79.7 ± 7.0	127.3 ± 89.2				
Pat+Rb	B24	13	A/B	81.0 ± 12.6	84.2 ± 54.1				

a: sd = standard deviation
b: RbF1 crosses: Rb4T1Wa/Rb4T1Wa (males) x Rb4T70H/Rb4T70H (females)
c: RbB1 and backcrosses in Mat+Rb and Pat+Rb stocks: Rb4T70H/Rb4T70H (males) x Rb4T70H/Rb4T1Wa (females)
1: RbB5 and RbB15 generations are probably produced by Rb4T70HRb4T70H father homozygous for the ancestral haplotype A because recombination is suppressed in the translocated chromosome 1 region near the T70H breakpoint in Rb4T70H/Rb4+ females (chapter 5).
2 : Pedigree analysis in combination with haplotyping of several parents revealed that most Rb4T70H/Rb4T70H fathers of the RbB20-RbB22 generations were homozygous for haplotype A. Only a few fathers were heterozygous for haplotypes A and F.

and "fertile" F1 families pooled). Variation in both parameters was however lower in the RbF1 versus F1 groups (data not shown).

Subsequently, we analysed the effect of one transmission of the 1^{13}Wa chromosome via double heterozygous females on male fertility of the double heterozygous progeny in relation to homozygosity for Rb4. Similar to the 1996 B1 generation, offspring of the first backcross generation (RbB1) backcross was also karyotyped by haplotype analysis (see table 2 and Mat. & Meth.). RbB1 was produced by crossing RbF1 Rb4T70H/Rb4T1Wa females (haplotypes A and B; tables 2, 3) with Rb4T70H/Rb4T70H males homozygous for haplotypes A or C (tables 2, 3). Fertility estimates of the RbB1 double heterozygous segregants generated by haplotype A fathers equalled those of the RbF1 males produced in the same family lineage (table 6). In contrast, RbB1 double heterozygous segregants from haplotype C fathers had significantly higher mean testis weights and sperm counts compared with RbF1 males produced in the same pedigree (table 6; $P < 0.0007$ and $P < 0.0011$, respectively).

In a similar way, Rb4T70H homozygous segregants carrying haplotypes A and C had significantly higher testis weights and sperm counts than homozygous haplotype A RbB1 segregants (table 6; $P < 0.025$ for both parameters). Sperm counts of these RbB1 haplotype A/C segregants were also significantly higher in comparison with the Rb4T70H homozygous controls from the Rb4T70H stock (mostly haplotypes A/A; few males with haplotypes A/F (table 3); table 6; $P < 0.01$). However, this haplotype F effect was not restricted to RbB1 offspring as a similar trend was observed for haplotype A/C versus haplotype A/A segregants from homozygous Rb4T70H parents (table 6).

To summarize, in contrast to the "1996 fertile F1-B1" situation, we observed no decrease in male fertility of Rb4T70H/Rb4T1Wa males after one meiotic passage of the 1^{13}Wa chromosome via Rb4T70H/Rb4T1Wa females. Moreover, the presence of haplotype C correlates with a high degree of male fertility, irrespective of the karyotype.

Transmission of the Rb4T1Wa genome over 25 successive generations through meiosis of double heterozygous females.

In the Mat+Rb stock, the Rb4T1Wa translocation chromosomes have been transmitted over 25 successive generations in crosses between double heterozygous females and Rb4T70H homozygous males (table 1). This stock enables us to study the effect of multi-generational transmission of the 1^{13}Wa chromosome through a heteromorphic bivalent configuration on male fertility of the double heterozygous progeny. Here we present fertility-related characteristics of the two balanced types of progeny (fig. 2) of several different backcross generations of this stock.

Backcross generations RbB20-RbB24 of the Mat+Rb stock were predominantly sired by fathers homozygous for the ancestral haplotype A (tables 2, 3; chapter 5). A few fathers

heterozygous for haplotypes A and F (table 2) were used at Rb20 to Rb22 generations. Presumably, RbB1 to RbB19 generations were also bred on 11-13^{1}H chromosomes with haplotype A as recombination is suppressed in regions around the T70H breakpoints in Rb4T70H/Rb4+ multivalents (chapter 5).

Fertility of Rb4T70H/Rb4T1Wa males from the successive backcrosses of the Mat+Rb stock was not constant in time (tables 7, 8). At backcross generations RbB5 and RbB15 testis weights and sperm counts were higher compared with RbF1 and RbB1 from the previous section (table 7). At RbB15, the double heterozygous and homozygous segregants had a similar mean testis weight and sperm count, a situation not encountered in any other generation (table 7). Fertility of the double heterozygous segregants produced at RbB20 and successive generations was decreased compared with RbB15 (table 7: testis weights and sperm counts; table 8: fractions of fertile males and number of implants). Breeding pairs of all different Rb4T70H/Rb4T1Wa pedigrees produced a varying percentage of fertile double heterozygous sons in each generation.

Generation RbB25 was produced by fathers homozygous for haplotype C. Similar to the RbB1 double heterozygous males with haplotype C, testis weights and sperm counts of the RbB25 double heterozygous males were elevated compared with testis weights and sperm counts of the males of the previous generations carrying haplotype A on chromosome 11-13^{1}H. Also the mean testis weight and sperm counts of the Rb4T70H homozygous haploype A/C segregants were high. Therefore, the presence of haplotype C seems to favour the spermatogenic process.

Since RbB20, the Rb4T1Wa translocation chromosomes have also been transmitted through double heterozygous males to analyse the effect of multi-generational *paternal* transmission of the 1^{13}Wa chromosome through a heteromorphic bivalent configuration on male fertility of the double heterozygous progeny (Pat+Rb stock). Similar to the Mat+Rb stock, most Rb4T70H/Rb4T70H mothers were homozygous for haplotype A (table 2). Despite the fact that the mean testis weight and sperm count of the maternally and paternally derived double heterozygous offspring did not differ, the percentage of fertile males and the number of implants produced was higher with paternal transmission over the last five generations (table 8).

In summary, male fertility of Rb4T70H/Rb4T1Wa progeny produced at different backcross generations of crosses between double heterozygous females and Rb4T70H homozygous males (Mat+Rb and Pat+Rb) varied in time but was almost always elevated over the RbF1 and RbB1 generations. The percentage fertile males is higher after paternal than maternal transmission of the T1Wa translocation chromosomes.

Table 8: Fertility indices of Rb4T70H/Rb4T1Wa double heterozygous males from RbF1 and successive backcross generations.

Backcross Generation	*Number of Analysed Males*	*% Fertile Males according to Mating Test*[1]	*Sperm Count*[2]	*Number of Implants*[3] *mean ± sd (n females)*	*% Large Moles*[3]	*% Small Moles*[3,4]
Maternal transmission of the Rb4T1Wa genome:						
RbF1	45	35	35	9.4 ± 4.6 (30)	0	24.1
RbB1	27	41	52	10.7 ± 3.3 (17)	1.8	20.5
RbB11	25	96	-	13.5 ± 3.6 (47)	0.8	22.8
RbB15	20	75	80	12.7 ± 1.9 (18)	0	25.9
RbB20-RbB24[5]	70	57	66	10.1 ± 4.0 (54)	0.4	24.7
RbB25	21	43	71	10.0 ± 3.9 (13)	0.8	23.1
Paternal transmission of the Rb4T1Wa genome:						
RbB20-RbB24[6]	45	73	87	11.4 ± 2.8 (64)	0.3	26.2

sd = standard deviation
1: A male is declared fertile when at least one conceptus, expressed as a decidual reaction, is produced after two matings with NMRI females.
2: Correction for males which have a sperm count > 100 but do not produce offspring in two matings with NMRI females.
3: Results from uterine inspection of NMRI females at day 13 of pregnancy mated with fertile double heterozygous males (vaginal plug formation at day 0 of development).
4: Caused by early embryonic death of the segregating deletion carriers of the chromosome 1 region between the T70H and T1Wa breakpoints.
5: Data of generations B20 (n = 32), B22 (n = 21), B23 (n = 9) and B24 (n = 8).
6: Data of generations B20 (n = 18), B22 (n = 6), B23 (n = 8) and B24 (n = 13).

Male fertility of the T70H/T1Wa double heterozygotes is improved by introduction of a single copy of Rb(11.13)4Bnr.

Data presented in tables 5 to 9 show that homozygosity for Rb4 improves fertility of the T70H/T1Wa males, especially after successive backcrosses. To analyse the effects of (*i*) a single dosis of Rb4 and (*ii*) repeated backcrossing of the T1Wa translocation chromosomes via Rb4T70H/Rb4T1Wa females on male fertility of the T70H/T1Wa offspring several additional crosses were performed.

Cross A: The RbB19 double heterozygous males from the Mat+Rb stock which had been used as founders of the Pat+Rb stock were mated with T70H homozygous females. Mean testis weight and sperm count of the +T70H/Rb4T1Wa offspring were slightly increased compared with the RbB20 double heterozygous segregants sired by the same fathers in the Pat+Rb stock (N.S.; tables 7, 9).

Table 9: Testis weights and epididymal sperm counts of double heterozygous and homozygous offspring produced in crosses A to D as described in the tekst.

Cross Code	*Cross* *Male*		*Female*	*+T70H/Rb4T1Wa* *Number of Mice*	*Testis Weight mean ± sd*[a]	*Sperm Count mean ± sd*	*+T70H/Rb4T70H* *Number of mice*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*
A	Rb4T70H/Rb4T1Wa	x	T70H/T70H	13	85.4 ± 13.6	174.2 ± 92.4	9	92.5 ± 9.6	224.2 ± 76.4
B	T70H/T70H	x	Rb4T70H/Rb4T1Wa	13	65.2 ± 6.8	58.3 ± 35.5			

a: sd = standard deviation

Cross C: +T70H/Rb4T1Wa (male) x +T70H/+T70H (female)

+T70H/Rb4T1Wa			*+T70H/Rb4T70H*			*+T70H/+T1Wa*			*+T70H/+T70H*		
Number of Mice	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*	*Number of Mice*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*	*Number of Mice*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*	*Number of Mice*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*
6	60.8 ± 18.5[1]	72.0 ± 62.1[2]	5	83.9 ± 9.5	-	10	34.9 ± 8.3[1]	1.1 ± 1.2[2]	13	108.1 ± 13.1	-

Mann-Whitney U tests: 1: $P < 0.01$ 2: $P < 0.028$

Cross D: Rb4T70H/Rb4T70H (male) x T70H/T1Wa (female) T70H/T70H (male) x T70H/T1Wa (female)

Rb4T70H/+T1Wa			*Rb4T70H/+T70H*			*+T70H/+T1Wa*			*+T70H/+T70H*		
Number of Mice	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*	*Number of Mice*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*	*Number of Mice*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*	*Number of Mice*	*Testis Weight mean ± sd*	*Sperm Count mean ± sd*
8	65.7 ± 13.2[1]	150.6 ± 52.7[2]	13	80.2 ± 10.4	205.1 ± 87.7	10	40.5 ± 3.7[1]	0.8 ± 2.4[2]	14	120.0 ± 9.5	425.3 ± 113.9

Mann-Whitney U tests: 1: $P < 0.001$ 2: $P < 0.001$

Cross B: In contrast, crosses between RbB19 double heterozygous females and T70H/T70H males produced +T70H/Rb4T1Wa males which had significantly smaller testes compared with the double heterozygous segregants of the RbB20 generation in the Mat+Rb stock ($P < 0.01$; tables 7, 9). Sperm counts did not significantly differ among males of both karyotypes.

Cross C: Several +T70H/Rb4T1Wa males of cross B were mated with T70H homozygous females. Both male fertility estimates were significantly lower in the T70H/T1Wa versus the +T70H/Rb4T1Wa littermates (table 9; $P < 0.01$; $P < 0.028$).

Cross D: Finally, T70H/T1Wa females from the first and second generation of the Mat-Rb stock were mated with either T70H or Rb4T70H homozygotes. Again, fertility estimates were significantly lower for the double heterozygotes in the absence of Rb4 than the presence of one copy of Rb4 ($P < 0.001$ for both parameters; table 9). Apparently, male infertility which is characteristic for the Mat-Rb heterozygotes can be restored by introducing a single copy of Rb4 into the T70H/T1Wa karyotype.

In accordance with the reduced male fertility of Rb4 heterozygotes, fertility of the +T70H/Rb4T70H segregants which were produced in any of the crosses A, C or D was significantly impaired compared with the contemporary T70H or Rb4T70H homozygous controls (table 9).

The T1Wa translocation chromosomes of the T70H/T1Wa male segregants produced in cross D had been transmitted for 18 generations in the Mat+Rb stock and 1 or 2 generations in the Mat-Rb stock. Testes of these males were significantly smaller than testes of the males produced in the 1996 B1 cross after one maternal transmission ($P < 0.001$). In the same way, testis weights of the T70H/T1Wa males sired in cross C by the +T70H/Rb4T1Wa males were significantly lower compared with the same karyotype produced in the 1996 B1 generation after one paternal transmission ($P < 0.001$). Thus, multi-generation transmission of the T1Wa translocation chromosomes through double heterozygotes seems to have a negative effect on testis development in the double heterozygous segregants lacking Rb4.

Influence of Rb4 on the synaptic behaviour of the small heteromorphic bivalent in double heterozygous males.

To be able to correlate male fertility data with chromosome behaviour at meiotic prophase we analysed the SC configurations of the small heteromorphic bivalent in spermatocytes of sterile and fertile Rb4T70H/Rb4T1Wa males of the RbF1, RbB5 and RbB19 generations. In a previous study, the testis weights and sperm counts of five F1 T70H/T1Wa males were positively correlated with the percentage of spermatocytes showing a fully synapsed small heteromorphic bivalent which had a symmetrical SC configuration (fig. 1c; Peters *et al.*, 1997b; chapter 3). In the present study on Rb4T70H/Rb4T1Wa males, fully synapsed

Karyotype	T70H / T1Wa			Rb4T70H / Rb4T1Wa		
Cross	F 1	B22/B21	B21	RbF1	RbB5	RbB19
Transmission	-	Mat/Pat	Pat	-	Mat	Mat
n Mice	5	3	1	5	3	4
Testis Weight (mean ± sd)	78.9 ± 37.7	39.6 ± 2.9	45.8	63.2 ± 20.4	-	71.8 ± 16.0
Sperm Count (mean ± sd)	77.4 ± 99.8	0 ± 0	1	42.8 ± 80.1	210.7 ± 29.2	68.0 ± 61.4
n Cells	255	80	27	206	200	149

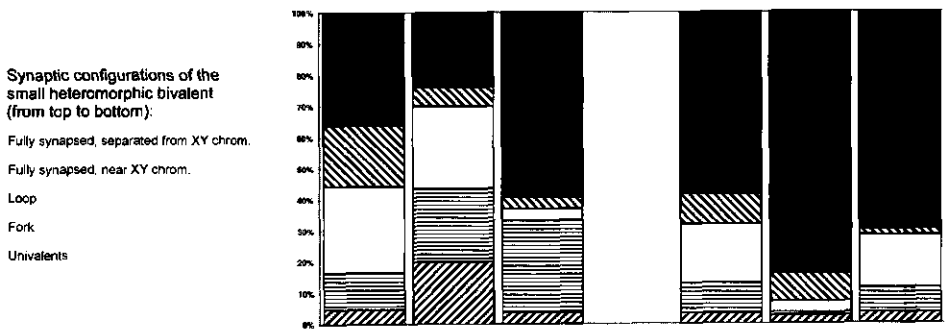


Figure 3: Fertility indices and synaptic behaviour of the small heteromorphic bivalent in (Rb4) double heterozygous males produced in the (Rb)F1 crosses and several different backcrosses. F1 data are from Peters *et al.* (1997b, chapter 3).

bivalents with a symmetrical SC configuration were identified indirectly according to their position relative to the XY bivalent (see Peters *et al.*, 1997b, chapter 3).

Three sterile and two fertile Rb4T70H/Rb4T1Wa males which were produced by homozygous parents (RbF1) had significantly more fully synapsed small heteromorphic bivalents dislocated from the XY chromosomes than five F1 T70H/T1Wa males which even had a higher mean testis weight and sperm count (2 steriles and 3 fertiles; fig. 3; $\chi^2_1 = 21.67$; $P < 0.001$). The same conclusion arises from the comparison of individual RbF1 sterile and fertile males with F1 sterile and fertile males respectively (data not shown). Three RbB5 double heterozygous males of the Mat+Rb stock (table 1, 7) had very high sperm counts. In these males, the percentage of fully synapsed bivalents (84% of 200 nuclei; fig. 3) was significantly higher compared with the two most fertile males of RbF1 (66% of 80 nuclei; $\chi^2_1 = 9.78$; $P < 0.001$). At RbB19, two fertile males had a similar percentage of fully synapsed bivalents (76% of 88 nuclei) as the RbB5 males ($\chi^2_1 = 2.02$). This percentage was significantly reduced in two sterile RbB19 males (61% of 61 nuclei) compared with RbB5 males ($\chi^2_1 = 13.76$; $P < 0.001$). In summary, we conclude that homozygosity for Rb4 favours

synapsis of the small heteromorphic bivalent. Moreover, backcross fertile males have a higher degree of synapsis than the fertile RbF1 males.

Finally, since the testis weights of the backcross males of the Mat-Rb stock lacking Rb4 were reduced compared with the testis of sterile 1996 F1 males, we wanted to find out whether synapsis was additionally impaired in the Mat-Rb males. Two sterile B22 T70H/T1Wa segregants of the Mat-Rb stock and one sterile T70H/T1Wa male sired by a +T70H/Rb4T1Wa male (cross C) had a similar low degree of fully synapsed heteromorphic bivalents (20% of 99 nuclei; fig. 3) compared with the two sterile F1 males (23% of 94 nuclei; males A and B in Peters *et al.*, 1997b, chapter 3). Sixty percent of 27 small bivalents were fully synapsed in a fourth sterile male sired by the same +T70H/Rb4T1Wa father (fig. 3). All four backcross males had significantly more 1^{13}-univalents and small bivalents with a fork configuration (41% of 107 nuclei; fig. 1c) compared with the sterile (16% of 94 nuclei) or all five analysed F1 (17% of 255 nuclei) males (fig. 3; $\chi^2_1 = 14.09$; $P < 0.001$; $\chi^2_1 = 23.94$; $P < 0.001$). Thus, in the male, synapsis of homologous chromosome segments seems to be affected after successive passages of the 1^{13}Wa chromosome through meiosis of double heterozygous females as is heterologous synapsis.

Influence of Rb4 on the synaptic behaviour of the small heteromorphic bivalent in double heterozygous females.

To study the influence of Rb4 on non-homologous chromosome synapsis during female meiosis as well as to investigate the effect of maternal transmission of the (Rb4)T1Wa translocation chromosomes on fertility of double heterozygous male offspring, we analysed the synaptic behaviour of the small heteromorphic bivalent during female meiotic prophase. Both, the double heterozygous and the duplication karyotypes, have one 1^{13}Wa and one 1^{13}H chromosome which are able to form a heteromorphic bivalent (fig. 2b). It was impossible to discern between these two karyotypes by analysis of the two large 13^1 chromosomes in fetal liver chromosome preparations. Therefore, we pooled the SC data of these females. (Rb4)T70H homozygotes could be positively identified by the presence of two very small 1^{13}H chromosomes.

Oocytes of RbB22/23 Mat+Rb and B24 Mat-Rb females were analysed. The T1Wa chromosomes of the B24 Mat-Rb karyotypes were maternally transmitted for 19 generations in the presence of Rb4 and for another 4 generations in the absence of Rb4. Early pachytene oocytes (day 16 of gestation) of B24 double heterozygous/duplication females displayed similar frequencies of the different synaptic configurations of the small bivalent as did 16 days old F1 females generated by homozygous parents (fig. 4; Peters *et al.*, 1997b, chapter 3). At day 18 however, late pachytene oocytes from 3 out of 4 B24 females had significantly

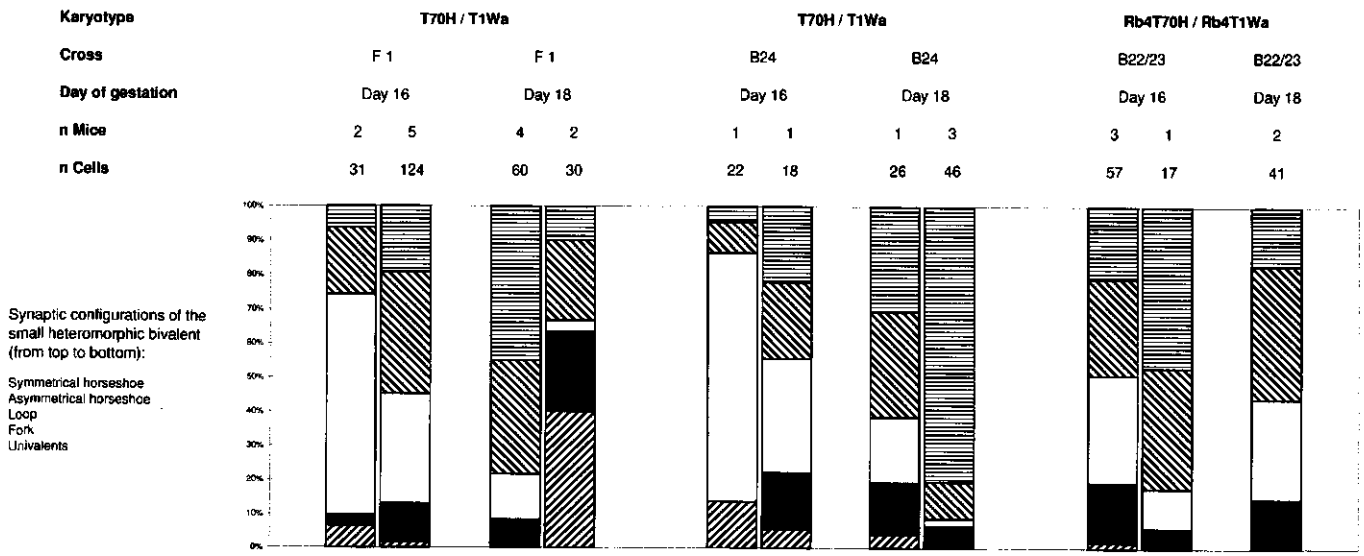


Figure 4: Percentages of different synaptic configurations of the small heteromorphic bivalent in pachytene nuclei of (Rb4) double heterozygous females at days 16 and 18 of gestation. (Rb4) backcross females were produced as described in the tekst. F1 data are from Peters *et al.* (1997b, chapter 3). Each column represents summed data of females with similar distributions of SC configurations for the 1^{13} bivalent.

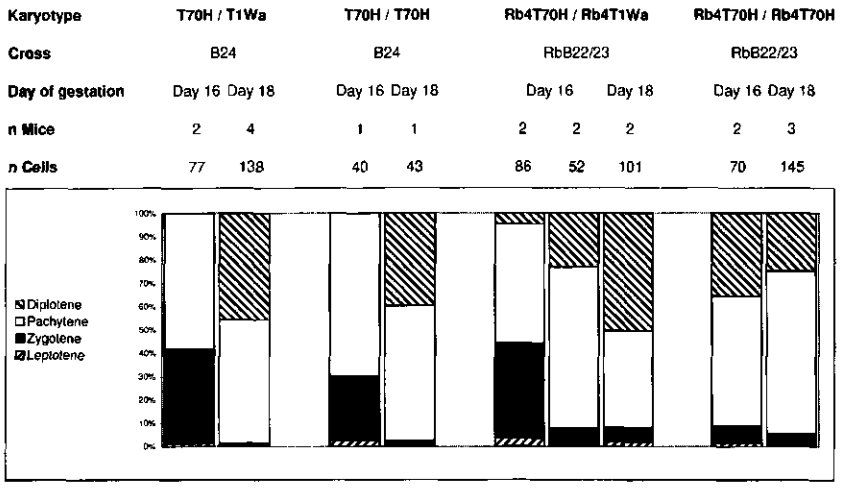


Figure 5: Distribution of foetal oocytes over successive meiotic prophase stages in (Rb4) double heterozygous backcross mice at days 16 and 18 of gestation.

more fully synapsed bivalents with a symmetrical horseshoe configuration than the 18 day old F1 females (fig. 4). The rate of synaptic adjustment was therefore higher in B24 compared with F1 females. The large heteromorphic bivalent behaved in a similar way (data not shown).

Compared with the karyotypes lacking Rb4 the degree of synapsis of the small bivalent was similar to or more advanced in oocytes of day 16 RbB22/23 heterozygous/duplication females. At day 18, the presumed late pachtene oocytes showed similar frequencies for the different synaptic configurations of the small bivalent as at day 16. Therefore, progression of non-homologous synapsis by synaptic adjustment seemed to be halted in the presence of the Rb4 (fig. 4).

A meiotic effect of Rb4 is also shown in the distribution of oocytes over the subsequent stages of the meiotic prophase (fig. 5). Oocytes of B24 heterozygous/duplication and homozygous littermates (fig. 5) progressed similarly through prophase as oocytes of F1 heterozygous or +/+ females (data not shown). In contrast, progression was more advanced in Rb4 containing heterozygotes/duplication carriers as well as homozygotes (of RbB22/23) when cells were sampled at the beginning of meiotic prophase (day 16 of gestation; fig. 5). Some females contained a few zygotene oocytes plus a considerable amount of diplotene oocytes (fig. 5). Over the subsequent two days of gestation meiotic progression occurred at a lower rate in the presence of Rb4 (fig. 5).

Discussion

Influence of the Robertsonian translocation Rb(11.13)4Bnr on chromosome synapsis and fertility of males, double heterozygous for the T(1;13)70H and T(1;13)1Wa translocations.

One object of this study was to analyse the influence of Rb(11.13)4Bnr on male fertility of mice double heterozygous for two nearly identical reciprocal translocations T70H and T1Wa in comparison with T70H homozygous controls. Crosses between +T70H/Rb4T1Wa males and T70H homozygous females and between T70H/T1Wa females and Rb4T70H or T70H homozygous males revealed that one copy of Rb4 is necessary and sufficient to retain or restore male fertility of the double heterozygous karyotype (crosses C and D in table 9).

These findings are unexpected since male fertility is reduced in both Rb4 heterozygotes and homozygotes as well as heterozygous and homozygous compounds with T70H (Nijhoff and de Boer, 1979; Gropp and Winking, 1981; Nijhoff, 1981; Wessels-Kaalen *et al.*, 1986; Hansmann *et al.*, 1988; Redi and Capanna, 1988; Everett *et al.*, 1996). Similarly, we observed that both testis weights and sperm counts of the +T70H/Rb4T70H males are reduced compared with either T70H or Rb4T70H homozygous controls (tables 5, 6, 9). In addition, sperm counts of Rb4T70H homozygotes are significantly lower in comparison with T70H homozygous controls (tabels 5, 6).

In order to determine the role of Rb4 on synapsis of the reciprocal translocation chromosomes in relation to male fertility, we analyzed SC configurations of the small heteromorphic bivalent in spermatocytes of sterile and fertile Rb4T70H/Rb4T1Wa males of different backcross generations. Similar to T70H/T1Wa males, the percentage of nuclei with a fully non-homologously synapsed small heteromorphic bivalent in the Rb4T70H/Rb4T1Wa males was positively correlated with testis weight and sperm count though the variation is higher in the Rb4 containing karyotype (fig. 6). Thus, Rb4 clearly favours non-homologous synapsis of the small heteromorphic bivalent (figs. 3, 6) thereby improving male fertility. However, despite a higher degree of synapsis, the testis weights and sperm counts of the Rb4T70H/Rb4T1Wa males were relatively low in comparison with the same karyotype lacking Rb4 (fig. 6). Rb4 must therefore also exert a deleterious influence on spermatogenesis in the double heterozygous karyotype, presumably of similar nature as in Rb4 and Rb4T70H homozygous males (see Everett *et al.*, 1996).

Like in T70H/T1Wa males (Peters *et al.*, 1997b, chapter 3), the synaptic configuration of the small heteromorphic bivalent in spermatocytes of Rb4T70H/Rb4T1Wa males seemed to be defined before or at the onset of pachytene as only a minor progression in synapsis was observed from mid to late pachytene substages (data not shown). From this we conclude that Rb4 is not involved in promoting synaptic adjustment during the pachytene stage but that it functions as a modifier of chromatin reorganization of the small 1^{13} translocation

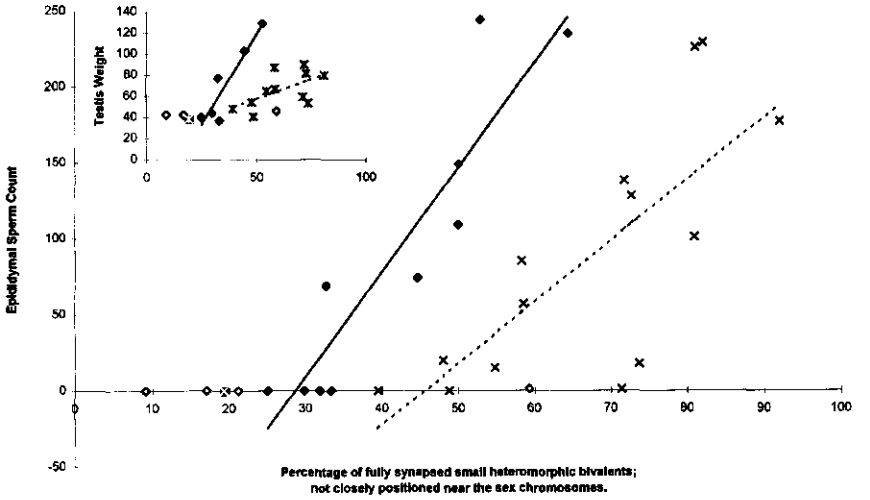


Figure 6: Epididymal sperm count and testis weight (in mg; insert) as a function of the percentage of fully synapsed small heteromorphic bivalents dislocated from the sex chromosomes in T70H/T1Wa (diamonds) and Rb4T70H/Rb4T1Wa (crosses) males. Linear regression analyses were performed using the data represented by filled diamonds and crosses. This figure includes data from de Boer *et al.* (1986), Peters *et al.* (1997b, chapter 3) and the present study.

chromosomes during the leptotene and/or zygotene stages allowing immediate non-homologous synapsis at the late zygotene and/or early pachytene stages. Two non-mutually exclusive mechanisms can be envisaged for how Rb4 mediates this function: (*i*) genetic "Rb4" modifiers of *Mus poschiavinus* origin or (*ii*) prolongation of the zygotene stage due to the presence of Rb4.

Several linkage studies revealed suppression of recombination in the proximal region of chromosome 13 but not of chromosome 11 in Rb4 heterozygotes (Cattanach *et al.*, 1972; Cattanach and Moseley 1973; Philips *et al.*, 1980; Davisson and Akeson, 1993). Therefore, although all translocation stocks used in the present study are maintained in an outbreeding program on the Swiss random bred background, the promoting influence of Rb4 on non-homologous synapsis could be due to the presence of some modifier genes near the centromeres of the Rb4 chromosomes which retained *Mus poschiavinus* Rb alleles.

Alternatively, the Rb4 modifier function might be related to the duration of the period during which the pre-meiotically organized chromatin of the resting pre-leptotene primary spermatocytes is reorganized for meiotic chromosome pairing and synapsis. For the mouse, several different chromosomal aberrations, including Rb4, have been described in which the duration of the period between pre-meiotic S-phase and diakinesis is increased in at least a portion of the spermatocytes (Forest, 1982; Speed and de Boer, 1983; Wauben-Penris *et al.*,

1985; Hansmann *et al.*, 1988). Thus, the presence of Rb4 in the double heterozygous karyotype might increase the duration of the initial stages of meiotic prophase and consequently enable the chromatin of the two differently sized 1^{13} chromosomes to be conditioned for non-homologous synapsis as observed at the early pachytene stage. Similarly, even a single copy of Rb4 could fulfill this task resulting in fertile double heterozygous males.

Non-homologous chromosome synapsis in heteromorphic bivalents in successive backcross generations: genetic or epigenetic inheritance ?

This study shows that fertility of double heterozygous males lacking Rb4 is impaired after one or several successive (maternal and paternal) meiotic transmissions of the T1Wa translocation chromosomes in the presence of the T70H chromosomes, permitting non-homologous chromosome synapsis in heteromorphic bivalents (tables 5, 9). This raises the question whether male fertility of the T70H/T1Wa males is influenced by one or more genetic modifiers or that it is mediated through de novo genetic mutations or an epigenetic modification mechanism.

As shown previously (Peters *et al.*, 1997b, chapter 3; this study), fertility of the 1996 F1 males was family dependent. Pedigree analysis of the T1Wa translocation chromosomes of the four F1 fertile families revealed that these chromosomes originated from two crosses between T1Wa homozygous males (T/T) and T1Wa heterozygous (T/+) females. Similarly, T1Wa chromosomes of the three sterile F1 families originated from two different T/T x T/+ crosses. Furthermore, since the double heterozygous progeny of both the F1 and B1 "sterile" families were sterile (figs. 3, 11; 1996 data), these data suggest that the capability of the 1^{13}Wa chromosome to synapse non-homologously with the 1^{13}H chromosome (Peters *et al.*, 1997b, chapter 3) is closely linked to the breakpoint regions of the 13^{1}Wa and especially the 1^{13}Wa translocation chromosomes.

Both maternal and paternal transmission of the T1Wa translocation from the fertile 1996 F1 families resulted in a decrease in male fertility of almost all B1 double heterozygotes (table 5). A similar decrease was found for the 1991 B1 generation after paternal transmission (table 5). In contrast, homozygous T70H segregants of all these fertile families had higher sperm counts than the T70H/T70H males of the sterile families and the contemporary control stocks. If a hypothetical male fertility modifier is positioned on the 1^{13}Wa chromosome (to explain these fertility data), it must be located distal of the T1Wa breakpoint on the translocated chromosome 13 segment (see fig. 2) because of the following reasons: (*i*) the Δ1 segment which undergoes non-homologous synapsis in the small heteromorphic bivalent is almost or completely excluded from meiotic recombinational exchange (Wauben-Penris *et al.*, 1983; chapter 6); (*ii*) all double heterozygous and T70H homozygous offspring retained their smooth

and fuzzy hair coat respectively indicating that the obligate crossover in the small heteromorphic bivalent was positioned distal of the T1Wa breakpoint.

Statistical analysis revealed that both the decrease in male fertility of the double heterozygous segregants and the increase in sperm count of the T70H homozygous segregants in the 1996 B1 maternal and paternal fertile families occurred in a non-Mendelian way (T70H/T1Wa: $\chi^2_1 = 3.27$; $P < 0.05$ (fertility estimates of 19 out of 24 B1 T70H/T1Wa males were lower in comparison with F1 T70H/T1Wa males of the same fertile family lineages); T70H/T70H: $\chi^2_1 = 8.02$; $P < 0.005$ (sperm counts of 19 out of 20 T70H/T70H males of the B1 fertile families were higher compared with the males of the B1 sterile families or T70H control stock). Thus, the existence of an enhancer locus for male fertility on the 1^{13}Wa chromosome of the T70H/T1Wa males of the 1996 F1 fertile families is highly unlikely.

This conclusion is enforced by the observation that male fertility in none of the B1 T70H/T1Wa males of the sterile family was increased compared the F1 T70H/T1Wa males of the same lineage. If enhancer alleles of male fertility are located at the distal end of chromosome 13, the non-Medelian segregation of male fertility in the sterile 1996 family lineages and 1991 maternal crosses is unexpected as chiasma frequencies in the distal segments of the 1^{13}Wa and 1^{13}H chromosomes are high in meiotic quadrivalents of both female single translocation heterozygotes (T1Wa/+ and T70H/+ respectively; de Boer *et al.*, 1983; van den Berg, pers. communication) and also in double heterozygotes. Moreover, we did not perform selection for that distal segment in either the T70H/T1Wa or T70H/T70H karyotypes.

Because of the reduced chiasma frequency in the long heteromorphic bivalent of double heterozygous females (compared with T70H homozygous females; Wauben-Penris *et al.*, 1983), the hypothetical male fertility modifier locus is likely also not positioned near the T1Wa breakpoint on the large 13^{1}Wa chromosome.

Alternatively, non-homologous synapsis within the heteromorphic bivalent could be regulated by a locus located within or near the Δ1 segment on the 13^{1}H chromosome. However, males of the maternal and paternal fertile B1 families had significantly higher testes weight in comparison with males of the B1 sterile families though all males contained the same haplotype E on their 13^{1}H chromosome (fertile versus sterile families: $P < 0.0027$). Moreover, a similar non-Mendelian decrease in male fertility was also observed for the 1991 B1 double heterozygous progeny after paternal transmission (table 5).

In conclusion, it is hard to explain the variability in fertility in different F1 and B1 generations (Peters *et al.*, 1997b, chapter 3; this study) in terms of introgression of alleles into regions near the translocation breakpoints of any of the different translocation chromosomes.

Non-homologous chromosome synapsis in heteromorphic bivalents in successive backcross generations is likely mediated by an epigenetic modification mechanism.

In table 9 we showed that fertility of the T70H/T1Wa males from the B21/B22 backcross generations was restored by the introduction of a single copy of Rb4 (table 9). These findings exclude the gain and accumulation of genetic mutations on the T1Wa chromosomes during one or more successive transmissions via heteromorphic bivalents as the cause of the reduction of male fertility observed in the T70H/T1Wa progeny of the backcrosses.

Because testis weight and sperm counts are positively correlated with the percentage of fully synapsed bivalents (fig. 6), we interpret the decrease in testis weights and sperm counts of the backcross versus F1 males in terms of a decrease in the percentage of spermatocytes with a fully synapsed heteromorphic bivalent. The low percentage of fully synapsed configurations of the T70H/T1Wa males in the backcross generation B21/B22 support this interpretation.

The inheritance of the synaptic state over one or several generations is analogous to the inheritance of allelic blueprints for methylation patterns as described in humans and mice (Silva and White, 1988; Sasaki *et al.*, 1991). Cumulative epigenetic inheritance has been observed for the TKZ751 transgene when it was introduced into the BALB/C strain (Allen *et al.*, 1990). Successive maternal transmission of the initially hypomethylated and highly expressed TKZ751 transgene from the DBA/2 strain into BALB/C strain resulted in accumulation of methylation and inactivation of the transgene (Allen *et al.*, 1990). Reversal of the nonexpressing BALB/C phenotype by repeated backcrossing to the DBA/2 strain could only be achieved if the transgenome was not fully methylated. For the HBsAg transgene locus an irreversible repression was noticed in the first generation when an outbred transgenic male was crossed with a C57Bl/6 female (Hadchouel *et al.*, 1987).

In analogy with these transgenes, we propose that the 1^{13}Wa chromosome acquires an epigenetic modification as result of non-homologous pairing and/or synapsis in a heteromorphic bivalent during meiosis of both sexes. During the zygotene stage of the subsequent male meiotic prophase this "non homologous synapsis imprint" disturbs chromosome pairing and synapsis in the small heteromorphic bivalent (fig. 3). In females, it improves synaptic adjustment during pachytene (fig. 4). Interestingly, impairment of male fertility in the B1 generations was independent from the parental origin of the imprint (table 5). Likewise, the maternal and paternal imprints might be of an identical nature.

The 1^{13}H chromosome might also acquire an epigenetic modification during non-homologous synapsis in a small heteromorphic bivalent which functions like an enhancer of male fertility of the T70H homozygous segregants in the fertile B1 families.

In retrospect, differences in chromosome synapsis and fertility between F1 males of different families and/or of different generations (1982, 1991, 1996 from Peters *et al.*, 1997b,

chapter 3) might not have a genetic cause. Instead, it might be related to the quantitative and/or qualitative differences in the epigenetic load of 1^{13}Wa chromosomes resulting from non-homologous synapsis within meiotic quadrivalents of different T1Wa/+ females and subsequent homologous synapsis in the T1Wa homozygous males, that were used in the F1 crosses.

Rb4 functions as a temporal modifier of the "non-homologous chromosome synapsis imprint".

Because the introduction of a single copy of Rb4 into the T70H/T1Wa males from the B21/B22 backcross generations is sufficient to restore male fertility, Rb4 must function like a dominant modifier of the hypothesized non-homologous synapsis imprint. How does Rb4 mediate this function? As suggested above, prolongation of the early stages of meiotic prophase could give the chromatin of the 1^{13}Wa time to overcome the meiotic imprint and would enable the small translocation chromosomes to become conditioned for non homologous synapsis at the pachytene stage. Thus, according to this model, Rb4 functions as a temporal modifier of non-homologous chromosome synapsis in double heterozygous males. In double heterozygous and/or duplication females, Rb4 seems to delay synapsis during pachytene (fig. 4).

Male fertility of the T70H/T1Wa segregants produced in the second outcross generation from the Mat+Rb stock to the T70H background (cross C) was more severely impaired (table 9) than of the T70H/T1Wa males of 1996 B1 sterile families (table 5). This suggests that the 1^{13}Wa chromosome is also able to cumulatively gain an epigenetic imprint over successive female meiotic passages in the presence of Rb4 (Mat+Rb stock) which is functionally active in males after removal of Rb4.

Improvement of non-homologous synapsis in the presence of Rb4 is due to inheritance of the imprint.

The high fertility characteristics of the double heterozygous segregants during the first 15 backcross generations in the Mat+Rb stock (table 4; fig. 6) suggest a maternal accumulation of an imprint which consequently favours heterologous synapsis of the 1^{13}H and 1^{13}Wa chromosomes in Rb4 containing males. The decrease in male fertility during generations RbB20-RbB24 (fig. 6) and the low penetrance of male fertility within a pedigree over these generations might indicate that the heritable epigenetic modification becomes increasingly more expressed in a mosaic-like manner or gets lost completely in the female germline. This is in accordance with the SC data of the RbB22/23 females in which synaptic adjustment was not encountered during the progression of pachytene (fig. 4). Characteristics of male fertility and its penetrance were higher after paternal transmission (Pat+Rb stock) in comparison with

maternal transmission but decreased in time as well (tables 7, 8). Similarly, fertility of the +T70H/Rb4T1Wa males was higher after paternal than maternal transmission (table 9: crosses A and B respectively). Apparently, the germline of fertile males is more homogeneous for the accumulated imprint than the female germline at the RbB20-RbB24 generations. This might be due a stronger selection for germ cells with non-homologously synapsed small heteromorphic bivalents in males to sire the next generation. The decrease in male fertility over the last five generations is difficult to explain. It might be related to the general reduction in fitness in the Mat+Rb and Pat+Rb stocks as indicated by a decrease in body weight and postnatal viability (chapter 6).

Nature of the non-homologous synapsis imprint.

Assuming that the nature of the imprint is independent from its parental origin, it must resist the major chromatin rearrangements occurring during spermiogenesis and it must be heritable through the germline. Moreover, the imprint must be able to influence chromosome pairing and/or synapsis by regulating chromatin structure. Finally, the imprint must be able to be modified during meiotic prophase of both sexes. As for classical genomic imprinting (Searle and Beechey, 1978; Barlow, 1995) cytosine methylation might be a good candidate.

Cytosine methylation differences have been detected for several uniparentally expressed imprinted genes (Brandeis *et al.*, 1993; Norris *et al.*, 1994; Glen *et al.*, 1993; Sutcliff *et al.*, 1994; Bartolomei *et al.*, 1993; Ferguson-Smith *et al.*, 1993). However, by analyzing how these patterns are established in the embryo, it has become clear that most differential methylation, such as seen associated with Igf2 and the H19 genes, is not directly present in the gametes, but is rather added after implantation in a sex specific way (Brandeis *et al.*, 1993). Though, a maternal gametic methylation imprint has been detected in Igf2r (Stoger *et al.*, 1993) and Xist (Ariel *et al.*, 1995; Zuccotti and Monk, 1995).

At 12.5 days post coitum (dpc), before the onset of sexual differentiation of the primordial germ cells, tissue-specific genes and imprinted genes are unmethylated in these cells (Kafri *et al.*, 1992; Brandeis *et al.*, 1993; Ariel *et al.* 1994). *De novo* methylation is first observed at 15.5 dpc in the germline of both sexes which coincides with the onset of female meiotic prophase. Between 15.5 - 18.5 dpc, the time period of meiotic prophase in females, global methylation occurs in the gonads of both sexes. A bimodal pattern of methylation is formed meaning that CpG islands remain unmethylated and non-island sequences are invariably methylated much as in the embryo proper (Kafri *et al.*, 1992). Therefore, cytosine methylation of 1^{13}Wa sequences could function as the maternal imprint for non-homologous synapsis.

Recently, Jue *et al.* (1995) showed that the DNA methyltransferase enzyme is expressed during all stages of meiotic prophase except for the pachytene stage. Thus, methylation of

1[13]Wa sequences by this enzyme (which has *de novo* and maintenance methylation activities) could act as the paternal imprint if the imprint is acquired during the leptotene and zygotene stages. This is in accordance with the temporal profile of non-homologous synapsis.

Quantitative trait locus for male fertility on mouse chromosome 1?

The presence of haplotype C, a chromosome 1 recombinant of haplotype A and the Swiss +/+ genome, majorly increased male fertility of both double heterozygous and homozygous segregants in the RbB1 (1996) and RbB25 generations (tables 6, 7). Similar observations were made after segregation of haplotype F in the Rb4T70H "homozygous" stock (table 6) showing that haplotype C functions like a genetic dominant modifier. This might suggest the localization of a major quantitative trait locus (QTL) for male fertility distally from D1Mit122 on mouse chromosome 1.

Acknowledgments

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Chapter 8

General discussion

Discussion

In this thesis, I have analyzed meiotic chromosome synapsis in mice carrying reciprocal translocations between chromosome 1 and 13. Synapsis was studied in:
(*i*) mice heterozygous for the T(1;13)70H translocation (de Boer, 1976; de Boer *et al.*, 1983), and
(*ii*) mice that were double heterozygous for two near identical reciprocal translocations, namely the T70H and the T(1;13)1Wa translocation (de Boer *et al.*, 1986).

I paid special attention to synapsis of the segment on chromosome 1 between the T70H and T1Wa breakpoints (Δ1 segment) and studied the possible correlations between (a) synapsis within this segment, (b) male fertility, (c) meiotic recombination within this segment and (d) viability of carriers of this segment.

In the T70H/T1Wa double heterozygous karyotype, the Δ1 segments are positioned on two different heteromorphic bivalents which undergo extensive non-homologous synapsis during prophase of the first meiotic division. During zygotene stage, apposition of chromatin of the two ectopic Δ1 segments was observed (Peters *et al.*, 1997b, chapter 3). Yet, as quadrivalents have never been encountered during the subsequent meiotic prophase I and diakinesis-metaphase I stages, these segments are hindered in homologous interactions and are excluded from crossing over in the T70H/T1Wa karyotype (Wauben-Penris *et al.*, 1983; de Boer *et al.*, 1986; Peters *et al.*, 1997b, chapter 3).

In multivalent configurations of (Rb4)T70H heterozygotes, the region distal of the T70H breakpoint on chromosome 1 is also engaged in extensive non-homologous synapsis during prophase I (chapter 5). The extent of disturbed meiotic recombinational interactions between and within chromosome 1 and 13 sequences respectively in this region is not exactly known but likely encompasses the Δ1 segment of the T70H/T1Wa system.

The Robertsonian translocation Rb(11.13)4Bnr influenced synapsis of the Δ1 segment in mice with either one of the above mentioned chromosomal constitutions.

After induction of T70H on the genetic backcground of the C3H/HeH x 101/H inbred strains (Lyon *et al.*, 1964) and of T1Wa on the genetic background of a multiple translocation stock (homozygous for Rb(11.13)4Bnr, Rb(9.19)163H and Rb(6.15)1Ald) (de Boer *et al.*, 1977), these translocation have been outcrossed to the Swiss random bred stock (chapter 5). The founding and outcross history made it possible to use genetic variation near the breakpoints, as defined by microsatellite polymorphisms, to analyze introgression of alleles between the

Swiss genome and the translocation chromosomes and to study genomic stability for these sequences in case of heterologous synapsis.

In this chapter, I will discuss both genetic and potential epigenetic aspects of non-homologous interaction, also making use of information of pilot experiments executed on the sideline of the main experiments.

Chromosome stability in heteromorphic bivalents.

A significantly higher mutation frequency for the uninterrupted tetra-repeat microsatellite D1Mit122 was found in double heterozygous (Rb4)T70H/(Rb4)T1Wa than in homozygous (Rb4)T70H meiosis (for the localization of D1Mit122 in the Δ1 segment, see chapter 4). Alteration of the allele on the 1^{13}Wa chromosome into the corresponding allele of 13^{1}H chromosome and vice versa are indications for the occurrence of ectopic gene conversions despite the absence of an intact SC over the Δ1 segments which belong to the two different heteromorphic bivalents (chapters 3, 7).

To investigate chromosome pairing as one prerequisite for gene conversion we executed a pilot *in situ* hybridization experiment involving the St2 gene which harbours the D1Mit122 microsatellite in its 7th intron (Tominaga *et al.*, 1991). Spread early meiotic prophase nuclei of T70H/T1Wa males were selected that showed apposition of the two Δ1 segments (see fig. 5 of chapter 3). Hybridization with the 40 kb genomic probe for St2 usually results in one big or two smaller aligned discrete signals per individual heteromorphic bivalent, one for each sister chromatid (chapter 2). In one out of six zygotene nuclei displaying close apposition of the axial elements of the homologous Δ1 segments, the four St2 signals were fused into a clover-leaf like signal, suggestive for pairing (fig. 1). Thus, DNA sequences from the ectopic homologous Δ1 segments of double heterozygotes could be successful in the DNA homology search during early meiotic prophase.

In addition, bar-like signals of the Atm and Rpa proteins between the ectopic homologous Δ1 segments of zygotene and early pachytene spermatocyte nuclei (Plug, 1997) suggest that at least some initial steps of the meiotic recombinational process have preceded synaptonemal complex formation in the double heterozygous karyotype.

Taken together, these data could serve to imply that homologous interactions can be success-fully completed in the absence of a morphologically normal SC. However, this never results in forma-tion of crossovers (Wauben-Penris *et al.*, 1983; de Boer *et al.*, 1986; Peters *et al.*, 1997b, chapter 3).

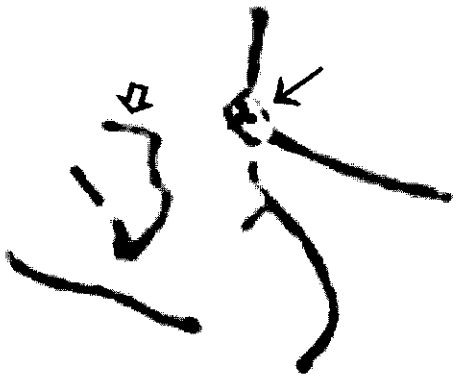

Figure 1: Ectopic homologous pairing between chromosome 1 sequences positioned on the large (arrow) and small (arrowhead) heteromorphic bivalent of a T70H/T1Wa male. Immunocytological detection of SCs (in grey) with a polyclonal anti-SC serum and subsequent in situ hybridization with a 40-kb genomic probe of the St2 gene (in black) was performed on surface sedimented nuclei as described in chapter 2.

Consequences of non-homologous chromosome synapsis for phenotypic characteristics of meiotic segregants.

As has been amply discussed in chapter 5, we interpret the slight but significant departure from a 1:1 segregation ratio in (Rb4)T70H/(Rb4)+ females, backcrossed to translocation homozygous males, as an indication for transmissable "damage" that followed from non-homologous interactions within the (Rb4)T70H multivalent during female meiosis.

One interpretation could be that, unlike the double heterozygous system, where homologous interactions for the Δ1 segment are only possible in early meiosis (heterosynapsis or "synaptic adjustment" follows immediately upon or jointly with normal synapsis, chapter 3), the non-homologous interactions around the centre of the translocation multivalents, where partner exchange takes place, are of a more gradual nature and maybe mixed with homologous interactions depending on the distance from the translocation breakpoints (chapter 5). As also indicated in chapter 5, T70H and Rb4T70H differ in this respect: synapsis in segments near the points of partner exchange (centre of the cross) is completed later during male meiotic prophase in the Robersonian translocation carriers which likewise have less recombination in these regions (chapter 5). T70H heterozygotes show more early non-homologous SC formation around the centre of the cross (the stalk region of fig. 9 in chapter 5). These synaptic configurations could predispose for an accumulation of recombination

Table 1: Fitness and fertility characteristics of matings between Rb4T1Wa homozygotes[a] during subsequent generations.

Intercross	% Fertile	Littersize[b]	% Death	% Death	Body Weight at Day 42	
Generation	Breeding Pairs	mean ± sd	(Days 0-5)	(Days 5-20)	Females	Males
-	-	-	-	-	23.8 ± 2.5 (10)	27.0 ± 2.7 (6)
1	85 (7)	7.1 ± 3.1[c]	7.8	32.4	19.8 ± 2.4 (20)	22.8 ± 4.1 (29)
2	70 (10)	5.2 ± 3.7[d]	9.4	44.8	19.6 ± 1.8 (17)	21.2 ± 5.0 (16)
3	20 (5)	5.0 ± 2.5[e]	0	33.3	16.7 ± 0.9 (5)	21.2 ± 5.9 (5)

a: Stock was set up with Rb4T1Wa homozygotes originating from the intercross between Rb4T70H/Rb4T1Wa homozygotes of the Mat+Rb stock (see chapter 6).
b: In most breeding pairs, the first litter was clearly larger than the following ones. If available, upto three litters were used for calculation
c: 1-3 Litters per breeding pair d: 1-2 Litters per breeding pair e: 3 Litters

intermediates that are not properly processed. Hence, this region is a candidate one for immunocytochemical analyses of proteins involved in recombination such as Rad51, Rpa and Mlh-1 (Plug, 1997), where the cytological timing of the latter one falls within the timespan of "stalk formation" for T70H/+ (Baker *et al.*, 1996).

Even after 25 generations of "female" transmission of the Δ1 segment through a situation of non-homologous synaptic adjustment (chapter 3), using the double translocation system, no phenotypic defects could be observed in either Rb4T70H/Rb4T1Wa double heterozygous (dominant effects) or Rb4T1Wa homozygous progeny (recessive effects) (chapter 6). Thus, based on this analysis, the situation of a disruption of homologous interactions resolved by speedy heterologous synapsis and symmetrical SC formation (chapter 3) is less detrimental for development than the "homology gradient" supposed to exist around the breakpoints of reciprocal translocations (chapter 5). For "male" transmission in double heterozygotes, the situation is not different (chapter 6).

Are the multi-generational consequences of non-homologous chromosome synapsis for chromosome stability only expressed in the homozygous state?

The homozygous Rb4T1Wa offspring from the double heterozygous intercross, as reported in chapter 6, was used to found a small homozygous breeding nucleus. As shown in table 1, both a decline in "breeding pair" fertility and in female body size was observed. As male sperm counts and testis weights of the Rb4T1Wa homozygotes (data not shown) could not explain the increase in sterility over successive generations, female fertility is suspected (first litters were markedly bigger than subsequent ones; table 1). Contemporary attempts to construct a

parallel Rb4T1Wa homozygous line originating from matings between Rb4 and T1Wa homozygotes (see chapter 6) likewise were not successful. Clearly, these results stimulate research into the effects of multi-generation structural meiotic homozygosity for regions that have adapted to a situation of partner exchange (and its consequences for a "homologous" SC, chapter 5), akin to the transmission of translocation carriers via heterozygotes only or via an alternation of homozygote and heterozygote generations (chapter 5).

In this respect, T70H and T1Wa are interesting reciprocal translocations as none of them could be made homozygous during the first generations after their induction (T70H: C.V. Beechey, pers. communication). For T70H, this was ascribed to a recessive lethal locus, induced in combination with the translocation but subsequently lost by recombination (Searle *et al.*, 1971). Position effects that were counteracted by epigenetic changes could likewise explain these findings.

Male fertility and meiotic pairing.

In mice, heterozygosity for reciprocal translocations characterized by one breakpoint at the very proximal and one at the very distal end of the chromosomes is often correlated with synaptic failure in the short interstitial chromosome segment and reduced or fully impaired male fertility (de Boer and de Jong, 1989). In this respect, T70H is a remarkable translocation as complete synapsis prevails in T70H/+ males which accordingly have a sperm count only marginally lower than normal (de Boer *et al.*, 1986; de Boer, 1986).

Chromatin that is not organised in the framework of an SC is thought to elicit a meiotic checkpoint function leading the spermatogenic cell into apoptosis (Peters *et al.*, 1997b, chapter 3; P.S. Burgoyne, pers. communication). Apparently, non-homologous synapsis fully substitutes for the fullfillment of requirements pertaining to this checkpoint that likely is Atm mediated (Xu *et al.*, 1996).

Immunocytochemical studies on T70H/T1Wa spermatocytes (see Plug, 1997) revealed the presence of Rad51 protein on the small 1^{13} heteromophic bivalents even after this protein had disappeared from normal bivalents during first meiotic prophase (data not shown). However, at the level of the fluorescence microscope, it is impossible to determine whether these small heteromorphic bivalents were symmetrically or asymmetrically synapsed (see chapter 3 for difference). Yet, the fact that a positive correlation for the frequency of symmetrically synapsed 1^{13} bivalents and the sperm count was found (chapter 3) points into the direction of the presence of unprocessed Rad51 intermediates on asymmetrically synapsed heteromorphic bivalents.

Surprisingly, the Robertsonian translocation Rb4 was found to boost male fertility of the double heterozygous karyotype though Rb4 on its own is known to act as a fertility reducer in both the heterozygous and homozygous condition (chapter 7). No straightforward genetic explanation for this phenomenon has been brought up in chapter 7. This finding, combined with the multi-generational trends in male fertilty of double heterozygotes with and without the Rb4 translocation, favours an epigenetic explanation for the modulation of the frequeny of small heteromorphic bivalents with a symmetrical SC (chapter 7). Akin to this explanation is the notion that meiotic chromosome behaviour contains a memory function that can be modified by an epigenetic mechanism.

Due to the relatively large size of the Δ1 chromosome segment (see chapter 3, 4), analysis at the molecular level is impossible. However, the system presented here is suitable for *in situ* investigation with candidate molecules that already are and will be the result of research in the areas of chromatin organisation, nuclear structure and gene expression (DNA methyation, protein acetylation; Hendrich and Willard, 1995).

Haplotype C

The fact that crossover suppression in the Δ1 segment of the Rb4T70H long marker chromosome led to the preservation of what most likely is the "founder (T70H) haplotype" (chapter 5), enabled us to study the consequences of a crossover between D1Mit20 and D1Mit 121, which resulted in haplotype C containing Swiss alleles distal of the site of recombination, for fertility and fittnes of progeny. The following effects were seen:

a) an increase in litter size of Rb4T70H homozygotes (both heterozygous and homozygous effects, chapter 5)

b) an improvement of the life expectancy of Δ1 duplication offspring from double heterozygous mothers in heterozygous condition (chapter 6)

c) a clear improvement of male fertility in Rb4 carrying double heterozygotes and T70H homozygotes.

When a qualitative genetic approach is followed, these results could be explained by supposing one or two alleles of Swiss origin of genes with pleiotrophic action. As candidate genes that could explain the effects observed are not known for this segment of chromosome 1 (Seldin, 1996; Mouse Genome Database), other explanations can be considered too.

When chromatin configuration has a hereditable aspect in the confinement of small segments (and not only through genetic polymorphisms of genes contributing to chromatin structure), interaction between newly imported chromatin (from +/+, see chapter 5) and the homologous segment from translocation origin could be at the origin of the pleiotrophic phenomena involved. Interestingly, positive effects ensue for traits where heterosis is easily observable (fertility, survival).

Especially the littersize data of the breeding lines in fig. 9 of chapter 5 are intriguing in that the phenomenon of paramutation (Jorgenson, 1994) is observable in the crosses between haplotype A/A Rb4T70H homozygotes, of which one of the parents is from haplotype A/C descent. As the one and multi-generation male fertility data of the double heterozygous stocks could most easily be explained by supposing a sequence independent mechanism (chapter 7), the powerfull influence of haplotype C gives more thought to the existence of these mechanisms in the framework of transmission.

Alberts, B., Bray D., Lewis j., Raff M., Roberts K. and Watson J.D. (1987) In: *Molecular Biology of the* Cell (Adams R. and Walker A., Eds) Second edition. Garland Publishing Incl., New York. pp 845-855

Albini, S.M. and Jones G.H. (1987) Synaplonemal complex spreading in *Alium cepa* and *A. fistulosum I.* The initiation and sequence of pairing. *Chromosoma* **95:** 324-338.

Allen, N.D., Norris M.L. and Surani M.A. (1990) Epigenetic control of transgene expression and imprinting by genotype-specific modifiers. Cell 61: 853-861.

Anderson, L.K., Offenberg H.H., Verkuijlen W.M.H.C. and Heyting C. (1997) RecA like proteins are components of early meiotic nodules in lily. *Proc. Natl. Acad. Sci. U.S.A.* **94:** 6868-6873.

Anderson, L.K. and Stack, S.M. (1988) Nodules associated with axial cores and synaptonemal complexes during zygotene in *Psilotum nudum. Chromosoma* **97:** 96-100.

Ariel, M., Cedar H. and McCarrey J. (1994) Developmental changes in methylation of spermatogenesis-specific genes include reprogramming in the epididymis. *Nat. Genet.* **7:** 59-63.

Ariel, M., Robinson E., McCarrey J.R. and Cedar H. (1995) Gamete-specific methylation correlates with imprinting of the murine Xist gene. *Nat. Genet.* **9:** 312-315.

Ashley, T., Moses M.J. and Solari A.J. (1981) Fine structure and behaviour of a pericentric inversion in the sand rat, *Psammomys obesus. J. Cell. Sci.* **50:** 105-119.

Ashley, T. (1984) Application of the spreading techniques to structural heterozygotes. In: Bennett MD, Gropp A, Wolf U, eds. *Chromosomes Today.* George Allen and Unwin Ltd London, pp 80-89.

Ashley, T. (1988) G-band position effects on meiotic synapsis and crossing over. *Genetics* **118:** 307-317.

Ashley, T. (1990) Prediction of mammalian meiotic synaptic and recombinational behavior of inversion heterozygotes based on mitotic breakpoint data and the possible evolutionary consequences. *Genetica* **83:** 1-7.

Ashley, T. and Cacheiro N.L. (1990) Correlation between meiotic behavior and breakpoints with respect to G-bands in two X-4 mouse translocations: T(X;4)7R1 and T(X;4)8R1. *Cytogenet. Cell. Genet.* **53:** 178-184.

Ashley, T. (1994) Mammalian meiotic recombination: a reexamination. *Hum. Genet.* **6:** 587-93

Ashley, T., Ried T. and Ward D.C. (1994) Detection of nondisjunction and recombination in meiotic and postmeiotic cells from XY^{Sxr} [XY,Tp(Y)1Ct] mice using multicolor fluorescence in situ hybridization. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 524-528.

Ashley, T., Plug AW., Xu J., Solari A.J., Reddy G., Golub E.I. and Ward D.C. (1995) Dynamic changes in Rad51 distribution on chromatin during meiosis in male and female vertebrates. *Chromosoma* **104:** 19-28.

Bähler, J., Wyler T., Loidl J. and Kohli J. (1993) Unusual nuclear structures in meiotic prophase of fission yeast: A cytological analysis. *J. Cell. Biol.* **121:** 241-256.

Baker, S.M., Plug A.W., Prolla T.A., Bronner C.E., Harris A.C., Yao X., Christie D.M., Monell C., Arnheim N., Bradley A., Ashley T. and Liskay R.M. (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nat. Genet.* **13:** 336-342.

Barlow, C., Hirotsune S., Paylor R., Liyanage M., Eckhaus M., Collins F., Shiloh Y., Crawley J.N., Ried T., Tagle D. and Wynshaw B.A. (1996) Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell* **86:** 159-171.

Barlow, D.P. (1995) Gametic imprinting in mammals. Science 270: 1610-1613.

Bartolomei M.S., Webber A.L., Brunkow M.E. and Tilghman S.M. (1993) Epigenetic mechanisms underlying the imprinting of the mouse H19 gene. *Genes Dev.* **7:** 1663-1673.

Beechey C.V. and Evans E.P. (1996). Chromosomal variants: numerical variants and structural rearrangements. In: Lyon M.F., Rastan S. and Brown S.D.M., ed. *Genetic variants and strains of the laboratory mouse.* Oxford University Press, pp 1452-1506.

Bell, G.I., Horita, S. and Karam, J.H. (1984) The polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. Diabetes 33: 176-183.

Bennett, S.T., Lucassen A.M., Gough S.C., Powell E.E., Undlien D.E., Pritchard L.E., Merriman M.E., Kawaguchi Y., Dronsfield M.J., Pociot F., et-al (1995) Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat. Genet.* **9:** 284-292.

Bishop, D.K. (1994) RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. *Cell* **79:** 1081-1092.

de Boer, P. (1973) Fertile tertiary trisomy in the mouse (*Mus muculus*). *Cytogenet. Cell. Genet.* **12:** 435-442.

de Boer, P. and van Gijsen M. (1974). The location of the positions of the breakpoints involved in the T26H and T70H mouse translocations with the aid of Giemsa-banding. *Can. J. Genet. Cytol.* **16:** 783-788.

de Boer, P. (1976) Male meiotic behaviour and male and female litter size in mice with the T(2;8)26H and T(1;13)70H reciprocal translocations. *Genet. Res.* **27:** 369-387.

de Boer, P., van Buul P., van Beek R., van der Hoeven F.A., and Natarajan T. (1977) Chromosomal radiosensitivity and karyotype in mice using cultured peripheral blood lymphocytes, and comparison with this system in man. *Mutat. Res.* **42:** 379-394.

de Boer, P., Nijhoff J.H. and van der Hoeven F.A. (1977) Translocation induction in a triple metacentric stock. *Mouse News Lett.* **57:** 40

de Boer, P. and Searle A.G. (1980) Summary and synthesis. Workshop on chromosomal aspects of male sterility in mammals. *J. Reprod. Fertil.* **60:** 257-265.

de Boer, P. and Nijhoff J.H. (1981) Incomplete sex chromosome pairing of oligospermic male hybrids of *Mus musculus* and *M. musculus molossinus* in relation to the source of the Y chromosome and the presence or absence of a reciprocal translocation. *J. Reprod. Fertil.* **62:** 235-243.

de Boer, P. and Speed R.M. (1982) Meiosis of T70H translocation trisomic male mice. II Meiotic rate, spermatocyte interactions and fertility. *Chromosoma* **87:** 315-325.

de Boer, P., Stam P., van Oosten L. and Wauben-Penris P.J.J. (1983). Chiasma frequency and position in male and female mice of chromosomes involved in homozygous and heterozygous translocations and tertiary trisomy. *Genetica* **61:** 205-214.

de Boer, P. (1986) Chromosomal causes for fertility reduction in mammals. In: *Chemical Mutagens, Volume 10* (de Serres F.J., ed.) Plenum Publishing Corporation. pp. 427-467.

de Boer, P., Searle A.G., van der Hoeven F.A., de Rooij D.G. and Beechey C.V. (1986) Male pachytene pairing in single and double translocation heterozygotes and spermatogenic impairment in the mouse. *Chromosoma* **93:** 326-336.

de Boer, P. (1979) Proximal chiasma localization within an interstitial chromosome segment, a likely correlate of adjacent-2 segregation of translocation causing multivalents in the mouse. *Environmental Health Perspectives* **31:** 137-140.

de Boer, P. and de Jong J.H. (1989) Chromosome pairing and fertility in mice. In: *Fertility and Chromosome Pairing: Recent Studies in Plants and Animals.* (Gilles C.B., ed.) Boca Raton: CRC Press, pp. 37-76.

de Boer, P. P. and van der Hoeven F.A. (1991) Chromosome damage and non-disjunction measured at the first cleavage division in normal and chromosomally mutant female mice irradiated at the diakinesis stage of female meiosis. *Mutat-Res.* **248:** 155-162.

Bondeson, M.L., Dahl N., Malmgren H., Kleijer W.J., Tonnesen T., Carlberg B.M. and Pettersson U. (1995) Inversion of the IDS gene resulting from recombination with IDS-related sequences is a common cause of the Hunter syndrome. *Hum. Mol. Gen.* 4, 615-621.

Borodin, P.M., Gorlov I.P. and Ladygina T.Y. (1990) Synapsis in single and double heterozygotes for partially overlapping inversions in chromosome 1 of the house mouse. *Chromosoma* **99:** 365-370.

Borodin, P.M., Gorlov I.P. and Ladygina T.Y. (1992) Synaptic interrelationships between the segments of the heteromorphic bivalent in double heterozygotes for paracentric inversions in chromosome 1 of the house mouse. *Chromosoma* **101:** 374-379.

Brandeis, M., Kafri T., Ariel M., Chaillet J.R., McCarrey J., Razin A. and Cedar H. (1993) The ontogeny of allele-specific methylation associated with imprinted genes in the mouse. *EMBO J.* **12:** 3669-77

Braun, R.E., Behringer R.R,. Peschon J.J., Brinster R.L. and Palmiter R.D. (1989) Genetically haploid spermatids are phenotypically diploid. *Nature* **337:** 373-376.

Brunet, J.F., Denizot F., Luciani M.F., Roux-Dosseto M., Suzan M., Mattei M.G. and Golstein P. (1987). A new member of the immunoglobulin superfamily--CTLA-4. *Nature* **328:** 267-270.

Buard, J. and Vergnaud G. (1994) Complex recombination events at the hypermutable minisatellite CEB1 (D2S90). *Embo J.* **13:** 3203-3210.

Buard, J. and Jeffreys A.J. (1997) Big, bad minisatellites. *Nat. Genet.* **15:** 327-328.

Burgoyne, P.S. and Baker T.G. (1984) Meiotic pairing and gametogenic failur. In *Controlling Evenst in Meiosis.* 38th Symposium of the Society for Experimental Biology (ed. C.W.Evans and M.G.Dickinson) pp 349-362. Cambridge: Compagny of Biologists.

Burgoyne, P.S. and Baker T.G. (1985) Perinatal oocyte loss in XO mice and its implications for the aetiology of gonadal dysgenesis in XO women. *J. Reprod. Fertil.* **75:** 633-645.

Burgoyne, P.S., Mahadevaiah S. and Mittwoch U. (1985) A reciprocal autosomal translocation which causes male sterility in the mouse also impairs oogenesis. *J. Reprod. Fertil.* **75:** 647-652.

Cao, L., Alani E. and Kleckner N. (1990) A pathway for generation and processing of double-strand breaks during meiotic recombination in *Saccharomyces cerevisiae. Cell* **61:** 1089-1102.

Carpenter, A.T.C. (1975) Electron microscopy of meiosis in *Drosophila melanogaster* females: II. The recombination nodule—a recombination-associated structure at pachytene? *Proc. Natl. Acad. Sci. U.S.A.* **72:** 3186-3189.

Carpenter, A.T.C. (1987) Gene conversion, recombination nodules, and the initiation of meiotic synapsis. *Bioessays* **6:** 232-236.

Carpenter, A.T.C. (1994) Chiasma Function. *Cell* **77:** 959-962.

Carter, T.C., Lyon M.F. and Phillips R.J.S. (1955) Gene-tagged chromosome translocations in eleven stocks of mice. *J. Genet.* **53:** 154-166.

Cattanach, B.M., Williams B.E. and Bailey H. (1972) Identification of the linkage groups carried by the metacentric chromosomes of the tobacco mouse (*Mus poschiavinus*). *Cytogenetics* **11:** 412-423

Cattanach B.M. and Moseley H. (1973) Nodisjunction and reduced fertility caused by the tobacco mouse metacentric chromosomes. *Cytogenet. Cell. Genet.* **12:** 264-287

Cattanach, B.M. (1974) Position effect variegation in the mouse. *Genet. Res.* **23:** 291-306.

Cattanach, B.M. (1975) Control of chromosome inactivation. *Annu. Rev. Genet.* **9:** 1-18

Cattanach, B.M., Barr J.A, Evans E.P., Burtenshaw M., Beechey C.V., Leff S.E., Brannan C.I., Copeland N.G., Jenkins N.A. and Jones J. (1992) A candidate mouse model for Prader-Willi syndrome which shows an absence of Snrpn expression. *Nat-Genet.* **2:** 270-274.

Chandley, A.C. (1986) A model for effective pairing and recombination at meiosis based on early replicating sites (R-bands) along chromosomes. *Hum. Genet.* **72:** 50-57.

Chakraborty, R., Kimmel M., Stivers D.N. Davison L.J. and Deka R. (1997) Relative mutation rates at di-, tri-, and tetranucleotide microsatellite loci. *Proc. Natl. Acad. Sci. U.S.A.* **94:** 1041-1046.

Chikashige, Y., Ding D.Q., Funabiki H., Haraguchi T., Mashiko S., Yanagida M. and Hiraoka Y. (1994) Telomere-led premeiotic chromosome movement in fission yeast. *Science* **264:** 270-273.

Cole, T.J., Copeland N.G., Gilbert D.J., Jenkins N.A., Schutz G. and Ruppert S. (1992). The mouse CREB (cAMP responsive element binding protein) gene: structure, promoter analysis, and chromosomal localization. *Genomics* **13:** 974-982.

Counce, S.J. and Meyer G.F. (1973) Differentiation of the synaptonemal complex and the kinetochore in *Locusta* spermatocytes studied by whole mount electron microscopy. *Chromosoma* **44:** 231-253.

Coverley, D., Kenny M.K., Lane D.P. and Wood R.D. (1992) A role for the human single-stranded DNA binding protein HSSB/RPA in an early stage of nucleotide excision repair. *Nucl. Acids. Res.* **20:** 3873-3880.

Crooijmans, R.P., van Kampen A.J., van der Poel J.J. and Groenen M.A. (1994). New microsatellite markers on the linkage map of the chicken genome. *J. Hered.* **85:** 410-413.

Davisson, M. T. and Akeson E.C. (1993) Recombination suppression by heterozygous Robertsonian chromosomes in the mouse. *Genetics* **133**, 648-667.

Dickie, M.M. and Woolley G.W. (1950) Fuzzy mice. *J.Hered.* **:** 193-197.

Dietrich, A.J. and de Boer P. (1983) A sequential analysis of the development of the synaptonemal complex in spermatocytes of the mouse by electron microscopy using hydroxyurea and agar filtration. *Genetica* **61:** 119-129.

Dietrich, A.J. and Mulder R.J. (1983) A light- and electron microscopic analysis of meiotic prophase in female mice. *Chromosoma* **88:** 377-385.

Dietrich, W.F., Miller J.C., Steen R.G., Merchant M., Damron D., Nahf R., Gross A., Joyce D.C., Wessel M., Dredge R.D., Marquis A., Stein L.D. Goodman N., Page D.C. and Landler E.S. (1994). A genetic map of the mouse with 4,006 simple sequence length polymorphisms. *Nat. Genet.* **7:** 220-245.

Dym, M. and Fawcett D.W. (1971) Further observations on the numbers of spermatogonia, spermatocytes, and spermatids connected by intercellular bridges in the mammalian testis. *Biol. Reprod.* **4:** 195-215.

Edelmann, W., Cohen P.E., Kane M., Lau K., Morrow B., Bennett S., Umar A., Kunkel T., Cattoretti G., Chaganti R., Pollard J.W., Kolodner R.D. and Kucherlapati R. (1996) Meiotic pachytene arrest in MLH1-deficient mice. *Cell* **85:** 1125-1134.

Evans, E.P., Burtenshaw, M.D. and Ford C.E. (1972) Chromosomes of mouse embryos and newborn young: preparations from membranes and tail tips. *Stain Techn.* **47:** 229-234.

Everett, C.A., Searle J.B. and Wallace B.M.N. (1996) A study of meiotic pairing, nondisjunction and germ cell death in laboratory mice carrying Robertsonian translocations. *Genetical Research* **67:** 239-247.

Fairman, M.P. and Stillman B. (1988) Cellular factors required for multiple stages of SV40 DNA replication in vitro. *Embo J.* **7:** 1211-1218.

Fang, J.S. and Jagiello G.M. (1988) An analysis of the chromomere map and chiasmata characteristics of human diplotene spermatocytes. *Cytogenet. Cell. Genet.* **47:** 52-57.

Felsenstein, J. (1974) The evolutionary advantage of recombination. *Genetics* **78:** 737-756.

Ferguson-Smith, A.C., Sasakai H., Cattanach B.M. and Surani, M.A. (1993) Parental origin-specific epigentic modification of the mouse H19 gene. *Nature* **383:** 751-755

Forejt, J. (1974) Non-random association between a specific autosome and the X chromosome in meiosis of the male mouse: possible consequences of homologous centromeres' separation. *Cytogenet. Cell. Genet.* **19:** 369-383.

Forejt, J. and Gregorova S. (1977) Meiotic studies of translocations causing male sterility in the mouse. I. Autosomal reciprocal translocations. *Cytogenet. Cell. Genet.* **19:** 159-179.

Forejt, J., Gregorova S. and Goetz P. (1981) XY pair associates with the synaptonemal complex of autosomal male-sterile translocations in pachytene spermatocytes of the mouse (*Mus musculus*). *Chromosoma* **82:** 41-53.

Forejt, J. (1996) Hybrid sterility in the mouse. *Trends Genet* **12:** 412-417.

Gabriel-Robez, O. and Rumpler Y. (1994) The meiotic pairing behaviour in human spermatocytes carrier of chromosome anomalies and their repercussions on reproductive fitness. I: Inversions and insertions. A European collaborative study. *Ann. Genet.* **37:** 3-10.

Gabriel-Robez, O. and Rumpler Y. (1996) The meiotic pairing behaviour in human spermatocytes carrier of chromosome anomalies and their repercussions on reproductive fitness. II: Robertsonian and reciprocal translocations. A European collaborative study. *Ann. Genet.* **39:** 17-25.

Game, J.C., Sitney K.C., Cook V.E. and Mortimer R.K. (1989) Use of a ring chromosome and pulsed-field gels to study interhomolog recombination, double-strand DNA breaks and sister-chromatid exchange in yeast. *Genetics* **123:** 695-713.

Gillies, C.B. (1989) Preface. In: Gilles CB, ed. *Fertility and Chromosome Pairing: Recent Studies in Plants and Animals*. Boca Raton: CRC Press.

Gourdon, G., Radvanyi F., Lia A. S., Duros C., Blanche M., Abitbol M., Junien C. and Hoffmann R.H. (1997) Moderate intergenerational and somatic instability of a 55-CTG repeat in transgenic mice. *Nat. Genet.* **15:** 190-192.

Gropp, A., Tettenborn U. and Lehmann E. (1970) Chromosomenvariation vom Robertson'schen Typus bei der Tabakmaus, *M. poschiavinus* und ihren Hybriden mit der Laboratoriumsmaus. *Cytogenetics* **9:** 9-23.

Gropp, A. and Winking, H. (1981) Robertsonian translocations: cytology, meiosis, segregation patterns and biological consequences of heterozygosity. *Symp. Zool. Soc. Lond.* **47:** 141-181.

Guioli, S., Incerti B., Zanaria E., Bardoni B., Franco B., Taylor K., Ballabio A. and Camerino G. (1992) Kallmann syndrome due to a translocation resulting in an X/Y fusion gene. *Nat. Genet.* **1:** 337-340.

Guitart, M., Coll M.D., Ponsa M. and Egozcue J. (1985) Sequential study of synaptonemal complexes in mouse spermatocytes by light and electron microscopy. *Genetica* **67:** 21-30.

Hadchouel, M., Farza H., Simon D., Tiollais P. and Pourcel C. (1987) Maternal inhibition of hepatitis B surface antigen gene expression in transgenic mice correlates with de novo methylation. *Nature* **329:** 454-6

Haigh, J. (1978) The accumulation of deleterious genes in a population - Muller's ratchet. *Theoret. Pop. Biol.* **14:** 251-267.

Hansmann, I., de Boer P. and Speed, R.M. (1988) Aneuplody-related delay of meiotic development in the mouse and the Djungarian hamster. In: *The Cytogenetics of Mammalian Autosomal Rearrangements,* pp 295-314.

Harada, Y.N., Matsuda Y., Shiomi N. and Shiomi T. (1995). Complementary DNA sequence and chromosomal localization of xpg, the mouse counterpart of human repair gene XPG/ERCC5. *Genomics* **28:** 59-65.

Hasenkampf, C.A. (1984) Synaptonemal complex formation in pollen mother cells of *Tranescantia. Chromosoma* **90:** 275-284.

Hastbacka, J., de la Chapelle A., Kaitila I., Sistonen P., Weaver A. and Lander E. (1992) Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. *Nat. Genet.* **3:** 204-211.

Havekes, F.W., de Jong J., Heyting C. and Ramanna M.S. (1994) Synapsis and chiasma formation in four meiotic mutants of tomato (*Lycopersicon esculentum*). *Chrom. Res.* **2:** 315-325.

Hawley, R.S. and Friend S.H. (1996) Strange bedfellows in even stranger places: the role of ATM in meiotic cells, lymphocytes, tumors, and its functional links to p53. *Genes. Dev.* **10:** 2383-2388.

Hendrich, B.D. and Willard H.F. (1995) Epigenetic regulation of gene expression: the effect of altered chromatin structure from yeast to mammals. *Hum. Mol. Genet.* **4:** 1765-1777.

Heng, H.H.Q., Tsui L.C. and Moens P.B. (1994) Organization of heterologous DNA inserts on the mouse meiotic chromosome core. *Chromosoma* **103:** 401-407.

Heyer, W.D., Rao M.R.S., Erdile L.F., Kelly T.J. and Kolodner R.D. (1990) An essential *Saccharomyces cerevisiae* single-stranded DNA binding protein is homologous to the large subunit of human RP-A. *Embo J.* **9:** 2321-2330.

Heyting, C. (1996) Synaptonemal complexes: structure and function. *Curr. Opin. Cell. Biol.* **8:** 389-396.

Howell, W.M., and Black D.A. (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* **36:** 1014-1015.

Hunter, A, Jacob P., O'Hoy K., MacDonald I., Mettler G., Tsilfidis C. and Korneluk R.G. (1993) Decrease in the size of the myotonic dystrophy CTG repeat during transmission from parent to child: implications for genetic counselling and genetic anticipation. *Am. J. Med. Genet.* **45:** 401-407.

Jeffreys, A.J., MacLeod A., Tamaki K., Neil D.L. and Monckton D.G. (1991) Minisatellite repeat coding as a digital approach to DNA typing. *Nature* **354:** 204-209.

Jeffreys, A.J., Tamaki K., MacLeod A., Monckton D.G., Neil D.L. and Armour J.A.L. (1994) Complexe gene conversion events in germline mutation at human minisatellites. *Nat. Genet.* **6:** 136-145.

Jeffreys, A.J. and Neumann R. (1997) Somatic mutation processes at a human minisatellite. *Hum. Mol. Genet.* **6:** 129-136.

Jorgensen, R. (1994) Developmental significance of epigenetic impositions on the plant genome: a paragenetic function for chromosomes. *Dev. Genet.* **15:** 523-532.

Jue, K., Bestor T.H. and Trasler J.M. (1995) Regulated synthesis and localization of DNA methyltransferase during spermatogenesis. *Biol. Reprod.* **53:** 561-569.

Kafri, T., Ariel M., Brandeis M., Shemer R., Urven L., McCarrey J., Cedar H. and Razin A. (1992) Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. *Genes. Dev.* **6:** 705-714

Keegan, K.S., Holtzman D.A., Plug A.W., Christenson E.R., Brainerd E.E., Flaggs G., Bentley N.J., Taylor E.M., Meyn M.S., Moss S.B., Carr A.M., Ashley T. and Hoekstra M.F. (1996) The Atr and Atm protein kinases associate with different sites along meiotically pairing chromosomes. *Genes. Dev.* **10:** 2423-2437.

Keeney, S., Giroux C.N. and Kleckner N. (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88:** 375-384.

Kennedy, G.C., German M.S. and Rutter W.J. (1995) The minisatellite in the diabetes susceptibility locus IDDM2 regulates insulin transcription. *Nat. Genet.* **9:** 293-298.

Kleckner, N. (1996) Meiosis: how could it work? *Proc. Natl. Acad. Sci. U.S.A.* **93:** 8167-8174.

Klemenz, R., Hoffmann S. and Werenskiold A.K. (1989) Serum- and oncoprotein- mediated induction of a gene with sequence similarity to the gene encoding carcinoembryonic antigen. *Proc. Natl. Acad. Sci. U. S. A.* **86:** 5708-5712.

Kluin, P.M., Kramer M.F. and de Rooij D.G. (1982) Spermatogenesis in the immature mouse proceeds faster than in the adult. *Int. J. Androl.* **5:** 282-294.

Kodama, Y., Yoshida M.C. and Sasaki M. (1980) An improved silver staining technique for nucleolus organizing regions by using nylon cloth. *Jpn. J. Hum. Genet.* **25:** 229-233.

Kohli, J. (1994) Telomeres lead chromosome movement. *Current Biology* **4:** 724-727.

Kohli, J. and Bähler J. (1994) Homologous recombination in fission yeast: Absence of crossover interference and synaptonemal complex. *Experientia* **50:** 295-306.

Koike, M., Matsuda Y., Mimori T., Harada Y.N., Shiomi N. and Shiomi T. (1996). Chromosomal localization of the mouse and rat DNA double-strand break repair genes *Ku p70* and *Ku p80/XRCC5* and their mRNA expression in various mouse tissues. *Genomics* **38:** 38-44.

Kowalczykowski, S.C., Dixon D.A., Eggleston A.K., Lauder S.D. and Rehrauer W.M. (1994) Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.* **58:** 401-465.

Lafreniere, R.G., Rochefort D.L., Chretien N., Rommens J.M., Cochius J.I., Kalviainen R., Nousiainen U., Patry G., Farrell K., Soderfeldt B., Federico A., Hale B.R., Hernandez C.O., Sorensen T., Pouliot M.A., Kmiec T., Uldall P., Janszky J., Pranzatelli M.R., Andermann F., Andermann E. and Rouleau G.A.

(1997) Unstable insertion in the 5' flanking region of the cystatin B gene is the most common mutation in progressive myoclonus epilepsy type 1, EPM1. *Nat. Genet.* **15:** 298-302.

Lagerstedt, K., Karsten S.L., Carlberg B.M., Kleijer W.J., Tonnesen T., Pettersson U. and Bondeson M.L. (1997) Double-strand breaks may initiate the inversion mutation causing the Hunter syndrome. *Hum. Mol. Genet.* **6:** 627-633.

Lakich, D., Kazazian H.H.,Jr., Antonarakis S.E. and Gitschier J. (1993) Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. *Nat. Genet.* **5:** 236-241.

Lammers, J.H.M., Offenberg H.H., van Aalderen M., Vink A.C., Dietrich A.J. and Heyting C. (1994) The gene encoding a major component of the lateral elements of synaptonemal complexes of the rat is related to X-linked lymphocyte-regulated genes. *Mol. Cell. Biol.* **14:** 1137-1146.

Lichter, P., Chang C., Call K., Hermanson G., Evans G.A., Housman D. and Ward D.C. (1990) High-resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. *Science* **247:** 64-69.

Lifschytz, E. and Lindsley D.L. (1972) The role of X-chromosome inactivation during spermatogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **69:** 182-186.

Loidl, J. (1994) Cytological aspects of meiotic recombination. *Experientia* **50:** 285-294.

Loidl, J., Klein F. and Scherthan H. (1994) Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. *J. Cell. Biol.* **125:** 1191-1200.

Lynch, M. and Gabriel W. (1990) Mutation load and the survival of small populations. *Evolution* **44:** 1725-1737.

Lyon, M.F., Phillips R.J.S. and Searle A.G. (1964). The overall rates of dominant and recessive lethal and visible mutation induced by spermatogonial X-radiation of mice. *Genet. Res. Camb.* **5:** 448-467.

Lyon, M.F. and Hawker S.G. (1973) Reproductive lifespan in irradiated and unirradiated chromosomally XO mice. *Genet. Res.* **21:** 185-194.

Lyon, M.F., Cocking Y. and Gao X. (1996). Mouse chromosome atlas. *Mouse genome* **94:** 30-73.

Mahadevaiah, S., Mittwoch U. and Moses M.J. (1984) Pachytene chromosomes in male and female mice heterozygous for the Is(7;1)40H insertion. *Chromosoma* **90:** 163-169.

Malo, D., Vidal S., Lieman J.H., Ward D.C. and Gros P.(1993). Physical delineation of the minimal chromosomal segment encompassing the murine host resistance locus Bcg. *Genomics* **17:** 667-675.

Mangiarini, L., Sathasivam K., Mahal A., Mott R., Seller M. and Bates G.P. (1997) Instability of highly expanded CAG repeats in mice transgenic for the Huntington's disease mutation. *Nat. Genet.* **15:** 197-200.

Messier, P.E., Jean P. and Richer C.L. (1986) Easy transfer of selected mitoses from light to electron microscopy. *Cytogenet. Cell. Genet.* **43:** 207-210.

Metsaranta, M., Toman D., De Crombrugghe B. and Vuorio E. (1991). Specific hybridization probes for mouse type I, II, III and IX collagen mRNAs. *Biochem. Biophys. Acta* **1089:** 241-243.

Miklos, G.L. (1974) Sex-chromosome pairing and male fertility. *Cytogenet. Cell. Genet.* **13:** 558-577.

Mittwoch, U. and Mahadevaiah S.K. (1992) Unpaired chromosomes at meiosis: cause or effect of gametogenic insufficiency? *Cytogenet. Cell. Genet.* **59:** 274-279.

Moens, P.B., Heyting C., Dietrich A.J.J., van-Raamsdonk W. and Chen Q. (1987) Synaptonemal complex antigen localization and conservation. *J. Cell. Biol.* **105:** 93-103.

Monckton, D.G., Neumann R., Guram T., Fretwell N., Tamaki K., MacLeod A. and Jeffreys A.J. (1994) Minisatellite mutation rate variation associated with a flanking DNA sequence polymorphism. *Nat. Genet.* **8:** 162-170.

Monckton, D.G., Coolbaugh M.I., Ashizawa K.T., Siciliano M.J. and Caskey C.T. (1997) Hypermutable myotonic dystrophy CTG repeats in transgenic mice. *Nat. Genet.* **15:** 193-196.

Mongelard, F., Poras I., Usson Y., Batteux B., Robert-Nicoud M., Avner P. and Vourc'h C. (1996). A mouse chromosome-specific YAC probe collection for *in situ* hybridisation. *Genomics* **38:** 432-434.

Moore, S.P., Erdile L., Kelly T. and Fishel R. (1991) The human homologous pairing protein HPP-1 is specifically stimulated by the cognate single-stranded binding protein hRP-A. *Proc. Natl. Acad. Sci. U. S. A.* **88:** 9067-9071.

Moormann, R.J.M., den Dunnen J.T., Heuyerjans J., Jongbloed R.J.E., van Leen R.W., Lubsen N.H. and Schoenmakers J.G.G. (1985). Characterization of the rat γ-crystallin gene family and its expression in the eye lens. *J. Mol. Biol.* **182:** 419-430.

Moses, M.J. (1969) Structure and function of the synaptonemal complex. *Genetics* **61:** Suppl. 41-51.

Moses, M.J. (1968) Synaptonemal complex. *Annu. Rev. Genet.* **2:** 363-412.

Moses, M.J. (1977) Microspreading and the synpptonemal complex in cytogenetic studies. *Chromosomes Today* **6:** 71-82.

Moses, M.J. (1980) New cytogenetic studies on mammalian meiosis. In: Serio M, Martini L, eds. *Animal Models in Human Reproduction.* Raven Press, New York pp 169-190.

Moses, M.J. and Poorman P.A. (1981) Synaptosomal complex analysis of mouse chromosomal rearrangements. II. Synaptic adjustment in a tandem duplication. *Chromosoma* **81:** 519-35

Moses, M.J. and Poorman P.A. (1981) Synaptonemal complex analysis of mouse chromosomal rearrangements. II. Synaptic adjustment in a tandem duplication. *Chromosoma* **81:** 519-535.

Moses, M.J., Poorman P.A., Roderick T.H. and Davisson M.T. (1982) Synaptonemal complex analysis of mouse chromosomal rearrangements. IV. Synapsis and synaptic adjustment in two paracentric inversions. *Chromosoma* **84:** 457-474.

Moses, M.J., Dresser M.E. and Poorman P.A. (1984) Composition and role of the synaptonemal complex. *Symp. Soc. Exp. Biol.* **38:** 245-270.

Muller, H.J. (1964) The relation of recombination to mutational advantage. *Mut. Res.* **1:** 2-9.

Nag, D.K., Scherthan H., Rockmill B., Bhargava J. and Roeder G.S. (1995) Heteroduplex DNA formation and homolog pairing in yeast meiotic mutants. *Genetics* **141:** 75-86.

Naylor, J., Brinke A., Hassock S., Green P.M. and Giannelli F. (1993) Characteristic mRNA abnormality found in half the patients with severe haemophilia a is due to large DNA inversions. *Hum. Mol. Genet.* **2:** 1773-1778.

Nicolas, A., Treco D., Schultes N.P. and Szostak J.W. (1989) An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae. Nature* **338:** 35-39.

Nijhoff, J.H. and de Boer P. (1979) A first exploration of a Robertsonian translocantion heterozygote in the mouse for its usefulness in cytological evaluation of radiation-induced meiotic autosomal non-disjunction. *Mut. Res.* **61:** 77-86.

Nijhoff, J.H. (1981) Meiotic behaviour and spermatogenesis in male mice heterozygous for translocation types also occuring in man. *Thesis* at the *Agricutural University Wageningen.*

Norris, D.P., Patel D., Kay G.F., Penny G.D., Brockdorff N., Sheardown S.A. and Rastan S. (1994) Evidence that random and imprinted Xist expression is controlled by preemptive methylation. *Cell* **77:** 41-51

O'Hoy, K.L., Tsilfidis C., Mahadevan M.S., Neville C.E., Barcelo J., Hunter A.G.W. and Korneluk R.G. (1993) Reduction in size of the myotonic dystrophy trinucleotide repeat mutation during transmission. *Science* **259:** 809-812.

Offenberg, H.H., Dietrich A.J.J. and Heyting C. (1991) Tissue distribution of two major components of synaptonemal complexes of the rat. *Chromosoma* **101:** 83-91.

van den Ouweland A.M., Deelen W.H., Kunst C.B., Uzielli M.L.G., Nelson D.L., Warren S.T., Oostra B.A. and Halley D.J.J. (1994) Loss of mutation at the FMR1 locus through multiple exchanges between maternal X chromosomes. *Hum. Mol. Genet.* **3:** 1823-1827.

Owerbach, D. and Gabbay K.H. (1993) Localization of a type I diabetes susceptibility locus to the variable tandem repeat region flanking the insulin gene. *Diabetes* **42:** 1708-1714.

Padmore, R., Cao L. and Kleckner N. (1991) Temporal comparison of recombination and synaptonemal complex formation during meiosis in *Saccharomyces cerevisiae*. *Cell* **66:** 1239-1256.

Peters, A.H.F.M., Plug A.W., van Vugt M.J. and de Boer P. (1997a) A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chrom. Res.* **5:** 66-68.

Peters, A.H.F.M., Plug A.W. and de Boer P. (1997b) Meiosis in carriers of heteromorphic bivalents: sex differences and implications for male fertility. *Chrom. Res.* **5:** 313-324.

Plug, A.W., Xu J., Reddy G., Golub E.I. and Ashley T. (1996) Presynaptic association of Rad51 protein with selected sites in meiotic chromatin. *Proc. Natl. Acad. Sci. U.S.A.* **93:** 5920-5924.

Plug, A.W. (1997) Visualization of molecular mechanisms during mammalian meiosis: temporal and spacial resolution by antibody localization. *Thesis* at the *University of Utrecht.*

Poorman, P.A., Moses M.J., Davisson M.T. and Roderick T.H. (1981a) Synaptonemal complex analysis of mouse chromosomal rearrangements. III. Cytogenetic observations on two paracentric inversions. *Chromosoma* **83:** 419-429.

Poorman, P.A., Moses M.J., Russell L.B. and Cacheiro N.L. (1981b) Synaptonemal complex analysis of mouse chromosomal rearrangements. I. Cytogenetic observations on a tandem duplication. *Chromosoma* **81:** 507-518.

Radding, C.M. (1991) Helical interactions in homologous pairing and strand exchange driven by RecA protein. *J. Biol. Chem.* **266:** 5355-5358.

Redi, C.A. and Capanna, E. (1988) Robertsonian heterozygotes in the house mouse and the fate of their germ cells. In: *The Cytogenetics of Mammalian Autosomal Rearrangements,* pp 315-359.

Rice, W.R. (1987) Genetic hitchhiking and the evolution of reduced genetic activity of the Y sex chromosome. *Genetics* **116:** 161-167.

Rocco V., De Massy B. and Nicolas A. (1992) The *Saccharomyces cerevisiae* ARG4 initiator of meiotic gene conversion and its associated double-strand DNA breaks can be inhibited by transcriptional interference. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 12068-12072.

Rockmill, B., Sym M., Scherthan H. and Roeder G.S. (1995) Roles of two RecA homologs in promoting meiotic chromosome synapsis. *Genes. Dev.* **9:** 2684-2695.

Roeder, G.S. (1995) Sex and the single cell: Meiosis in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **92:** 10450-10456.

Rouyer, F., Simmler M.C., Page D.C. and Weissenbach J. (1987) A sex chromosome rearrangement in a human XX male caused by Alu-Alu recombination. *Cell* **51:** 417-25

Saitoh, Y. and Laemmli U.K. (1994) Metaphase chromosome structure: bands arise from a differential folding path of the highly AT-rich scaffold. *Cell* **76:** 609-622.

Sambrook, J., Fritsch E.F. and Maniatis T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sasaki, H., Hamada T., Ueda T., Seki R., Higashinakagawa T. and Sakaki Y. (1991) Inherited type of allelic methylation variations in a mouse chromosome region where an integrated transgene shows methylation imprinting. *Development* **111:** 573-81

Scherthan, H., Weich S., Schwegler H., Heyting C., Haerle M. and Cremer T. (1996) Centromere and telomere movements during early meiotic prophase of mouse and man are associated with the onset of chromosome pairing. *J. Cell. Biol.* **134:** 1109-1125.

Schmekel, K., Wahrman J., Skoglund U. and Daneholt B. (1993) The central region of the synaptonemal complex in Blaps cribrosa studied by electron microscope tomography. *Chromosoma* **102:** 669-681.

Schmekel, K. and Daneholt B. (1995) The central region of the synaptonemal complex revealed in three dimensions. *Trends in Cell Biology* **5:** 239-242.

Schwacha, A. and Kleckner N. (1995) Identification of double Holliday junctions as intermediates in meiotic recombination. *Cell* **83:** 783-791.

Searle, A.G., Ford C.E. and Beechey C.V. (1971). Meiotic disjunction in mouse trans-locations and the determination of centromere position. *Genet. Res. Camb.* **18:** 215-235.

Searle, A.G. and Beechey, C.V. (1974) Sperm-count, egg-fertilization and dominant lethality after X-irradiation of mice. *Mutat. Res.* **1:** 63-72

Searle, A.G. and Beechey C.V. (1978) Complementation studies with mouse translocations. *Cytogenet. Cell. Genet.* **20:** 282-303.

Searle, A.G., Beechey C.V., de Boer P., de Rooij D.G., Evans E.P. and Kirk M. (1983) A male-sterile insertion in the mouse. *Cytogenet. Cell. Genet.* **36:** 617-626.

Seldin, M.F. (1996). Encyclopedia of the mouse genome V. Mouse chromosome 1. *Mamm. Genome* **6:** S28-S50.

Setterfield, L.A., Mahadevaiah S. and Mittwoch U. (1988a) Chromosome pairing and germ cell loss in male and female mice carrying a reciprocal translocation. *J. Reprod. Fertil.* **82:** 369-379.

Setterfield, L.A., Mahadevaiah S. and Mittwoch U. (1988b) Pachytene pairing in relation to sperm and oocyte numbers in a male-fertile reciprocal translocation in the mouse. *Cytogenet Cell Genet* **49:** 293-299.

Sherman, J.D. and Stack S.M. (1995) Two-dimensional spreads of synaptonemal complexes from solanaceous plants. VI. High-resolution recombination nodule map for tomato (*Lycopersicon esculentum*). *Genetics* **141:** 683-708.

Siegel, S. (1956) Nonparametric statistics for the behavioral sciences. McGraw-Hill Kogakusha, Ltd.

Silva, A.J. and White R. (1988) Inheritance of allelic blueprints for methylation patterns. *Cell* **54:** 145-52

Smithies, O. and Powers P.A. (1986) Gene conversions and their relation to homologous chromosome pairing. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **312:** 291-302.

Speed, R.M. (1982) Meiosis in the foetal mouse ovary. I. An analysis at the light microscope level using surface-spreading. *Chromosoma* **85:** 427-437.

Speed, R.M. and Chandley A.C. (1983) Meiosis in the foetal mouse ovary. II. Oocyte development and age-related aneuploidy. Does a production line exist? *Chromosoma* **88:** 184-189.

Speed, R.M. and de-Boer P. (1983) Delayed meiotic development and correlated death of spermatocytes in male mice with chromosome abnormalities. *Cytogenet. Cell. Genet.* **35:** 257-262

Speed, R.M. (1986) Oocyte development in XO foetuses of man and mouse: The possible role of heterologous X-chromosome pairing in germ cell survival. *Chromosoma* **94:** 115-124.

Stack, S.M. and Roelofs D. (1996) Localized chiasmata and meiotic nodules in the tetraploid onion *Allium porrum*. *Genome* **39:** 770-783.

Stack, S.M, Sherman J.D., Anderson L.K. and Herickhoff L.S. (1993) in *Chromosomes Today,* eds. Sumner A.T. and Chandley A.C. (Chapman and Hall, London), Vol. 11, pp 301-311.

Stoger, R., Kubicka P., Liu C.G., Kafri T., Razin A., Cedar H. and Barlow D.P. (1993) Maternal-specific methylation of the imprinted mouse Igf2r locus identifies the expressed locus as carrying the imprinting signal. *Cell* **73:** 61-71

Storlazzi, A., Xu L., Schwacha A. and Kleckner N. (1996) Synaptonemal complex (SC) component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes. *Proc. Natl. Acad. Sci. U.S.A.* **93:** 9043-9048.

Strachan, T. and Read A.P. (1996) Human Molecular Genetics. Bios Scientific Publishers Ltd.

Suijkerbuijk, R.F., Looijenga L., de Jong B., Oosterhuis J.W., Cassiman J.J. and Geurts van Kessel A. (1992) Verification of isochromosome 12p and identification of other chromosome 12 aberrations in gonadal and extragonadal human germ cell tumors by bicolor double fluorescence in situ hybridization. *Cancer. Genet. Cytogenet.* **63:** 8-16.

Sun, H., Treco D., Schultes N.P. and Szostak J.W. (1989) Double-strand breaks at an initiation site for meiotic gene conversion. *Nature* **338:** 87-90.

Takiyama, Y., Sakoe K., Soutome M., Namekawa M., Ogawa T., Nakano I., Igarashi S., Oyake M., Tanaka H., Tsuji S. and Nishizawa M. (. (1997) Single sperm analysis of the CAG repeats in the gene for machado joseph disease (MJD1): evidence for non mendelian transmission of the MJD1 gene and for the effect of the intragenic cgg/ggg polymorphism on the intergenerational instability. *Hum. Mol. Genet.* **6:** 1063-1068.

Terasawa, M., Shinohara A., Hotta Y., Ogawa H. and Ogawa T. (1995) Localization of RecA-like recombination proteins on chromosomes of the lily at various meiotic stages. *Genes. Dev.* **9:** 925-934.

Tominaga, S. (1989). A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. *FEBS Lett.* **258:** 301-304.

Tominaga, S., Jenkins N.A., Gilbert D.J. Copeland N.G and Tetsuka T. (1991) Molecular cloning of the murine ST2 gene. Characterization and chromosomal mapping. *Biochem. Biophys. Acta.* **1090:** 1-8.

Tres, L.L. (1977) Extensive pairing of the XY bivalent in mouse spermatocytes as visualized by whole-mount electron microscopy. *J. Cell. Sci.* **25:** 1-15.

Vergnaud, G., Mariat D., Apiou F., Aurias A., Lathrop M. and Lauthier V. (1991) The use of synthetic tandem repeats to isolate new VNTR loci: Cloning of a human hypermutable sequence. *Genomics* **11:** 135-144.

Virtaneva, K., D'Amato E., Miao J., Koskiniemi M., Norio R., Avanzini G., Franceschetti S., Michelucci R., Tassinari C.A., Omer S., Pennacchio L.A., Myers R.M., Dieguez L.J.L., Krahe R., Chapelle A.D.L. and Lehesjoki A.L. (1997) Unstable minisatellite expansion causing recessively inherited myoclonus epilepsy, EPM1. *Nat. Genet.* **15:** 393-396.

Warren, S.T. (1996) The expanding world of trinucleotide repeats. *Science* **271:** 1374-1375.

Wauben-Penris, P.J., van der Hoeven F.A. and de Boer P. (1983) Chiasma frequency and nondisjunction in heteromorphic bivalents: meiotic behavior in T(1;13)70H/T(1;13)1Wa mice as compared to T(1; 13)70H/T(1;13)70H mice. *Cytogenet. Cell. Genet.* **36:** 547-553.

Wauben-Penris, P.J. and Prins J.B. (1983) Meiotic behavior of alloxan-treated diabetic and nondiabetic T(;13)70H/+ mice. *Hum. Genet.* **63:** 268-73.

Wauben-Penris, P.J., and van der Hoeven F.A. (1985) Meiotic nondisjunction and translocation segregation in dwarf (dw/dw) and control T(1;13)70H heterozygous mice. *J. Hered.* **76:** 341-344.

Wauben-Penris, P.J., van-Meel G.P. and deBoer P. (1985) Spermatogenic delay and increased chiasma frequency in T70H/+ male mice with hydroxyurea-Trenimon limited spermatocyte populations. *Can. J. Genet. Cytol.* **27:** 192-199.

Weber, J.L. and Wong C. (1993) Mutation of human short tandem repeats. *Hum. Mol. Genet.* **2:** 1123-1128.

Weil, D., Wang I., Dietrich A., Poustka A., Weissenbach J. and Petit C. (1994) Highly homologous loci on the X and Y chromosomes are hot-spots for ectopic recombinations leading to XX maleness. *Nat. Genet.* **7:** 414-419.

Weiner, B.M. and Kleckner N. (1994) Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. *Cell* **77:** 977-991.

Weith A. and Traut, W. (1980) Synaptonemal complexes with associated chromatin in moths, *Ephestia kuehniella Z. Chromosoma* **78:** 275-291.

Wessels-Kaalen, K. M., Bakker R. and de Boer P. (1986) Transmission of X-ray-induced reciprocal translocations in normal male mice and in male mice with a reduced sperm count due to translocation homozygosity. *Mutat. Res.* **163:** 181-191.

von Wettstein, W. D., Rasmussen S.W. and Holm P.B. (1984) The synaptonemal complex in genetic segregation. *Annu. Rev. Genet.* **18:** 331-413.

White, M.A., Detloff P., Strand M. and Petes T.D. (1992) A promoter deletion reduces the rate of mitotic, but not meiotic, recombination at the HIS4 locus in yeast. *Current Genetics* **21:** 109-116.

Winking, H., Reuter C. and Traut W. (1993) Meiotic synapsis of homogeneously staining regions (HSRs) in chromosome 1 of *Mus musculus*. *Chrom. Res.* **1:** 37-44.

Wobbe, C.R., Weissbach L., Borowiec J.A., Dean F.B., Murakami Y., Bullock P. and Hurwitz J. (1987) Replication of simian virus 40 origin-containing DNA in vitro with purified proteins. *Proc. Natl. Acad. Sci. U. S. A.* **84:** 1834-1838.

Wold, M.S. and Kelly T. (1988) Purification and characterization of replication protein A, a cellular protein required for in vitro replication of simian virus 40 DNA. *Proc. Natl. Acad. Sci. U. S. A.* **85:** 2523-2527.

Wu, T.C. and Lichten M. (1994) Meiosis-induced double-strand break sites determined by yeast chromatin structure. *Science* **263:** 515-518.

Xu, L., Ajimura M., Padmore R., Klein C. and Kleckner N. (1995) NDT80, a meiosis-specific gene required for exit from pachytene in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15:** 6572-6581.

Xu, Y., Ashley T., Brainerd E.E., Bronson R.T., Meyn M.S. and Baltimore D. (1996) Targeted disruption of ATM leads to growth retardation, chromosomal fragmentationduring meiosis, immune defects, and thymic lymphoma. *Genes. Dev.* **10:** 2411-2422.

Yen, P.H., Tsai S.P., Wenger S.L., Steele M.W., Mohandas T.K. and Shapiro L.J. (1991) X/Y translocations resulting from recombination between homologous sequences on Xp and Yq. *Proc. Natl. Acad. Sci. U.S.A.* **88:** 8944-8948.

Zahn , L.M. and Kwaitkowski D.J. (1995) *Genomics* **28:** 140-146.

Zhao, G.Q., Deng K., Labosky P.A., Liaw L. and Hogan B.L. (1996) The gene encoding bone morphogenetic protein 8B is required for the initiation and maintenance of spermatogenesis in the mouse. *Genes. Dev.* **10:** 1657-1669.

Zickler, D., Moreau P.J., Huynh A.D. and Slezec A.M. (1992) Correlation between pairing initiation sites, recombination nodules and meiotic recombination in *Sordaria macrospora*. *Genetics* **132:** 135-148.

Zneimer, S.M. and Womack J.E. (1988). Regional localization of the fibronectin and gamma crystallin genes to mouse chromosome 1 by in situ hybridization. *Cytogenet. Cell. Genet.* **48:** 238-241.

Zuccotti, M. and Monk M. (1995) Methylation of the mouse Xist gene in sperm and eggs correlates with imprinted Xist expression and paternal X-inactivation. *Nat. Genet.* **9:** 316-320.

Summary

In the mouse, heterozygosity for several reciprocal and Robertsonian translocations is associated with impairment of chromosome synapsis and suppression of crossover formation in segments near the points of exchange during prophase of meiosis. This thesis describes the analysis of the consequences of the occurrence of non-homologous synapsis and/or suppression of meiotic crossover formation over many successive generations for male fertility and viability of the progeny.

For studying chromosome synapsis, we modified a drying down technique which results in high yields of nuclei of all first meiotic prophase stages in both male and female from only small amounts of tissue (chapter 2). Preparations are suitable for synaptonemal complex (SC) analysis by normal light and electron microscopy (chapters 2, 3 and 7), for fluorescence immunocytochemistry and *in situ* hybridization (chapters 2, 8).

In the study presented in *chapter 3*, we analysed the variation in male fertility of mice double heterozygous for two near identical reciprocal translocations T(1;13)70H and T(1;13)1Wa in relation to the synaptic behaviour of two differently sized heteromorphic bivalents during meiotic prophase. Male fertility rises when non-homologous synapsis in the small 1^{13} heteromorphic bivalent, leading to a "symmetrical" SC, is more frequent at the initial prophase stages. Based on the data presented, we favour the "unsaturated pairing site" model as the primary cause for male sterility.

In T70H/T1Wa females not all heterologous synapsis within the small heteromorphic bivalent is effectuated during the early stages of meiosis; some is achieved lateron by the mechanism of "synaptic adjustment" (chapter 3). Each heteromorphic bivalent contains a copy of the chromosome 1 region between the T70H and T1Wa breakpoints which is about 10 cM in size (Δ1 segment). Although axial elements representing these Δ1 segments are seen to approach each other during early meiotic prophase stages, they never successfully constitute a synaptonemal complex in either sex (chapter 3). This agrees with the fact that in earlier cytogenetic studies quadrivalents were never seen at both male and female diakinesis-metaphase I.

In *chapter 7*, we demonstrate that male fertility of the T70H/T1Wa mice is not only determined by the chromosomal constitution of the carrier but is additionally influenced by the pairing or synaptic history in previous meioses of especially the T70H and T1Wa short translocation chromosomes. Fertility of T70H/T1Wa males is more impaired after one or

more successive transmissions of the T1Wa translocation chromosomes through a heteromorphic bivalent configuration, irrespective of the sex of the transmitting parent.

Furthermore, we show that the introduction of the Robertsonian translocation Rb(11.13)4Bnr into the T70H/T1Wa karyotype restores fertility of double heterozygous males by stimulating non-homologous synapsis of the small heteromorphic bivalent. We speculate that this Rb4Bnr effect is mediated by a prolongation of the early stages of meiotic prophase I.

Successive female transmissions of the T1Wa translocation chromosomes in the presence of Rb4Bnr inititially resulted in an increase of the capacity for early meiotic non-homologous synapsis within the small heteromorphic bivalent, leading to a restoration of fertility for the majority of carriers. Subsequently, a decrease of the capacity of the small heteromorphic bivalent to fully synapse was noticed, although a higher than original (F1) background level of male fertility remained.

These variations in male fertility are most likely based on epigenetic variance, reflected as the capacity to engage into non-homologous synapsis early in male meiosis leading to a "symmetrical" SC, despite the different amounts of chromatin to accommodate.

In *chapter 4*, the localization of several microsatellite markers and single copy genes relative to the T70H and T1Wa breakpoints, using quantitative PCR, quantitative Southern blotting and *in situ* hybridization, is described.

In *chapter 5*, we investigated the level of suppression of meiotic recombination and impairment of chromosome synapsis in T70H heterozygotes in relation to the viability of the progeny. For T70H/+ females, the introgression of the D1Mit4, D1Mit20 and D1Mit122 microsatellite marker alleles positioned distal of the T70H breakpoint on the normal chromosome 1 into the 13^1 T70H long translocation chromosome was suppressed in a distance dependent manner. This effect was more pronounced in T70H/+ females, additionally homozygous for Rb4Bnr. The delay in introgression was paralleled by a reduction of the frequency and extent of non-homologous synapsis in segments near the T70H breakpoints of the pachytene translocation multivalents in T70H/+ and Rb4BnrT70H/Rb4Bnr+ males. The extend of non-homologous synapsis around the centre of the synaptic cross configuration in these males correlated with fluctuations in prenatal viability of segregating translocation homozygotes in crosses between (Rb4Bnr)T70H homozygous males and heterozygous females when meiotic drive at the female second meiotic division is excluded. The reduction in viability is explained by the gain of mutations resulting from incorrect processing of recombination intermediates which is due to non-homologous synapsis around the translocation breakpoints.

In *chapter 6*, we analysed the consequences of the absence of crossing over for regions between the T70H and T1Wa breakpoints (Δ1 and Δ13 segments) of the Rb4BnrT1Wa translocation chromosomes, which have been transmitted for over 20 generations via heteromorphic bivalents in Rb4BnrT70H/Rb4BnrT1Wa females. Survival of heterozygous and homozygous carriers for these segments was taken as the phenotypic endpoint. The viability of progeny of crosses between Rb4BnrT70H homozygous males and Rb4BnrT70H/Rb4BnrT1Wa females, of which the latter principally produce 4 types of gametes, was estimated using a haplotype analysis of microsatellites in the Δ1 segment for genotyping (see chapter 4). We observed no differences in the pre- and postnatal survival rates of the double heterozygous and 13^{1}H, 13^{1}H, 1^{13}Wa 1^{13}H "duplication" progeny in which the Δ1 and Δ13 segments of the T1Wa translocation chromosomes had either no, an one-generation or a multi-generation history of non-homologous synapsis in heteromorphic bivalents during previous female meioses. In addition, intercrossing of Rb4BnrT70H/Rb4BnrT1Wa double heterozygotes after genetic isolation of these Δ1 and Δ13 segments for 20 to 22 generations, showed that the viability of the Rb4BnrT1Wa homozygotes was not different from the Rb4BnrT70H homozygous and Rb4BnrT70H/-Rb4BnrT1Wa karyotypes generated by this cross. Thus, exclusion of the Δ1 and Δ13 segments from meiotic crossing over within non-homologous synapsed heteromorphic bivalents during 20 to 25 successive generations does not result in an accumulation of recessive lethal mutations or an increased susceptibility for gaining dominant lethal mutations.

For the D1Mit122 microsatellite used in offspring haplotyping a higher mutation frequency was observed after transmission through a double heterozygous than after transmission through a T70H homozygous karyotype (chapter 6). On the basis of the identity of the mutations, the ectopic pairing of the St2 gene copies (containing D1Mit122) during meiosis of T70H/T1Wa males (chapter 8) and the observation of ectopic homologous contacts of the Δ1 segments during the zygotene stage without SC formation (chapter 3), we speculate that these mutations are the result of ectopic homologous gene conversion events most likely occurring in the absence of a synaptonemal complex.

The crossover suppressive influence of the Rb translocation on the Δ1 segment (chapter 5) enabled us to analyze the effects of introgression of genetic material from the Swiss +/+ stock into the translocation karyotypes. Introgression of "new" genetic material correlated with an increase in littersize of Rb4BnrT70H homozygotes (chapter 5), an improvement of the life expectancy of Δ1 duplication offspring from double heterozygous mothers (chapter 6) and a clear improvement of male fertility in double heterozygous and T70H homozygous males also

carrying Rb4Bnr (chapter 7). These pleiotrophic findings are discussed in chapter 8 in terms of genetic versus epigenetic mechanisms of inheritance.

Finally, when T1Wa was backcrossed for many generations to the Rb4BnrT70H/-Rb4BnrT70H karyotype, essentially precluding genetic recombination in the Δ1 and Δ13 segments, or when T1Wa was combined with Rb4Bnr after many successive transmissions via alternating T1Wa heterozygotes and homozygotes, stable Rb4BnrT1Wa homozygous lines could not be bred (chapter 8). Especially female reproductive performance decreases after repeated male and female homologous meiosis. As non-homologous synapsis in the centre of the synaptic cross configuration in T1Wa/+ males is common too (unpublished results), more work into the genetic stability of chromosome segments, that have a history of hindered homologous interaction, is indicated (chapter 8).

Samenvatting

Samenvatting

Bij sommige reciproke and Robertsonische translocaties wordt in de heterozygote conditie een verstoring van chromosoom synapsis en een onderdrukking van crossing over in regio's rondom breukpunten tijdens de profase van de eerste meiotische deling gevonden. Bij de muis zijn dergelijke verstoringen het meest bekend. In dit proefschrift worden de gevolgen van niet-homologe synapsis en/of onderdrukking van recombinatie gedurende vele opeenvolgende generaties met betrekking tot de mannelijke en vrouwelijke vruchtbaarheid en de levensvatbaarheid van het nageslacht geanalyseerd.

Voor de studie van chromosoom synapsis hebben we een bestaande cel spreidings methode gemodificeerd. Met deze techniek kunnen uit kleine hoeveelheden weefsels van zowel de mannelijke als vrouwelijke gonaden grote aantallen cellen afkomstig uit alle stadia van de eerste meiotische profase worden verkregen (hfd. 2). De preparaten zijn geschikt voor de analyse van synaptonemale complexen (SC) via licht of electronen microscopie (hfd. 2, 3, 7). Tevens zijn ze ook geschikt voor immunocytochemie en *in situ* hybridizatie (hfd 2, 8).

In *hoofdstuk 3* hebben we de variatie in mannelijke vruchtbaarheid, welke karakteriserend is voor muizen die heterozygoot zijn voor de twee bijna identieke reciproke translocaties T(1;13)70H and T(1;13)1Wa, onderzocht in relatie tot het synaptisch gedrag van twee heteromorfe bivalenten die gedurende de eerste meiotische profase bij deze muizen aanwezig zijn. De mannelijke vruchtbaarheid is positief gecorreleerd aan de frequentie van primaire spermatocyten waarin gedurende de begin stadia van de meiotische profase (zygoteen en vroeg pachyteen) niet-homologe synapsis in het kleine 1^{13} heteromorfe bivalent heeft geleid tot de vorming van een "symmetrische" SC configuratie. We concluderen we dat de gevonden resultaten het model van "unsaturated pairing sites" (Miklos, 1974; Burgoyne and Baker, 1984), als negatieve beinvloeders van de ontwikkeling van pachyteen spermatocyten, ondersteunen.

In T(1;13)70H/T(1;13)1Wa vrouwen voltrekt de niet-homologe synapsis in het kleine heteromorfe bivalent zich niet volledig gedurende de vroege meiotische profase stadia. Synapsis wordt gedurende het pachyteen stadium voltooid volgens het zogenaamde "synaptic adjustment" mechanisme (hfd. 3).

Zowel het kleine (1^{13}) als het grote (13^{1}) heteromorfe bivalent bevatten een kopie van een ongeveer 10 cM grote chromosoom 1 regio, die gelegen is tussen de T70H and T1Wa breukpunten (Δ1 segment; zie fig. 1b uit hfd. 3). Hoewel de axiale elementen van deze Δ1 segmenten elkaar tijdens de vroege meiotische profase stadia lijken te naderen, vormen ze nooit een SC gedurende de latere stadia van de mannelijke of vrouwelijke meiose (hfd. 3).

Deze observatie is in overeenstemming met het feit dat men in vroegere cytogenetische studies nooit quadrivalent configuraties heeft waargenomen tijdens de diakinese and metafase I stadia van beide geslachten.

In ***hoofdstuk 7*** laten we zien dat de mannelijke vruchtbaarheid van T70H/T1Wa muizen niet alleen wordt bepaald door het synaptisch gedrag van de desbetreffende translocatie chromosomen maar ook door de parings en/of synapsis geschiedenis van speciaal de kleine T70H en T1Wa translocatie chromosomen in voorafgaande meiotische situaties. De vruchtbaarheid van deze dubbel heterozygote T70H/T1Wa mannen gaat achteruit wanneer de T1Wa chromosomen één of meerdere malen in een meiotische situatie met heteromorfe bivalenten hebben vertoefd, ongeacht de sexe van de ouder.

Tevens laten we zien dat de vruchtbaarheid van T70H/T1Wa mannen positief wordt beinvloed door de aanwezigheid van de Robertsonische translocatie Rb(11.13)4Bnr. De verbetering van de vruchtbaarheid gaat samen met een verhoging van het percentage primaire spermatocyten met een niet-homoloog gesynapsed klein heteromorf bivalent. We speculeren dat dit Rb4Bnr effect wordt veroorzaakt door een verlenging van de duur van de eerste stadia van de meiotische profase I.

Herhaaldelijke transmissie van de T1Wa chromosomen via T70H/T1Wa vrouwen die homozygoot waren voor Rb4Bnr leidde eerst tot een verhoging van de capaciteit tot niet-homologe synapsis in het kleine heteromorfe bivalent en van de vruchtbaarheid van het mannelijke nageslacht. Na voortzetting van deze herhaalde transmissie zakte de capaciteit tot niet-homologe synapsis weer in en daarmee ook de sperma produktie. Toch was de vruchtbaarheid van dubbel heterozygote mannen na 20-25 herhaalde transmissies van de Rb4BnrT1Wa translocatie chromosomen via heteromorfe bivalenten hoger dan die van mannen met Rb4BnrT70H en Rb4BnrT1Wa translocatie chromosomen zonder geschiedenis van niet-homologe synapsis in heteromorfe bivalenten.

De hier waargenomen variaties in mannelijke vruchtbaarheid hebben het meest waarschijnlijk een epigenetische grondslag, welke tot uiting komt in het vermogen tot niet-homologe synapsis tijdens de eerste stadia van de meiotische profase I. Dit leidt tot de vorming van een "symmetrische" SC configuratie ondanks de verschillen in hoeveelheden chromatine tussen de 1^{13}Wa en 1^{13}H translocatie chromosomen.

In ***hoofdstuk 4*** wordt de localizatie van enkele microsatelliet merkers en " single copy" genen ten opzichte van de T70H en T1Wa breukpunten beschreven. In deze studies hebben we gebruik gemaakt van kwantitatieve PCR and Southern blott technieken en van *in situ* hybridizatie.

In *hoofdstuk 5* hebben we de onderdrukking van meiotische recombinatie en de verstoring van chromosoom synapsis in T70H heterozygoten onderzocht in relatie tot de levensvatbaarheid van het nageslacht. De introgressie van Swiss (+/+) allelen van de microsatelliet merkers D1Mit4, D1Mit20 en D1Mit122, die distaal van T70H op chromosoom 1 gelegen zijn, is onderdrukt tijdens de vrouwelijke meiose van T70H heterozygoten. Dit effect is sterker naarmate de betreffende merker dichter bij het T70H breukpunt ligt. De mate van recombinatie onderdrukking was hoger in Rb4BnrT70H/Rb4Bnr+ vrouwen. Tevens correleerde de mate van recombinatie onderdrukking in vrouwen met de frequentie en mate van niet-homologe synapsis in het centrum van de synaptische kruis configuratie in T70H/+ en Rb4BnrT70H/Rb4Bnr+ mannen. In dit gebied wisselen de chromosomen van paringspartner. Tenslotte waren zowel de recombinatie frequentie (in vrouwen) als de mate en frequentie van niet-homologe synapsis (in mannen) positief gecorreleerd met de mate van prenatale sterfte van T70H en Rb4BnrT70H homozygoten, die verkregen waren uit kruisingen tussen heterozygote vrouwen en homozygote mannen. Een verklaring voor de vermindering in levensvatbaarheid van T70H homozygote translocatie dragers kan zijn gelegen in de combinatie van complete SC vorming in het centrum van het "pachyteen kruis" met niet homologe chromosoom paring. Hierdoor kunnen recombinatie intermediairen ontstaan die niet "genetisch" correct worden verwerkt en zo kunnen leiden tot recessief lethale factoren.

In *hoofdstuk 6* hebben we onderzocht wat de gevolgen zijn voor de levensvatbaarheid van heterozygote en homozygote dragers van chromosoom segmenten die gedurende meer dan 20 generaties uitgesloten zijn geweest van crossing over. Deze segmenten worden begrensd door de T70H en T1Wa breukpunten (Δ1 en Δ13 segmenten) en liggen op de Rb4BnrT1Wa translocatie chromosomen die herhaaldelijk zijn doorgegegeven via heteromorfe bivalenten in Rb4BnrT70H/Rb4BnrT1Wa vrouwen. De levensvatbaarheid van het nageslacht dat geproduceerd was in kruisingen tussen Rb4BnrT70H homozygote mannen en Rb4BnrT70H/ Rb4BnrT1Wa vrouwen, werd bepaald nadat het genotype vastgesteld was via een haplotype analyse met microsatellieten die gelegen zijn in de Δ1 regio (zie hierboven en hfd. 4). We hebben geen verschillen gevonden in de pre- en postnatale overleving van het dubbel heterozygote en het $13^{1}H$, $13^{1}H$, $1^{13}H$, $1^{13}Wa$ "duplicatie" nageslacht waarin de Δ1 en Δ13 segmenten van de T1Wa translocatie chromosomen of geen, of een één-generatie of een multi-generatie geschiedenis van niet-homologe synapsis in heteromorfe bivalenten gedurende voorafgaande meiotische passages hadden gehad. Tevens was na 20-22 generaties uitkruisen van de Rb4BnrT1Wa translocatie chromosomen via dubbel heterozygote vrouwen, de levensvatbaarheid van het Rb4BnrT1Wa homozygote nageslacht niet verschillend van die van het Rb4BnrT70H/Rb4BnrT1Wa en Rb4BnrT70H homozygote nageslacht, dat geproduceerd was in kruisingen tussen de desbetreffende dubbel heterozygoten. Wij concluderen dat de

afwezigheid van crossing over in de Δ1 en Δ13 segmenten gedurende 20 tot 25 generaties in de context van een niet-homoloog gesynapsed heteromorf bivalent niet heeft geleid tot een opeenhoping van recessieve lethale mutaties of tot een verhoogde gevoeligheid voor het verkrijgen van dominante lethale mutaties.

Voor de microsatelliet merker D1Mit122 hebben we na transmissie via een dubbel heterozygote ouder een hogere mutatie graad waargenomen dan na transmissie via een Rb4BnrT70H homozygote ouder (hfd. 6). In ogenschouwnemende de identiteit van de mutaties, het voorkomen van paring (=geen synapsis) tussen de ectopisch gelegen kopien van het St2 gen (waarin D1Mit122 ligt) gedurende de eerste meiotische profase van T70H/T1Wa mannen (hfd. 8) en het waarnemen van ectopisch homoloog chromatine contact tussen de Δ1 segmenten zonder de vorming van een SC gedurende het zygotene (hfd. 3), lijkt het het meest aannemelijk dat deze mutaties het gevolg zijn van ectopische homologe gen conversies.

Doordat de recombinatie sterk onderdrukt was in het Δ1 segment van Rb4BnrT70H/Rb4+ vrouwen, was het mogelijk om de effecten van introgressie van "nieuw" genetisch materiaal vanuit de Swiss +/+ lijn in de translocatie lijn te bestuderen. De introgressie van "nieuw" genetisch materiaal ging samen met een verhoging van de worpgrootte van kruisingen tussen Rb4BnrT70H homozygoten (hfd. 5), een verhoging van de levensduur van Δ1 duplicatie nakomelingen van dubbel heterozygote vrouwen (hfd. 6) en een duidelijke verbetering van de mannelijke vruchtbaarheid van dubbel heterozygote en Rb4BnrT70H homozygote mannen (hfd. 7). Deze observaties worden in hoofdstuk 8 besproken in termen van pleiotrofe werking van genen en/of een epigenetisch mechanisme van overerving.

Tenslotte hebben we geprobeerd Rb4T1Wa homozygote lijnen op te zetten (hfd. 8). Hiervoor zijn de Rb4BnrT1Wa homozygote dieren gebruikt die in het onderzoek naar recessief lethale factoren waren verkregen (hfd. 6). Opvallend was dat al in de eerste generaties het lichaamsgewicht en de vrouwelijke vruchtbaarheid terug liepen. Kruisingen tussen Rb4Bnr homozygoten en T1Wa homozygoten leverden na terugkruising Rb4BnrT1Wa homozygoten op (hfd. 6). Echter, bij het vervolgens inkruisen van deze dieren leken mannelijke en vrouwelijke dieren onderling steriel. Omdat tijdens het pachyteen ook niet-homologe synapsis in het centrum van T1Wa/+ kwadrivalenten voorkomt (ungepubliseerde waarnemingen), is meer onderzoek nodig naar de stabiliteit van chromosome segmenten die een geschiedenis van verstoorde meiotische homologe interacties hebben.

Zusammenfassung

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Bei Mäusen wird die Synapse von Chromosomen in der meiotischen Prophase durch heterozygote reziproke und Robertsonische Translokationen beeinträchtigt, sowie das Entstehen von Crossovers in Segmenten nahe der Bruchstellen unterdrückt. Diese Arbeit beschreibt die Konsequenzen von über mehrere aufeinanderfolgende Generationen erfolgter nicht-homologer Synapse und/oder Unterdrückung des meiotischen Crossovers für männliche Fruchtbarkeit und Lebensfähigkeit der Nachkommenschaft.

Eine "drying down"-Technik zur Analyse von Chromosomen-Synapsen wurde so modifiziert, daß eine hohe Ausbeute sowohl an männlichen als auch an weiblichen Zellkernen aller Stadien der meiotischen Prophase auch aus kleinen Mengen von Gewebe gewonnen werden kann (Kapitel 2). Diese Präparate sind für die Analyse von Synaptonemalen Komplexen bei normalem Licht und durch Elektronenmokroskopie (Kapitel 2, 3 und 7) ebenso brauchbar wie für fluoreszenzimmunocytochemische Färbungen und für *in situ* Hybridisierungen (Kapitel 2 und 8).

In Kapitel 3 werden die Abweichungen der männlicher Fruchtbarkeit von Mäusen, die doppelt-heterozygot für die beiden beinahe identischen reziproken Translokationen T(1:13)70H und T(1;13)1Wa sind, in Relation zu dem synaptischen Verhalten von zwei underschiedlich großen heteromorphen Bivalenten in der meiotischen Prophase analysiert. Nicht-homologe Synapse in dem kleinen 1^{13} heteromorphen Bivalent führt zu einem "symmetrischen" SC und hat bei erhöter Frequenz in den ersten Stadien der meiotischen Prophase eine Steigerung der männlichen Fruchtbarkeit zur Folge. Wegen diese Beobachtungen favorisieren wir das "unsaturated pairing site"-Modell als primäre Ursache für männliche Sterilität.

Bei T70H/T1Wa-Weibchen sind nicht alle heterologen Synapsen des kleinen heteromorphen Bivalents in den frühen Stadien der Meiose gestört, manche erreichen das Stadium der vollständigen Synapse später und zwar über den Mechanismus des "synaptic adjustments" (Kapitel 3).

Jedes heteromorphe Bivalent enthält eine Kopie der ungefähr 10 cM langen Region von Chromosom 1 zwischen den T70H- und T1Wa-Bruchstellen (D1-Segment). In keinem der beiden Geschlechter wird ein synaptonemaler Komplex ausgebildet, obwohl beobachtet werden kann, daß sich die axialen Elemente, die das D1-Segment representieren, während der frühen meiotischen Prophase einander nähern (Kapitel 3). Dies stimmt mit früheren zytogenetischen Studien überein, in denen weder in männlicher noch in weiblicher Diakinese-Metaphase I Quadrivalente beobachtet wurden.

In Kapitel 7 zeigen wir, daß männliche Fruchtbarkeit nicht nur von der chromosomalen Konstitution des Trägers bestimmt wird, sondern zusätzlich auch unter dem Einfluß der Geschichte von Paarung und Synapse vor allem des kurzen T70H- und T1Wa-Translokationschromosoms in früheren Meiosen steht. Werden die T1Wa-Translokationschromosomen ein oder mehrere Male nacheinander über eine heteromorphe Bivalent-Konformation vererbt, so ist die Fruchtbarkeit von T70H/T1Wa-Männchen deutlicher gestört, ungeachtet des Geschlechts des vererbenden Elternteils.

Weiters zeigen wir, daß durch die Einführung der Robertsonischen Translokation Rb(11.13)4Bnr in den T70H/T1Wa-Karyotyp die Fruchtbarkeit von doppelt-heterozygoten Männchen durch Stimulation von nicht-homologer Synapse des kleinen heteromorphen Bivalents wiederhergestellt wird. Wir vermuten, daß dieser Rb4Bnr-Effekt auf einer Verlängerung der frühen Stadien der meiotischen Prophase beruht.

Wiederholte mütterliche Vererbung des T1Wa-Translokationschromosoms in Gegenwart von Rb4Bnr führte innerhalb des kleinen heteromorphen Bivalents zuerst zu einer Zunahme der Fähigkeit nicht-homologe Synapse in der frühen Meiose auszubilden, was wiederum die Fruchtbarkeit bei den meisten Trägern wiederherstellt. Später wurde für das kleine heteromorphe Bivalent eine Abnahme der Fähigkeit zur vollständigen Synapse festgestellt, obwohl die männliche Fruchtbarkeit höher als im originalen (F1) genetischen Hintergrund war.

Diese Abweichungen in der männlichen Fruchtbarkeit basieren sehr wahrscheinlich auf einer epigenetischen Varianz, die sich als die Fähigkeit, während der Meiose beim Männchen nicht-homologe Synapse (einen "symmetrischen" SC) auszubilden, zeigt.

In Kapitel 4 wird die Lokalisierung von verschiedenen Mikrosatellitenmarkern und "single copy"-Genen relativ zu den T70H- und T1Wa-Bruchstellen mit Hilfe von quantitativer PCR, quantitativem Southern Blotting und *in situ* Hybridisierung beschrieben.

In Kapitel 5 stellen wir das Ausmaß der Unterdrückung von meiotischer Rekombination sowie der Störung der Chromosomen-Synapse in T70H-heterozygoten Tieren in Relation zur Lebensfähigkeit der Nachkommenschaft. In T70H/+ -Weibchen wurde die Introgression der D1Mit4-, D1Mit20- und D1Mit22-Mikrosatellitenmarker-Allele (welche sich distal von der T70H-Bruchstelle auf dem normalen Chromosom 1 befinden) in das lange 13^{1} T70H-Translokationschromosom abhängig vom Abstand unterdrückt. Dieser Effekt war in T70H/+ -Weibchen, die zusätzlich homozygot für Rb4Bnr waren, noch deutlicher. Bei T70H/+- und Rb4BnrT70H/Rb4Bnr+ -Männchen lief die Verzögerung der Introgression parallel mit einer Reduktion von Frequenz und Ausmaß der nicht-homologen Synapse in Segmenten, die nahe den T70H-Bruchstellen der pachytenen Translokations-Multivalente liegen. Sowohl die

Rekombinationsfrequenz bei Weibchen, als auch die Frequenz und das Ausmaß der nicht-homologe Synapse bei Männchen korrilierten mit einer Fluktuation der prenatalen Lebensfähigkeit von segregierenden T70H- und Rb4BnrT70H Translokations-Homozygoten stammend aus die Kreuzung von heterozygoten Weibchen und homozygoten Männchen. Wir erklären die Abnahme der Lebensfähigkeit durch eine Zunahme von Mutationen auf Grund von fehlerhaftem Auflösen von Rekombinations-Zwischenprodukten wegen nicht-homologer Synapse rundum die Translokationsbruchstellen.

In Kapitel 6 analysieren wir die Konsequenzen von fehlenden Crossovers in der Region zwischen den T70H- und T1Wa-Bruchstellen (D1- und D13 -Segment) des Rb4BnrT1Wa Translokationschromosoms, welches in Rb4BnrT70H/Rb4BnrT1Wa Weibchen 20 Generationen lang via heteromorphe Bivalente vererbt wurde. Das Überleben von heterozygoten und homozygoten Trägern dieser Segmente wurde als phenotypischer Endpunkt angenommen. Die Lebensfähigkeit der Nachkommenschaft aus Kreuzungen von RbBnrT70H-homozygoten Männchen mit Rb4BnrT70H/Rb4BnrT1Wa Weibchen (welche im Prinzip 4 verschiedene Typen von Gameten produzieren können) wurde durch Genotypisierung mittels Haplotypen-Analyse der Mikrosatelliten im D1-Segment bestimmt (siehe Kapitel 4). Wir stellten keine Unterschiede in den pre- und postnatalen Überlebensraten bei doppelt-heterozygoten Tieren und der Nachkommenschaft mit $13^{1}H$, $13^{1}H$, $1^{13}Wa$ $1^{13}H$ - "Duplication" fest, wobei die D1- und D13-Segmente des T1Wa-Translokationschromosoms entweder in keiner, einer oder mehreren früheren Meiosen beim Weibchen über nicht-homologe Synapse der heteromorphen Bivalente vererbt worden waren. Zusätzlich zeigte eine Kreuzung von Rb4BnrT70H/Rb4BnrT1Wa doppelt-heterozygoten Tieren nach einer 20 bis 22 Generationen lang dauernden genetischen Isolation der D1- und D13-Segmente, daß sich die Lebensfähigkeit der Rb4BnrT1Wa-homozygoten Tiere nicht von jener der Tiere mit Rb70H-homozygotem Karyotyp oder Rb4BnrT70H/Rb4BnrT1Wa -Karyotyp aus dieser Kreuzung unterscheidet. Werden die D1- und D13-Segmente 20 bis 25 aufeinanderfolgenden Generationen lang vom meiotischen Crossover innerhalb der nicht-homolog gepaarten heteromorphen Bivalente ausgeschlossen, so führt dies nicht zur Akkumulation von rezessiev-lethalen Mutationen oder zu erhöhter Anfälligkeit für dominant-lethale Mutationen.

Der D1Mit122-Mikrosatellit, der für das "offspring-haplotyping" verwendet wurde, weist eine höhere Mutationsfrequenz nach Vererbung über einen doppelt-heterozygoten Karyotyp auf als nach Vererbung über einen T70H-homozygoten Karyotyp (Kapitel 6). Auf Grund der Identität der Mutationen einerseits und der Tatsache, daß die Kopien des St2-Gens (welches D1Mit122 enthält) während der Meiose bei T70H/T1Wa -Männchen ektopisch paaren andererseits (Kapitel 8), sowie wegen der beobachteten ektopischen homologen Kontakte des

D1-Segments ohne die Ausbildung eines Synaptonemalen Komplexes während des Zygotens, vermuten wir, daß diese Mutationen das Resultat von ektopischen homologen Genkonversionen, die vermutlich ohne das Vorhandensein eines synaptonemalen Komplexes auftreten, sind.

Der Crossover-unterdrückende Einfluß der Rb-Translokation auf das D1-Segment von Chromosom 1 (Kapitel 5) ermöglichte es, die Effekte von Introgression von genetischem Material aus dem Swiss +/+ stock in den Translokationskaryoptyp zu analysieren. Introgression von "neuem" genetischem Material korrilierte mit einer Zunahme der Wurfgröße bei RbT70H-homozygoten Tieren (Kapitel 5), weiters mit einer Erhöhung der Lebenserwartung der Nachkommen mit D1-Duplikationen, welche von doppelt-heterozygoten Müttern stammten, und mit einer deutlichen Verbesserung der Fruchtbarkeit von doppelt-heterozygoten und T70H-homozygoten Rb4Bnr-Männchen (Kapitel 7). Diese pleiotrophischen Ergebnisse werden hinsichtlich der Mechanismen für genetische und epigenetische Vererbung in Kapitel 8 diskutiert.

Nach Rückkreuzung von T1Wa in Rb4BnrT70H/Rb4BnrT70H über mehrere Generationen (was genetische Rekombination in den D1- und D13-Segmenten im Wesen ausschließt), ebenso wie durch Kombination von T1Wa mit Rb4Bnr nach vielen aufeinanderfolgenden Vererbungen abwechselnd via T1Wa-heterozygote und -homozygote Tiere, stellten wir schließlich fest, daß keine stabilen Rb4BnrT1Wa-homozygoten Linien gezüchtet werden können (Kapitel 8). Nach wiederholter homologer Meiose bei beiden Geschlechtern nahm vor allem die reproduktive Kapazität der Weibchen ab. Wenn nicht-homologes Paaren im Zentrum der synaptischen Kreuz-Konfiguration bei T1Wa/+ -Männchen normal ist (unveröffentlichte Resultate), so muß zukünftig mehr Arbeit an der genetischen Stabilität der Chromosomen-Segmente, die in vergangenen Meiosen gestörte homologe Interaktionen mitgemacht haben, getan werden (Kapitel 8).

Nawoord

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Curriculum vitae

Antoine Hendrik Felix Marie Peters werd op 11 mei 1968 geboren in Krimpen aan den IJssel. In 1986 behaalde hij het Gymnasium-β diploma cum laude aan de scholengemeenschap Jerusalem te Venray en begon hij aan zijn studie Moleculaire Wetenschappen aan de Landbouwuniversiteit te Wageningen. De docteraalfase omvatte twee afstudeervakken aan de Landbouwuniversiteit (Genetica en Moleculaire Genetica), een afstudeervak aan de Erasmus Universiteit Rotterdam (Moleculaire Endocrinologie) en een stage aan The Jackson Laboratory in Bar Harbor, Maine, USA. In augustus 1992 studeerde hij cum laude af en trad hij als assistent in opleiding in dienst bij de vakgroep Erfelijkheidsleer van de Landbouwuniversiteit te Wageningen. Een NWO talent stipendium stelt hem in staat om vanaf december 1997 als postdoc werkzaam zijn bij het Department of Genetics, University of Washington, Seattle, USA.

List of publications

Leen J. Blok, Axel P.N. Themmen, **Antoine H.F.M. Peters**, Jan Trapman, Willy M. Baarends, Jos W. Hoogerbrugge and J. Anton Grootegoed (1992).
Transcriptional regulation of androgen receptor gene expression in Sertoli cells and other cell types. *Mol. Cell. Endocrinol.* **88**: 53-164.

John J. Eppig, **Antoine H.F.M. Peters**, Evelyne E. Telfer and Karin Wigglesworth (1993).
Production of cumulus expansion enabling factor by mouse oocytes grown in vitro. *Mol. Reprod. Dev.* **43**: 450-456.

Dirk G. de Rooij, Ans M.M. van Pelt, Henk J.G. van de Kant, Paul T. van der Saag, **Antoine H.F.M. Peters**, Christa Heyting and Peter de Boer (1994).
Role of retinoids in spermatogonial proliferation and differentiation and the meiotic prophase. *In function of somatic cells in the testis (editor A. Bartke). Springer Verlag, New York.*

Johannes H.M. Lammers, Mirjam van Aalderen, **Antoine H.F.M. Peters**, Ans A.M. van Pelt, Ingrid C. Gaemers, Dirk G. de Rooij, Peter de Boer, Hildo H. Offenberg, Axel J. Dietrich and Christa Heyting (1995).
A change in the phosphorylation pattern of the 30000-33000 M_r synaptonemal complex proteins of the rat between early and mid-pachytene. *Chromosoma* **104:** 154-163.

Peter de Boer, **Antoine H.F.M. Peters**, Frits A. van der Hoeven and Rene Bakker (1996).
Crosses between mouse translocations with semi-identical breakpoints. I: Choice of translocations and meiotic chromosome behaviour in F1 hybrids. *Genet. Res. Camb.* **68:** 83.

Antoine H.F.M. Peters, Jolanda Westerlaken, Frits A. van der Hoeven and Peter de Boer (1996).
Crosses between mouse translocations with semi-identical breakpoints. II: Genetic consequences of repeated non-homologous meiotic pairing and the development of runted phenotypes. *Genet. Res. Camb.* **68:** 77-78.

Antoine H.F.M. Peters, Annemieke W. Plug, Martine J. van Vugt and Peter de Boer (1997).
A drying-down technique for spreading of mammalian meiocytes from the male and female germline. *Chromosome Research* **5:** 66-68.

Antoine H.F.M. Peters, Annemieke W. Plug and Peter de Boer (1997).
Meiosis in carriers of heteromorphic bivalents: sex differences and implications for male fertility. *Chromosome Research* **5:** 313-324.

Annemieke W. Plug, **Antoine H.F.M. Peters**, Yang Xu, Kathleen S. Keegan, Merl F. Hoekstra, David Baltimore, Peter de Boer and Terry Ashley.
Involvement of ATM and RPA in meiotic chromosome synapsis and recombination. *Nature Genetics (in press).*

Annemieke W. Plug, **Antoine H.F.M. Peters**, Kathleen S. Keegan, Merl F. Hoekstra, Peter de Boer and Terry Ashley.
Changes in protein composition of meiotic nodules during mammalian meiosis. *Submitted.*