Genet. Res., Camb. (1989), **53**, *pp.* 77–86 *With 6 text-figures Printed in Great Britain*

The influence of the Robertsonian translocation Rb(*X*.2)2Ad on anaphase I non-disjunction in male laboratory mice

I.-D. ADLER[1], R. JOHANNISSON[2] AND H. WINKING[3]

[1] *Institut für Säugetiergenetik, Gesellschaft für Strahlen- und Umweltforschung, D-8042 Neuherberg* [2] *Institut für Pathologie* [3] *Institut für Biologie, Medizinische Universität zu Lübeck, D-2400 Lübeck, Federal Republic of Germany*

(*Received 12 June 1988 and in revised form 10 August 1988*)

Summary

A Robertsonian translocation in the mouse between the *X* chromosome and chromosome 2 is described. The male and female carriers of the Rb(*X*.2)2Ad were fertile. A homozygous/hemizygous line was maintained. The influence of the *X*-autosomal Robertsonian translocation on anaphase I non-disjunction in male mice was studied by chromosome counts in cells at metaphase II of meoisis and by assessment of aneuploid progeny. The results conclusively show that the inclusion of Rb2Ad in the male genome induces non-disjunction at the first meoitic division. In second metaphase cells the frequency of sex-chromosomal aneuploidy was 10·8%, and secondary spermatocytes containing two or no sex chromosome were equally frequent. The Rb2Ad males sired 3·9% sex-chromosome aneuploid progeny. The difference in aneuploidy frequencies in the germ cells and among the progeny suggests that the viability of XO and XXY individuals is reduced. The pairing configurations of chromosomes 2, Rb2Ad and *Y* were studied during meiotic prophase by light and electron microscopy. Trivalent pairing was seen in all well spread nuclei. Complete pairing of the acrocentric autosome 2 with the corresponding segment of the Rb2Ad chromosome was only seen in 3·2% of the cells analysed in the electron microscope. The pairing between the *X* and the *Y* chromosome in the Rb2Ad males corresponded to that in males with normal karyotype. Reasons for sex-chromosomal non-disjunction despite the normal pairing pattern between the sex chromosomes may be seen in the terminal chiasma location coupled with the asynchronous separation of the sex chromosomes and the autosomes. The Rb2Ad chromosome can be useful for studies of *X* inactivation, as a marker for parental derivation of the *X* chromosome and for mapping loci by *in situ* hybridization.

1. Introduction

The standard karyotype of the house mouse, *Mus musculus*, and of the derived laboratory mice contains 40 acrocentric chromosomes. During the last 20 years, however, Robertsonian translocations (hereafter referred to as Rb) have been found in both feral and laboratory mice. The first was detected in a laboratory mouse (Léonard & Deknudt, 1967), a finding which was followed by several other reports concerning the identification and characterization of Rb chromosomes in laboratory and in feral mice (Evans *et al.* 1967; Gropp *et al.* 1970; Capanna *et al.* 1976; Gropp & Winking, 1981; Adolph & Klein, 1983; Winking *et al.* 1988). As a consequence, over 100 examples have now been described and their frequency is about four times greater in feral mice than in laboratory mice (Winking *et al.* 1988). All but one of these Rb translocations were derived from autosome–autosome fusions. The only exception was the Rb(*X*.3) chromosome discovered in a female laboratory mouse (Arroyo Nombela & Rodriguez Murcia, 1977) but, since the carrier died before reproducing, the rare chromosome was lost without being characterized in more detail. Recently, we have briefly reported on another *X*-autosomal Rb translocation, the Rb(*X*.2)2Ad (Adler & Neuhäuser-Klaus, 1987). Since both male and female Rb2Ad carriers are fertile, this *X*-autosome translocation could be maintained in a homozygous/hemizygous line.

Rb(*X*.2)2Ad was first discovered in a son of a female mouse carrying the reciprocal translocation T(5;13)Ad (Adler *et al.* 1987) and was assumed to be of spontaneous origin since neither parent had been treated with any mutagenic agent. One characteristic of Rb chromosomes in the mouse is the association

Reprint requests to Dr Ilse-Dore Adler at above address.

between heterozygosity and the occurrence of differing frequencies of meiotic anaphase I non-disjunction for the constituents of the autosomal multivalents formed in prophase of meiosis (Cattanach & Moseley, 1973; Gropp & Winking, 1981). In this connection, Rb2Ad presents the unique opportunity of analysing the meiotic pairing pattern of a highly heterogeneous *X*-autosomal trivalent and to study its influence on meiotic anaphase I disjunction of the autosomal and sex chromosome constituents in male heterozygotes. This study describes the observations made of the synaptonemal complex (SC) by light and electron microscopy, of the trivalent at diakinesis, and of the frequency of non-disjunction as assessed at the second meiotic division and also in the viable progeny.

2. Materials and methods

The original Rb2Ad male was mated to a (102/E1 × C3H/E1)F_1 female and by intercrossing of heterozygous and hemizygous offspring a homozygous/hemizygous line was established.

To determine the rate of aneuploid progeny hemizygous Rb2Ad males were mated to karyotypically normal (102/E1 × C3H/E1)F_1 females and to heterozygous and homozygous Rb2Ad females. Male and female animals were 10–12 weeks old at the beginning of the breeding experiments and 3–6 litters were observed per couple. Progeny were counted and sexed at birth and all newborn were cytogenetically examined by tail–tip preparation (Evans *et al.* 1972) at the day of birth or 1 day after. Aneuploid individuals were raised beyond weaning and the diagnosis was confirmed by karyotype analysis of G- and C-banded bone marrow cells (Adler, 1984). G- and C-banding was performed according to Gallimore & Richardson (1973) and Sumner (1972), respectively.

Testes preparations for light-microscopic analysis of spermatocytes at first and second meiotic divisions were performed according to Evans *et al.* (1964). The segregation pattern of the trivalent constituents in anaphase I was evaluated by scoring the total number of chromosomes and identifying the sex chromosomes in metaphase II (M II) cells. The cross-over frequency in chromosome 2 within the trivalent was ascertained by counting chiasmata at diakinesis/metaphase I. Light and electron microscopic analysis of SCs of two Rb2Ad males were undertaken on specimens prepared according to Johannisson *et al.* (1983) and Johannisson (1984). Testes wet weights were taken from 15 adult Rb2Ad males and 15 litter-mates with the standard karyotype at the age of 29–38 weeks.

Testes histology of four sex chromosomal aneuploid males, two Rb2AdXY and two Rb2AdRb2AdY, was analysed on 5 μm thick sections of specimen after fixation with Bouin's fluid, embedding in paraffin and staining with hematoxylin-eosin.

Differences of testis weight and between litter sizes were tested for significance using the *t* test. For differences in the frequencies of aneuploid cells or progeny, Fisher's exact probability test was used (Sachs, 1974). For differences in expected and observed incidences the χ^2 test was applied (Weber, 1967).

3. Results

(i) *Characterization of Rb(X.2)2Ad*

The two arms of the metacentric marker chromosome show the banding pattern typical of chromosome 2 and the *X* chromosome (Fig. 1). The presence of two distinct pericentromeric C-band positive blocks is shown in Fig. 2. At diakinesis the chromosomes 2 in the trivalent exhibited one or two chiasmata (Fig. 3*a*, *b*). None of the 100 cells analysed at diakinesis showed a univalent 2 plus a bivalent Rb2AdY. One cell showed a Y univalent. A chiasma frequency of 1·5 was determined by the observation of 53 cells with one chiasma and 47 cells with two chiasmata in chromosome 2. In case of a single cross-over along chromosome 2 the chiasma was preferentially located in the distal half of the chromosome. In contrast, in trivalents with two chiasmata along the chromosomes 2, one chiasma was always located proximally and one distally and the chromosomes 2 formed a ring-like figure (Fig. 3*b*). The sex chromosomes were associated with their telomeric tips. The mean testis weight of the Rb2Ad males of 77·7 ± 2·6 mg was significantly reduced compared to 104 ± 2·6 mg of the litter mates with normal karyotype ($P < 0{\cdot}001$).

(ii) *Pachytene analysis*

The synaptonemal complexes at pachytene of 312 nuclei were examined, 218 silver-stained nuclei were analysed in the light microscope (LM) and 94 cells in the electron microscope (EM). Some trivalent pairing was seen in all well spread nuclei but in the absence of a pairing partner for the proximal *X* chromosome segment, an entire trivalent synaptonemal complex which is characteristic for autosomal trivalents was not found. For comparison both types of trivalents are shown in Fig. 4*a*, *b*.

Complete pairing of the acrocentric autosome 2 was seen in three of 94 cells (3·2%) analysed in the EM, whereas in the remaining cells the proximal segment of the acrocentric chromosome 2 was not completely paired with the corresponding part of the metacentric chromosome Rb2Ad (Fig. 5).

The length of the non-paired segment was measured in 45 cells by EM photographs. It ranged from 6–23% of the length of the acrocentric chromosome 2. This range was found in early and later stages of pachytene. For staging of pachytene the amount of pairing between *X* and *Y* was used (de Boer *et al.* 1986). The non-paired segment of the acrocentric chromosome 2 was bent at a right angle to the axis in a characteristic way in about 50% of the cells (Fig. 4*b*) and appeared thickened in the majority of these cells.

https://doi.org/10.1017/S0016672300027944 Published online by Cambridge University Press

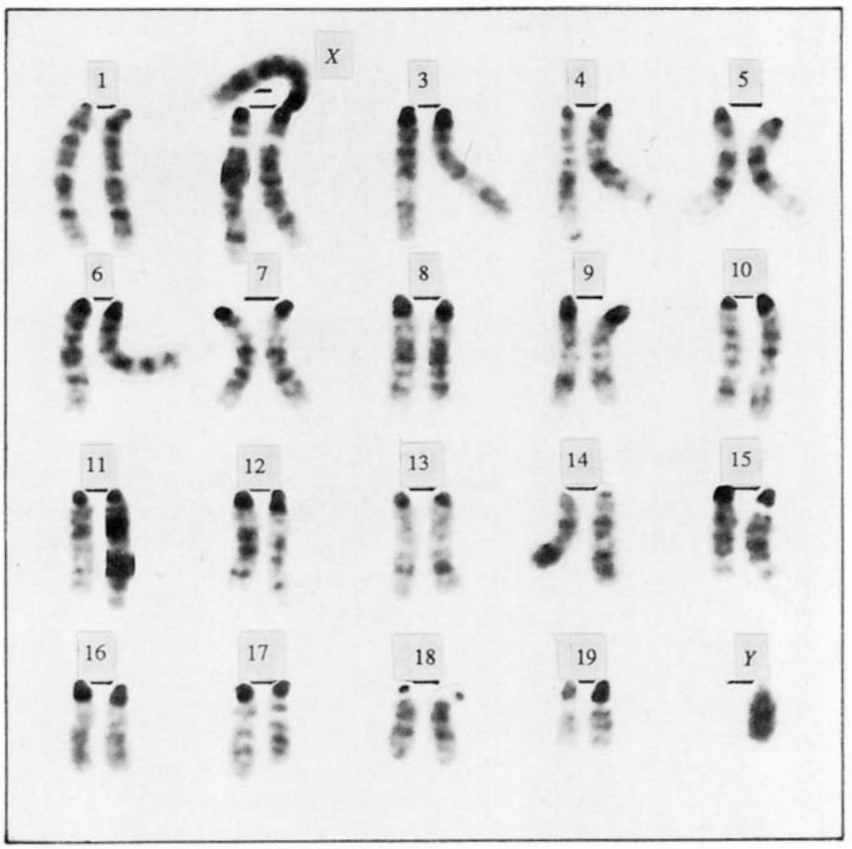

Fig. 1. Karyotype of a male mouse carrying the Robertsonian translocation between the *X* chromosome and chromosome 2 (Rb2Ad).

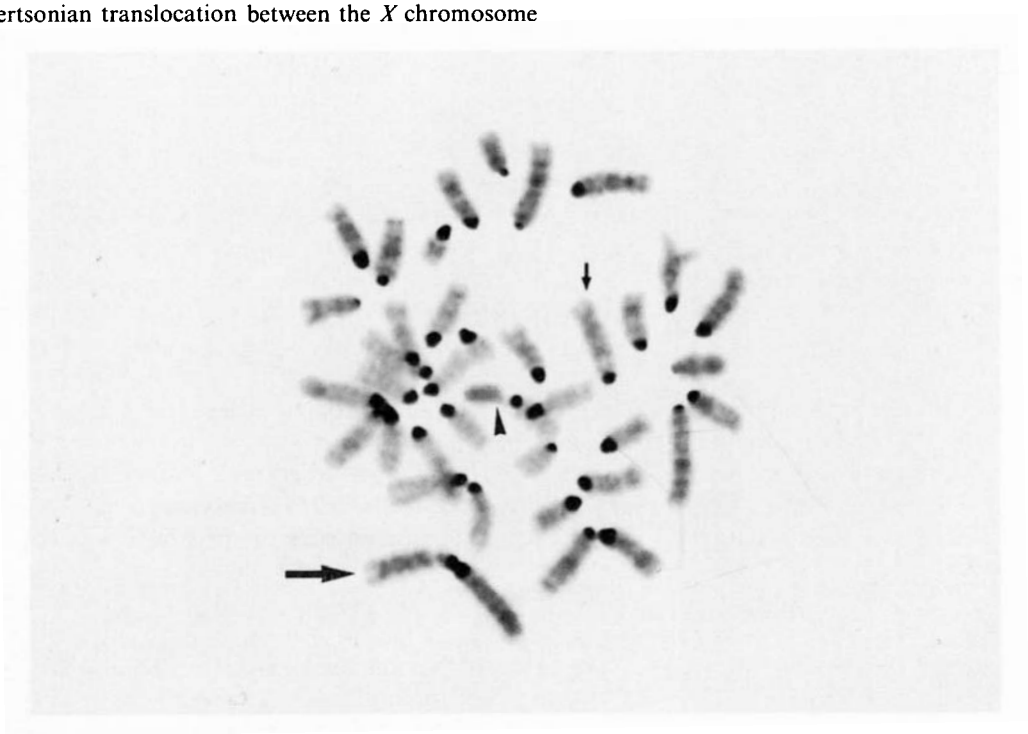

Fig. 2. C-banded mitotic cell from an Rb2Ad male. The large arrow indicates the Rb2Ad chromosome. The small arrow points to the acrocentric chromosome 2 and the arrow head to the *Y* chromosome.

The behaviour of the pairing segment of the *X* chromosome in the Rb2Ad and the *Y* chromosomes was in accordance to males with a normal karyotype. In 32 of 312 cells a secondary association was found between the proximal part of the *Y* chromosome and the non-paired proximal part of the acrocentric chromosome 2 (Fig. 6). Of these, 26 were classified as late pachytene.

https://doi.org/10.1017/S0016672300027944 Published online by Cambridge University Press

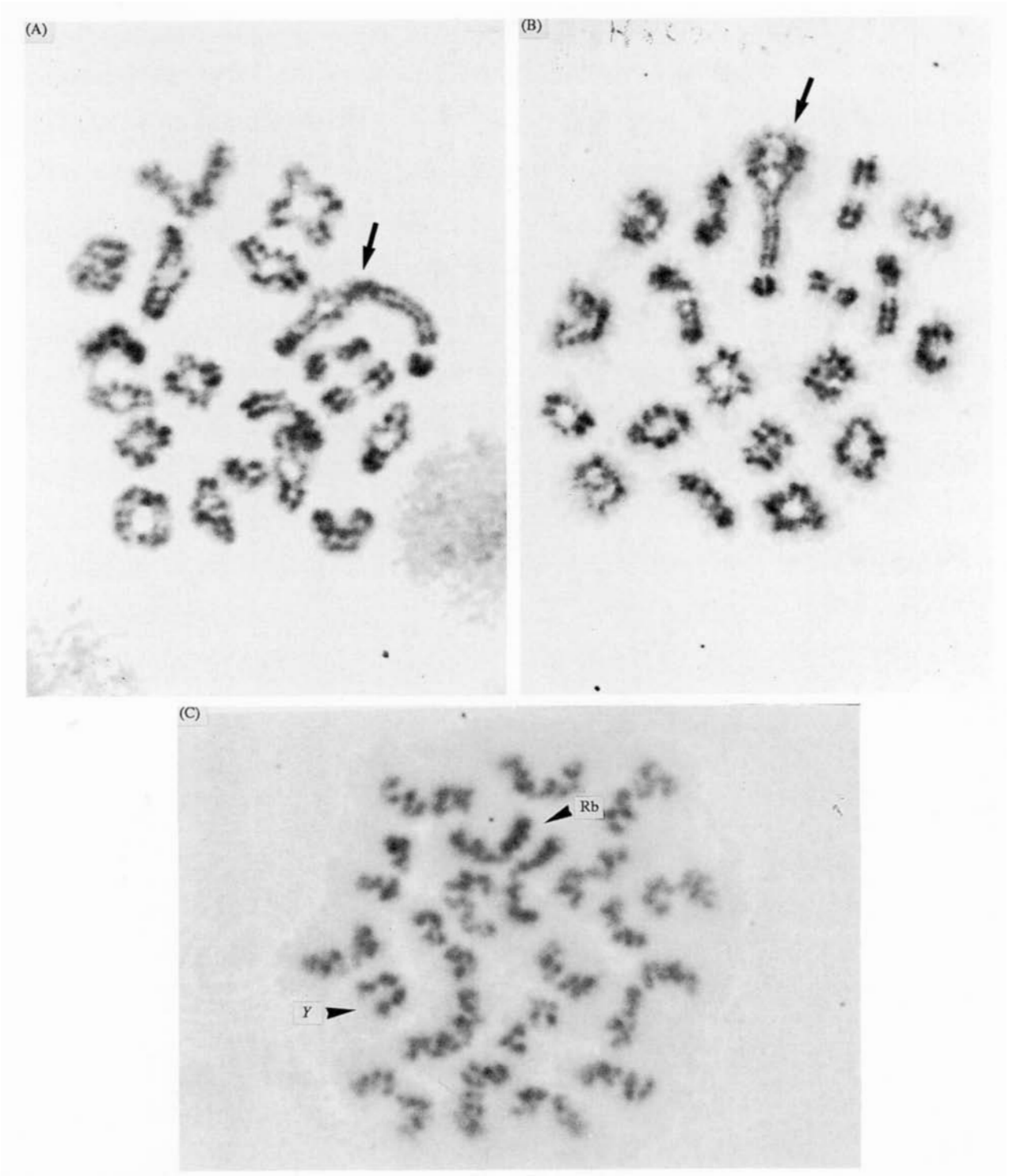

Fig. 3. Cells at first and second meiotic division of an Rb2Ad male. (A) Cell at diakinesis with one chiasma between the acrocentric chromosome 2 and the corresponding segment in the Rb2Ad chromosome (arrow). (B) Cell at diakinesis with two chiasmata in the acrocentric chromosome 2 and the 2 segment in the Rb2Ad chromosome (arrow). (C) Cell at metaphase II containing the Rb2Ad and the *Y* chromosome (arrows).

(iii) *Aneuploidy study in metaphase II of meiosis*

At metaphase of the second meiotic division (M II) the Rb2Ad and the *Y* chromosome are readily recognized by their morphology and darker appearance in orcein-stained preparations, viewed under phase contrast. The presence of both sex chromosomes in one cell is shown in Fig. 3*c*. Table 1 gives the results of the chromosome counts at M II. No deviation from the expected 1:1 ratio was observed for the *X*- and *Y*-bearing cells in the karyotypically normal and the Rb2Ad males. In M II cells of Rb2Ad males 4·5% of the cells possessed the Rb2Ad chromosome plus a *Y* chromosome, and 6·3% of the cells were without any sex chromosomes. The increase in hyperhaploid (Rb2AdY) M II cells is statistically significant

https://doi.org/10.1017/S0016672300027944 Published online by Cambridge University Press

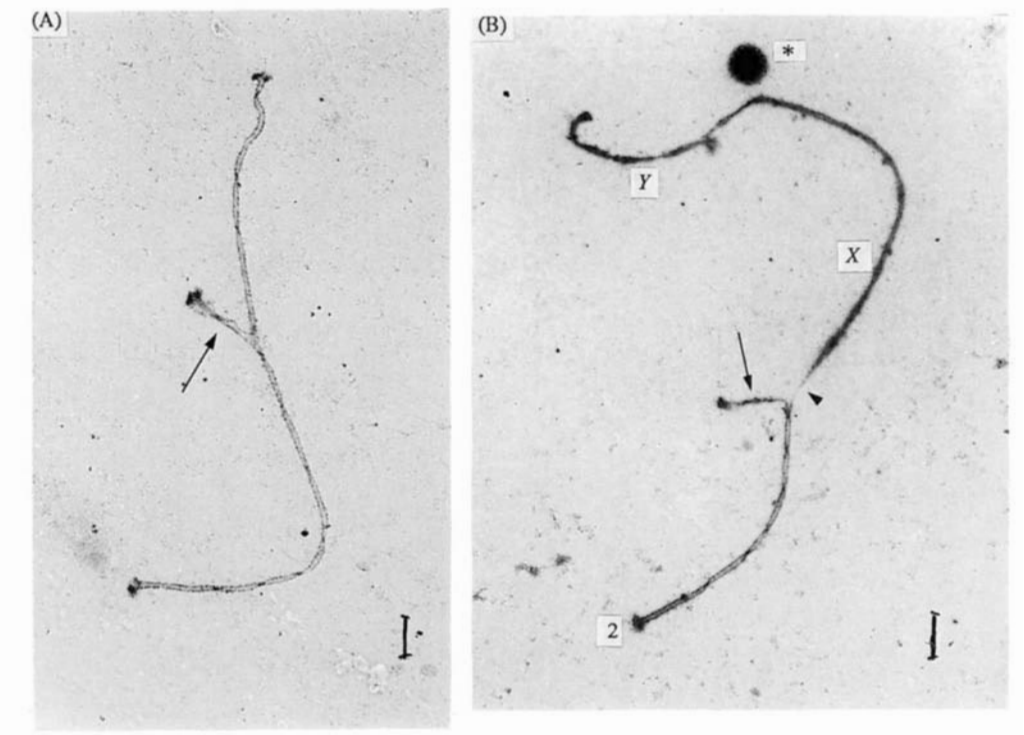

Fig. 4. Synaptonemal complexes of trivalents. Electron micrographs, bar = 1 μm. (A) Autosome–autosome trivalent showing a side arm (arrow) composed of the proximal parts of both acrocentric chromosomes. (B) Rb2Ad trivalent. The arrow head indicates a transitional region between the *X* and chromosome 2 segments of the Rb2Ad chromosome. The proximal part of the acrocentric chromosome 2 is bent at a right angle (arrow). The asterisk indicates the *X*-associated body.

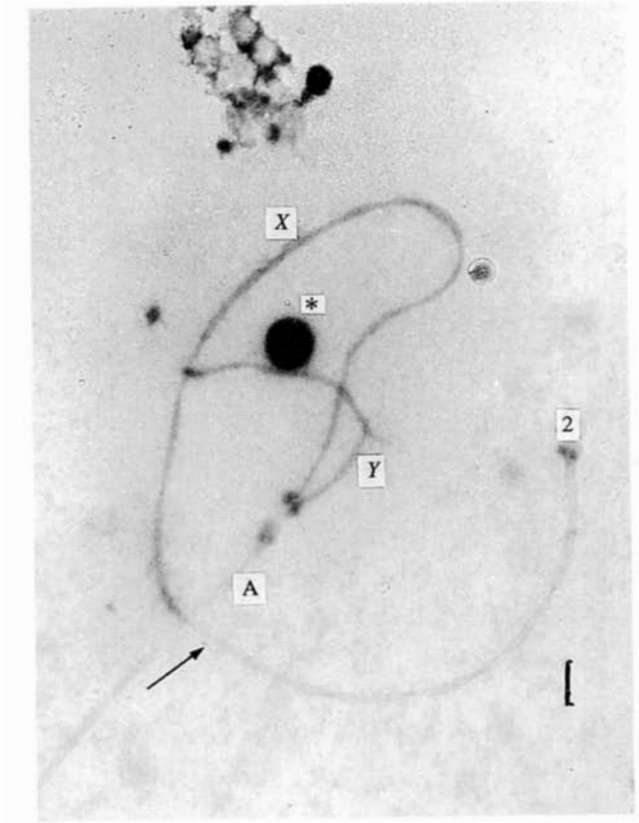

Fig. 5. Rb2Ad trivalent. Electron micrograph, bar = 1 μm. Complete pairing of the acrocentric chromosome 2 with the corresponding segment of the Rb2Ad chromosome. The arrow points to the proximal end of chromosome 2. The asterisk indicates the *X*-associated body. A = autosomal bivalent.

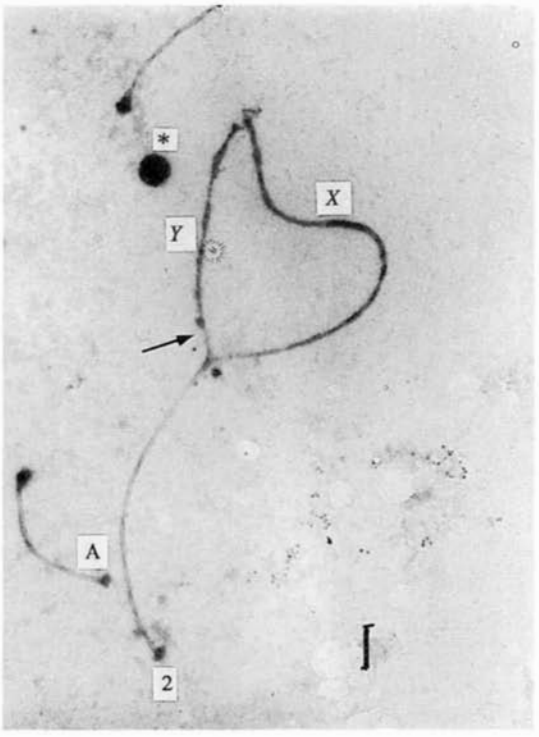

Fig. 6. Rb2Ad trivalent. Electron micrograph, bar = 1 μm. Late pachytene with secondary association between the *Y* chromosome and the proximal part of the acrocentric chromosome 2 (arrow). The asterisk indicates the *X*-associated body. A = autosomal bivalent.

https://doi.org/10.1017/S0016672300027944 Published online by Cambridge University Press

Table 1. *Chromosome counts of spermatocytes at second metaphase of meiosis (M II)*

Type of male[a]		No. of spermatocytes in M II							
		With normal		With aneuploid chromosome counts					
Rb*Y*	Total	18A + Rb	19A + *Y*	18A + Rb + *Y*	19A	17A + Rb	19A + Rb	18A + *Y*	20A + *Y*
	600	259	257	27	38	9	4	4	2
Percent	—	43·2	42·2	4·5	6·3	1·5	0·7	0·7	0·3
XY	Total	19A + *X*	19A + *Y*	19A + *X* + *Y*	19A	18A + *X*	20A + *X*	18A + *Y*	20A + *Y*
	600	284	271	0	0	21	0	23	1
Percent	—	47·3	45·2	0	0	3·5	0	3·8	0·2

[a] Four males, 150 cells at M II counted per male.
Rb = Rb(*X*.2)2Ad chromosome. A = autosomes.

Table 2. *Crosses to determine aneuploid progeny by chromosome analysis from tail-tip preparations of newborn mice*

Cross	Litter size [mean ± S.E. (*n*)]	Male offspring				Female offspring			
		Normal		Aneuploid		Normal		Aneuploid	
		XY	Rb*Y*	Rb*XY*	RbRb*Y*	Rb*X*	RbRb	RbO	*X*O
XX × Rb*Y*	7·4 ± 0·8 (17)	64	—	2	—	56	—	0	3
Rb*X* × Rb*Y*	5·1 ± 0·5* (22)	31	27	3	1	38	11	0	1
RbRb × Rb*Y*	6·7 ± 0·6 (15)	—	51	—	2	—	46	1	—
Total		95	78	5	3	94	57	1	4

* $P < 0{\cdot}05$ (*t* test) against a mean litter size of a random sample from (102/El × C3H/El)F_1 animals of 9·2 ± 0·2 (131 litters). Historical control frequency of *X*O females and *XXY* males is 0·07% (3 *X*O + 1 *XXY* among 5900 progeny). Rb = Rb2Ad chromosome.

($P < 0{\cdot}0001$). Autosomal aneuploidy was found in only 3·2% of the M II cells from the Rb2Ad males as compared to 7·5% of the M II cells from the normal males. Theoretically, the four hypohaploid cells with 18 autosomes plus *Y* and the four cells with 19 autosomes plus the Rb2Ad chromosome present at M II of Rb2Ad males can be interpreted as a result of non-disjunction of the Rb2Ad chromosome and the acrocentric chromosome 2. However, M II cells with 18 autosomes plus the *Y* chromosome were more frequent in the normal males ($P < 0{\cdot}0001$). While the hypohaploid M II cells may arise by loss of a chromosome due to preparational artefacts the hyperhaploid cells are most likely the result of non-disjunction during the first meiotic division. The rate of Rb2Ad autosome 2 non-disjunction as revealed by doubling the hyperhaploid M II cells amounts to 1·3%.

(iv) *Aneuploidy rate among live offspring*

Litter sizes and chromosomal constitution of the progeny of the three crosses are given in Table 2. Only the litter size of the cross between Rb2Ad males and heterozygous Rb2Ad females was significantly reduced compared to a random selection of progeny from (102/El × C3H/El)F_1 hybrids of the historical control for our heritable translocation assay ($P < 0{\cdot}05$). Among the progeny of Rb2Ad males and normal females, the total frequency of aneuploid progeny was 4·0%, whereby 2 of 66 males had a chromosome number of 40 (37 autosomes, *X*, Rb2Ad, *Y*) and 3 of 59 females were of an *X*O constitution.

The cross between the heterozygous Rb2Ad females and Rb2Ad males produced 4·5% aneuploid offspring, 3 Rb2AdXY and 1 Rb2AdRb2AdY among 62 males and one *X*O among 50 females. No triple-*X* female was found. It is noteworthy that less than one-third of the female progeny was homozygous for the Robertsonian translocation and the deviation from the expected 1 : 1 ratio is significant ($P < 0{\cdot}01$). Even when the males were included the progeny which had inherited the normal *X* from the mother were significantly more frequent than the Rb2Ad-bearing offspring ($P < 0{\cdot}05$).

Two aneuploid males (Rb2AdRb2AdY) among 53 males and one Rb2AdO female among 47 females were also found in the homozygous/hemizygous line. The total frequency of aneuploid progeny was 3·9%

https://doi.org/10.1017/S0016672300027944 Published online by Cambridge University Press

(13/337). In the large historical control group of the heritable translocation tests performed in the past years the spontaneous rate was 3 *X*Os among 2950 female progeny and one *XXY* among 2950 male progeny, which amounts to 0·07% (4/5900). The frequencies of aneuploid males and females among the progeny of the Rb2Ad crosses were significantly higher than in the historical control ($P < 0·001$).

(v) *Fertility of male progeny with sex chromosomal aneuploidy*

Rb2AdXY males and Rb2AdRb2AdY males were completely sterile judged by a total lack of spermatogenesis, even at the age of 4–5 weeks. Histologically only Sertoli cells were identified within the lumen of the testicular tubules. Testis weights ranged from 18 to 35 mg at 5 weeks of age. This observation is in accord with previous reports on *XXY* males (Russell & Chu, 1961; Russell, 1976).

4. Discussion

The results obtained from the chromosome analyses of the progeny and at the second metaphase of meiosis conclusively show that the inclusion of Rb2Ad in the male genome induces non-disjunction at the first meiotic division. Compared to $(102/E1 \times C3H/E1)F_1$ males with normal karyotype (0·07% sex-chromosomally abnormal offspring), the frequency of aneuploid progeny from Rb2Ad males was increased by a factor of 56, or to 3·9% of the total newborns screened in this study. At second metaphase, the total non-disjunction frequency of the sex chromosomes was 10·8%, and secondary spermatocytes containing two or no sex chromosomes were equally frequent. The difference between the estimates obtained from the secondary spermatocyte counts, as against those obtained from the progeny, is significant ($P < 0·05$) and the reduction to less than half probably results from the loss of zygotes with sex chromosomal aneuploidies (*X*O and *XXY*). Since *X*O and *XXY* animals are equally underrepresented among the newborn in comparison to the M II observations both sex-chromosome aneuploidies seem to be burdened with a similarly reduced viability. Reduced viability of *X*O female mice has been reported by Brook (1983). Similar losses of autosomal aneuploidies are well documented in studies involving purely autosomal Rb translocations (Gropp *et al.* 1976).

Without the inclusion of genetic markers on parental chromosomes it is largely impossible to determine whether aneuploidy arose at male or at female meiosis in crosses of Rb2Ad males to heterozygous Rb2Ad females. Only the Rb2AdRb2Ad males and *X*O females can be concluded to result from paternal non-disjunction whereas Rb2AdXY males could equally be the result of maternal and paternal non-disjunction. However, the frequency of aneuploid progeny observed (5 in 112, or 4·5%) is almost identical to the frequency obtained for normal females crossed to Rb2Ad males (5 in 125 or 4·0%). This result implies that the contribution to viable aneuploidy from the Rb2Ad females is negligible unless the result obtained is masked by prenatal loss of a large proportion of aneuploid progeny. Support for this assumption is obtained from the reduction in litter size of the hemizygous times heterozygous cross as compared to the other two crosses (Table 2) and the chief contributors to the loss are most likely to be the zygotes derived from the non-disjunction of chromosome 2 at female anaphase I. Monosomy or trisomy for this chromosome are known to act as early lethals with only a few trisomic embryos even reaching the postimplantation stage (Baranov, 1983).

As is evident from the studies of non-disjunction in mice homozygous for autosomal Rb translocations, little, if any, non-disjunction occurs at meiosis (Gropp & Winking, 1981). It is therefore assumed that the incidence of 3·0% aneuploid progeny observed in the homozygous/hemizygous Rb2Ad line results totally from male anaphase I non-disjunction and this frequency is not at variance with the other two frequencies obtained from crosses of hemizygous Rb2Ad males to karyotypically normal or Rb2Ad heterozygous females.

The lack of triple-*X* females in the recovered progeny has already been mentioned and this chromosome constitution in the mouse either does not occur or causes lethality before birth. The Rb2Ad chromosome appears to be preferentially distributed to the polar body, as suggested by the shortage of homozygous females among the progeny of Rb2Ad males crossed to heterozygous Rb2Ad females. The shortage of Rb2Ad homozygous females is likewise explainable by a reduced viability in utero of these females. However, among the homozygous/hemizygous Rb2Ad line, homozygous Rb2Ad females and hemizygous Rb2Ad males are equally represented (Table 2). A balanced sex ratio in this line does not support the assumption of a reduced viability of homozygous females. A preferential segregation of the Rb2Ad chromosome into the polar body could explain the lack of triple-*X* females. Such a preferential segregation of Rb chromosomes to the polar body has been found in females heterozygous for certain autosomal Rb translocations (Gropp & Winking, 1981). On the other hand, the preferential retention of the Rb chromosome in the oocyte nucleus has been described for one autosomal Rb translocation (Harris *et al.* 1986).

The pairing configurations of the chromosomes 2, Rb2Ad and *Y* were studied during meiotic prophase. In autosomal trivalents a complete synaptonemal complex is formed by the three pairing partners. In the heterosome/autosome trivalent a large part of the *X* chromosome remained unpaired. In addition the majority of cells showed lack of pairing between the

https://doi.org/10.1017/S0016672300027944 Published online by Cambridge University Press

proximal region of chromosome 2 and its homologue in the Rb2Ad chromosome. Despite these peculiarities the non-disjunction frequencies caused by the heterosome/autosome trivalent are in a range similar to those present in mice with Rb translocations of laboratory origin. Much higher rates have been reported in the literature for autosomal trivalents of ferally derived Rb chromosomes after introduction into a laboratory mouse strain (Gropp & Winking, 1981).

If the pairing failure seen in the proximal segment of chromosome 2 is associated in some way with the tendency for subsequent non-disjunctional events to occur in anaphase I, the acrocentric chromosome 2 should be preferentially involved. However, from the chromosome counts at MII it becomes evident that only 8 of 600 cells (1·3%) analysed showed an extra autosome in addition to the Rb2Ad chromosome or lack of an autosome in the presence of a *Y* chromosome. These cells are the candidates for non-disjunction of the Rb2Ad and the acrocentric autosome 2. In contrast, the chromosomal constitution of 65 of 600 (10·8%) MII cells with the Rb2Ad and the *Y* chromosome or no sex chromosome at all is explainable by non-disjunction of the Rb2Ad and the *Y* chromosomes. The two acrocentric constituents of the trivalent are therefore not equally involved in non-disjunctional events. Furthermore, there is a negative correlation between pairing irregularities seen in the proximal region of autosome 2, normal pairing between Rb2Ad and the *Y* chromosome and non-disjunction.

The reason for the relatively high incidence of sex-chromosomal non-disjunction, when compared to the Rb2Ad-2 non-disjunction frequency, may be seen in the two striking differences between the autosomal and heterosomal part of the trivalent. Firstly, in the heterosomal part chiasmata are exclusively located terminally, whereas in the autosomal part half of the cells showed proximal chiasmata. Secondly, in general the separation of the *X* and *Y* chromosome starts during pachytene much earlier than the separation of the autosomes, which move apart during diplotene. This asynchrony is also present between the pairing partners of the autosome/heterosome trivalent. It is not yet clear, whether these differences are responsible for the differing proneness of the pairing partners to non-disjunction. However, the asynchronous separation in conjunction with the highly unequal distribution of chiasmata on both sides of the centromere of the Rb2Ad chromosome seems to be well suited to cause this phenomenon. Therefore, the following model is suggested as an explanation of the Rb2Ad and *Y* chromosome non-disjunction. A precocious anaphase I disjunction of the *Y* chromosome from the Rb2Ad chromosome, which might be facilitated by the asynchronous separation and by the terminal chiasma position, renders it possible that the Rb2Ad-2 autosome trivalent fragment reorientates and disjoins later synchronously with the other autosomes. This enables the Rb2Ad chromosome to segregate independently from the *Y* chromosome, but not from the acrocentric chromosome 2.

An exceptional difference between the observations made in the autosomal Rb translocations and in the present *X*-autosomal Rb translocation is in the apparent contribution of non-disjunction at male and female meiosis to the incidence of viable aneuploid progeny. Of course, these are restricted to aneuploidy of the sex chromosomes since aneuploid individuals for autosomes are non-viable. A considerably higher meiotic non-disjunction rate in females with purely autosomal Rb trivalents seems to be a general phenomenon (Gropp & Winking, 1981). It has to be anticipated that in female meiosis of Rb2Ad heterozygotes a synaptonemal complex is formed along the entire length of the *X*-autosome trivalent, similar to the condition of autosome–autosome trivalents, while the major proximal part of the *X* chromosome in male meiosis remains unsynapsed due to the unequal length of the sex chromosomes (see Fig. 4*a*, *b*). However, in the Rb2Ad line the contribution of the female meiosis to the total incidence of viable aneuploid progeny seems negligible. Breeding experiments of heterozygous Rb2Ad females to normal males have been initiated to clarify this point. Furthermore, cytogenetic analyses of MII and first cleavage-division cells in heterozygous Rb2Ad females are presently conducted by I. Hansmann, Göttingen, to determine the total incidence of non-disjunction in heterozygous Rb2Ad females.

The Rb(*X*.2)2Ad translocation is an interesting tool for the study of non-disjunction processes as well as for the production of sex-chromosome aneuploid individuals. Another aspect of research with the Rb2Ad chromosome pertains to the fertility of male hybrids. Lifschytz & Lindsley (1972) postulated *X*-chromosome inactivation in primary spermatocytes of heterogametic males as a basic control mechanism for normal spermatogenesis. Following their hypothesis sterility of males carrying a reciprocal translocation between the *X* chromosome and an autosome results from *X*-chromosome reactivation during meiotic prophase. Lifschytz & Lindsley (1972) suggested that translocations between two chromosomes under asynchronous control (as *X* and autosomes) 'puts elements of each under dual and conflicting control resulting in the *X* chromosome reactivation'. This hypothesis was extended by Forejt & Gregorova (1977) to *X*-autosome contact in sterile mice with structural heterozygosity. Infertility in the human male was also attributed to *X*-autosome interaction (Johannisson *et al.* 1987). On the other hand, Viguie *et al.* (1982) and Ratomponirina *et al.* (1985) proposed autosomal inactivation in human *Y*-autosomal sterile translocations instead of the *X*-reactivation process (Lifschytz & Lindsley, 1972; Rosenmann *et al.* 1985).

In the Rb2Ad the *X*-chromosomal autosomal interaction in meiotic prophase can be expected to result in complete sterility on account of the joining of the *X* and autosome 2. However, normal fertility, despite the reduced testis weight, of Rb2Ad males estimated from litter size does not fit in this hypothesis. A similar discrepancy is known in *Drosophila*, namely fertile *X*4 and *XY* translocations. This was reconciled by postulating sequences in chromosomes 4 and *Y* which are analogous to those in the proximal heterochromatin of the *X* chromosome (Baker & Lindsley, 1982). Moreover, Burgoyne & Baker (1984) argue that incomplete pairing during pachytene is causally related to impairment of fertility with variable severity. As presented, this suggestion may have more bearing in the case of Rb2Ad since it could explain both its fertility and the reduction in testis weight.

Other aspects of research in which the Rb2Ad chromosome will prove useful include studies of *X* chromosome inactivation, which are in progress, as a readily identifiable marker for the determination of parental derivation of the *X* chromosome in both normal mice and in chimeras and also as a marker for mapping loci by *in situ* hybridization.

The authors would like to express their gratitude to Dr E. P. Evans, Oxford, for helpful and critical discussion of the manuscript. Ruth Schmöller and Beatrix Nether are gratefully acknowledged for their skilful conduct of the breeding experiments and the cytogenetic analysis of the tail-tip preparations.

References

Adler, I.-D. (1984). Cytogenetic tests in mammals. In *Mutagenicity Testing: A Practical Approach* (ed. S. Venitt and J. M. Parry), pp. 275–306. Oxford, Washington, D.C.: IRL Press.

Adler, I.-D. & Neuhäuser-Klaus, A. (1987). Seventeen stocks of mice with reciprocal or Robertsonian translocations. *Mouse News Letter* **77**, 139–142.

Adler, I.-D., Schmöller, R., Nether, B. & Johannisson, R. (1987). A Robertsonian translocation involving the *X*-chromosome of the mouse. *Genetical Research* **49**, 249.

Adolph, S. & Klein, J. (1983). Genetic variation of wild mouse populations in southern Germany. *Genetical Research* **41**, 117–134.

Arroyo Nombela, J. J. & Rodriguez Murcia, C. (1977). Spontaneous double Robertsonian translocations Rb(2.3) and Rb(*X*.3) in the mouse. *Cytogenetics and Cell Genetics* **19**, 227–230.

Ashley, T. (1983). Nonhomologous synapsis of the sex chromosomes in the heteromorphic bivalents of two *X*-7 translocations in male mice: R5 and R6. *Chromosoma* **88**, 178–183.

Baker, B. S. & Lindsley, D. L (1982). The genetic control of sex determination and male fertility in *Drosophila melanogaster*. In *Proceedings of the Serono Clinical Colloquia on Reproduction. 3. Genetic Control of Gamete Production and Function* (ed. P. G. Crosignani and B. L. Rubin), pp. 153–170. London: Academic Press/Grune and Stratton.

Baranov, V. S. (1983). Chromosomal control of early embryonic development in mice. I. Experiments on embryos with autosomal monosomy. *Genetica* **61**, 165–177.

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

Brook, J. D. (1983). *X*-chromosome segregation, maternal age and aneuploidy in the *XO* mouse. *Genetical Research* **41**, 85–95.

Burgoyne, P. S. & Baker, T. C. (1984). Meiotic pairing and gametogenic failure. In *Controlling Events in Meiosis* (ed. C. W. Evans and H. G. Dickson), 38th Symposium of SFEB, pp. 349–362. Company of Biologists, Cambridge.

Capanna, E., Gropp, A., Winking, H., Noak, G. & Civitelli, M.-V. (1976). Robertsonian metacentrics in the mouse. *Chromosoma* **58**, 341–353.

Cattanach, B. M. & Moseley, H. (1973). Non-disjunction and reduced fertility caused by the tabacco mouse metacentric chromosomes. *Cytogenetics and Cell Genetics* **12**, 264–287.

Evans, E. P., Breckon, G. & Ford, C. E. (1964). An air-drying method for meiotic preparations for mammalian testes. *Cytogenetics* **33**, 289–294.

Evans, E. P., Lyon, M. F. & Daglish, M. (1967). A mouse giving a metacentric marker chromosome. *Cytogenetics* **6**, 105–119.

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

Forejt, J. & Gregorova, S. (1977). Meitoic studies of translocations causing male sterility in the mouse. I. Autosomal reciprocal translocations. *Cytogenetics and Cell Genetics* **19**, 159–179.

Gallimore, P. H. & Richardson, C. R. (1973). An improved banding technique exemplified in the karyotype analysis of two strains of rats. *Chromosoma* **41**, 259–263.

Gropp, A., Tettenborn, U. & Lehmann, E. von (1970). Chromosomenvariationen vom Robertson'schen Typus bei der Tabakmaus M. poschiavius und ihren Hybriden mit der Laboratoriumsmaus. *Cytogenetics* **9**, 9–23.

Gropp, A. & Winking, H. (1981). Robertsonian translocations: cytology, meiosis, segregation patterns and biological consequences of heterozygosity. *Symposium of the Zoological Society London* **47**, 141–181.

Gropp, A., Putz, B. & Zimmerman, U. (1976). Autosomal monosomy and trisomy causing developmental failure. *Current Topics of Pathology* **62**, 177–192.

Harris, M. J., Wallace, M. E. & Evan, E. P. (1986). Aneuploidy in the embryonic progeny of females heterozygous for the Robertsonian chromosome (9.12) in genetically wild Peru-Coppock mice (*Mus musculus*). *Journal of Reproduction and Fertility* **76**, 193–203.

Johannisson, R., Gropp, A., Winking, H., Coerdt, W., Rehder, H. & Schwinger, E. (1983). Down's syndrome in the male. Reproductive pathology and meiotic studies. *Human Genetics* **63**, 132–138.

Johannisson, R. (1984). Methoden der Chromosomenspreitung zur Darstellung synaptonemaler Komplexe in der meiotischen Prophase von Säugern. *GIT Fachzeitschrift für das Laboratorium* **28**, 68–675.

Johannisson, R., Löhrs, U., Wolff, H. H. & Schwinger, E. (1987). Two different XY-quadrivalent associations and impairment of fertility in men. *Cytogenetics and Cell Genetics* **45**, 222–230.

Léonard, A., Deknudt, G. (1967). A new marker for chromosome studies in the mouse. *Nature* **214**, 504–505.

Lifschytz, E. & Lindsley, D. L. (1972). The role of *X*-chromosome inactivation during spermatogenesis. *Proceedings of the National Academy of Science, USA* **69**, 182–186.

Ratomponirina, C., Couturier, J., Gabriel-Robez, O., Rumpler, Y., Dutrillaux, B., Croquette, M., Rabache, Q. & Leduc, M. (1985). Aberrations of the synaptonemal complexes in a male 46,XY, −14, +der(14)t(Y;14). *Annals of Genetics* **28**, 214–218.

Rosenmann, A., Wahrman, J., Richler, C., Voss, R., Persitz, A. & Goldman, B. (1985). Meiotic association between the *XY* chromosomes and unpaired autosomal elements as a cause of human male sterility. *Cytogenetics and Cell Genetics* **39**, 19–29.

Russell, L. B. & Chu, E. H. Y. (1961). An *XXY* male in the mouse. *Proceedings of the National Academy of Science, USA* **47**, 571–575.

Russell, L. B. (1976). Numerical sex-chromosome anomalies in mammals: their spontaneous occurrence and use in mutagenesis studies. In *Chemical Mutagens. Principles and Methods of their Detection*, vol. 4 (ed. A. Hollaender), pp. 55–91. New York, London: Plenum Press.

Sachs, L. (1974). *Angewandte Statistik*. Berlin: Springer.

Sumner, A. T. (1972). A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research* **75**, 304–306.

Viguie, F., Romani, F. & Dadoune, J. P. (1982). Male infertility in a case of (Y;6) balanced reciprocal translocation. Mitotic and meiotic study. *Human Genetics* **62**, 255–277.

Weber, E. (1967). *Mathematische Grundlagen der Genetik*. Jena: G. Fischer.

Winking, H., Dulic, B. & Bulfield, G. (1988). Robertsonian karyotype variation in the European house mouse, *M. musculus*. Survey of present knowledge and new observations. *Zeitschrift für Säugetierkunde* **53**, 148–161.