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Two new X-autosome Robertsonian translocations in the mouse.
I. Meiotic chromosome segregation in male hemizygotes and female heterozygotes

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Summary

Two new X-autosome Robertsonian (Rb) translocations, Rb(X.9)6H and Rb(X.12)7H, were found during the course of breeding the Rb(X.2)2Ad rearrangement at Harwell. The influence of these new Rbs on meiotic chromosome segregation was investigated in hemizygous males and heterozygous females and compared to that of Rb(X.2)2Ad. Screening of metaphase II spermatocytes gave incidences of sex chromosome aneuploidy of 9·2% in Rb(X.2)6H/Y and 9·6% in Rb(X.9)2Ad/Y males; no metaphase II cells were present in the testes of the Rb(X.12)7H/Y males examined and no males with this karyotype have so far proved fertile. In breeding tests, 5% of the progeny of Rb(X.2)2Ad/Y males were sex chromosome aneuploids compared to 10% of the Rb(X.9)6H/Y offspring. The difference was not significant, however. Cytogenetic analyses of metaphase II stage oocytes showed elevated rates of hyperhaploidy ($n+1$) in Rb heterozygous females over chromosomally normal mice: 4·2% for Rb(X.2)2Ad/+; 2·1% for Rb(X.9)6H/+; 2·2% for Rb(X.12)7H/+ and 1·1% for normal females. There was, however, no statistically significant difference in the rates of hyperhaploidy between the three different Rb types, nor overall between Rb/+ and normal females. Karyotypic analyses of liveborn offspring of Rb heterozygous females revealed low incidences of X0 animals but no other type of sex chromosome aneuploidy. Intercrosses of heterozygous females and hemizygous males yielded 5·5% aneuploidy for Rb(X.2)2Ad and 5·4% for Rb(X.9)6H. In heterozygous females, there was evidence from the metaphase II and breeding test data for all three rearrangements, of preferential segregation of the Rb metacentric to the polar body resulting in a deficiency of cells and progeny carrying a translocation chromosome.

1. Introduction

Spontaneous chromosome rearrangements such as Robertsonian (Rb) translocations are believed to occur at a low incidence in the laboratory mouse. Accurate estimates of the rates of such events are not available due to the lack of large-scale, systematic karyotyping of laboratory populations. However, some indication of their rate can be obtained from information reported in mutation studies. For example, Ford (1970) summarized observations from a number of studies and reported that in a sample of 5460 mice, no animal with a *de novo* Rb translocation was found. These data therefore indicate an extremely low rate of spontaneous formation of this type of chromosome rearrangement. Nevertheless, some 21 such Rb translocations have been identified in laboratory strains of mice (see Searle, 1989). A considerably larger number of Rb translocations has been found in wild populations of mice, although it is not clear whether their rate of formation differs from that in laboratory strains. Overall, of the possible 171 autosomal arm combinations, approximately 120 have been found in laboratory and wild populations of mice (Redi & Capanna, 1988). In contrast, Rb translocations involving the sex chromosomes are extremely rare. Arroyo Nombela & Rodriguez Murcia (1977) described an Rb involving the X and chromosome 3. Unfortunately, identification was only made in a *post-mortem* analysis. More recently, Adler, Johannisson & Winking (1989) recovered a rearrangement involving the X and chromosome 2 (now formally identified as Rb(X.2)2Ad) in a fertile male and were thus able to breed the first stock of laboratory

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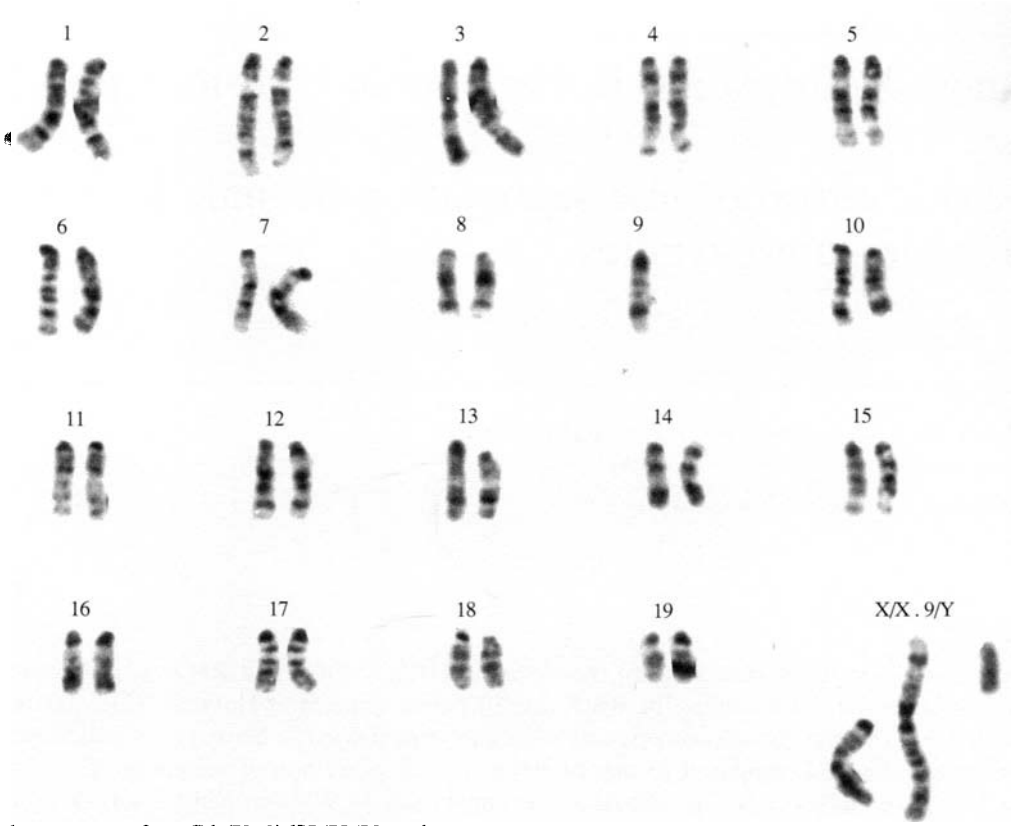

Fig. 1. G-banded karyotype of an Rb(X.9)6H/X/Y male.

mice with an Rb translocation involving a sex chromosome. They found that hemizygous males had a higher rate of nondisjunction of the sex chromosomes at meiosis compared to their chromosomally normal counterparts. As a consequence, an increased proportion of the progeny of hemizygous males were X0 or XXY aneuploids. Although they did not analyse directly the influence of the Rb on chromosome segregation in female meiosis, they were able to infer from breeding data that the effect was either small or masked by prenatal loss of aneuploid embryos. This inference was drawn from the similar rates of sex chromosome aneuploidy among the progeny of hemizygous males, regardless of whether they were mated to chromosomally normal or Rb heterozygous females.

In the course of breeding the Rb(X.2)2Ad translocation at Harwell, two new Rb rearrangements were found. Both proved to involve the X chromosome but different autosomes, 9 and 12. This paper describes the origin and identification of these new Rb translocations, and also examines the influence of these new rearrangements on chromosome segregation in both male and female meiosis.

2. Materials and methods

Rb(X.2)2Ad (abbreviated hereafter to R2A) heterozygous females and hemizygous males were bred from animals kindly supplied by Dr Ilse-Dore Adler. The two new Rb translocations were found as described below in the Results section in the course of breeding R2A. Identification of the chromosomes involved in the new rearrangements was carried out using G-banded lymphocyte preparations (Gallimore & Richardson, 1973; Triman, Davisson & Roderick, 1975).

Nondisjunction rates were assessed in these new Rbs both cytogenetically and through breeding tests in hemizygous males and heterozygous females. Cytogenetic analysis of chromosome segregation at anaphase I in male meiosis was investigated in 5 Rb males of each type and also in 5 normal males. The males were given 0·1 ml of 0·04% colchicine 2 h prior to killing to enhance the numbers of metaphase II cells in the preparations. Spermatocyte preparations were made using the standard method (Evans, Breckon & Ford, 1964).

Oocytes at metaphase II were obtained by inducing ovulation with 5 iu PMS followed 48 h later by 5 iu HCG. The cells were collected from the oviducts and prepared for microscopy in the standard fashion (Tarkowski, 1966).

Chromosome numbers were screened in metaphase II cells from male and female mice after C-banding (Sumner, 1972).

In some of the breeding tests, the X-linked coat marker genes tabby (*Ta*) and blotchy (Mo^{blo}) were used to aid identification of progeny with sex chromosome aneuploidy as described in reviews by Russell (1976) and Tease & Cattanach (1986). The various matings initiated to screen for sex chromosome

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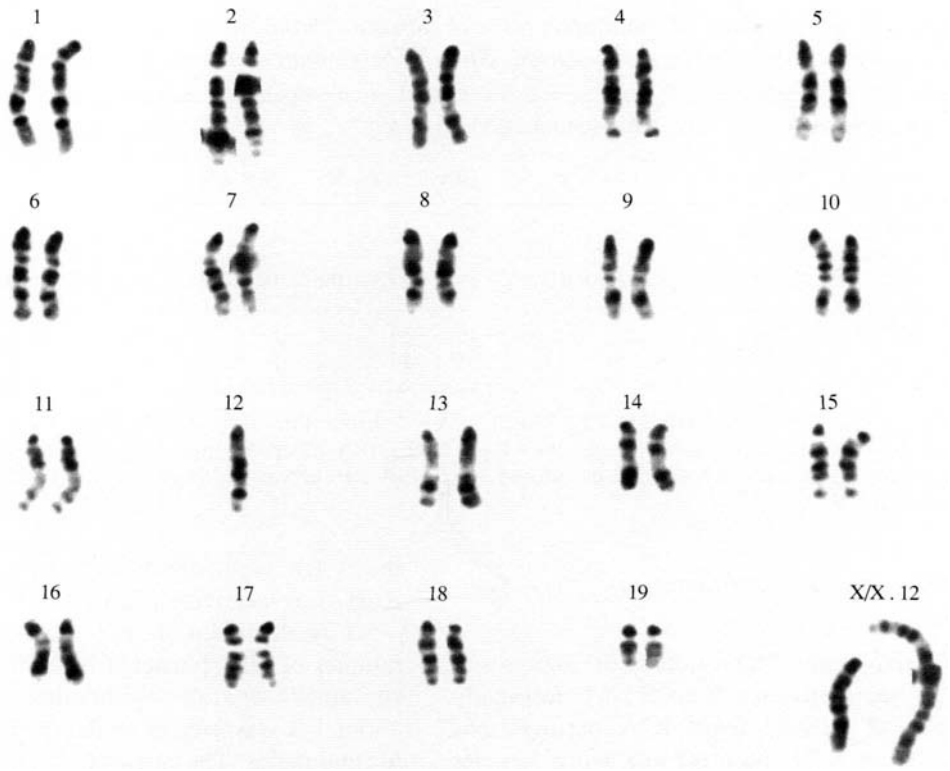

Fig. 2. G-banded karyotype of a female heterozygous for Rb(X.12)7H.

aneuploidy among the progeny of hemizygous males and heterozygous females are detailed below in the Results section. Post-weaning-age animals were karyotyped principally using lymphocyte chromosome preparations, although in a few instances bone marrow cells were used (Adler, 1984).

3. Results

(i) *Origin and identification of the new Rb translocations*

The first of the new Rb translocations was found among the progeny of an R2A/Y male mated to a chromosomally normal female mouse of the genotype $Ta+/+Mo^{blo}$. Paternal chromosome nondisjunction attributable to R2A resulted in progeny with sex chromosome aneuploidy that were in the majority of instances phenotypically recognizable, i.e. *Ta*0, $Mo^{blo}0$, $Ta/+/Y$, and $Mo^{blo}/+/Y$. Phenotypic classification was confirmed by karyotypic analysis. Unexpectedly, however, one male with the Mo^{blo}/Y phenotype of normal chromosome segregation, was found to carry an Rb metacentric chromosome. Examination of G-banded chromosomes showed the rearrangement to involve the X and chromosome 9, and this translocation has been designated Rb(X.9)6H (Fig. 1; abbreviated hereafter to R6H). Since the animal carried the marker gene Mo^{blo}, the new rearrangement must have involved the *maternally* derived X chromosome and was therefore apparently independent of the paternal Rb chromosome. There was no indication of mosaicism for the rearrangement, suggesting that it occurred either in the maternal germ line or immediately following conception and before the first cleavage division.

The R6H/Y male was mated to a female of the F_1 hybrid type C3H/HeH × 101/H to maintain the translocation; offspring were karyotyped to identify carriers. In the first litter, a female was found that carried 2 Rb translocations; examination of G-banded chromosomes showed that not only did this female carry R6H but also another involving the X chromosome and autosome 12. The latter rearrangement has been designated Rb(X.12)7H (Fig. 2; abbreviated hereafter to R7H). Since the female carried R6H, this second new Rb must again have involved the *maternally* derived acrocentric X chromosome. No evidence of mosaicism was found in the female.

Subsequent progeny from matings involving the R6H/Y male and the R6H/R7H female were karyotyped; however, no further instances of new Rb translocation appeared.

Hemizygous males and heterozygous females for R7H were recovered from the progeny of the R6H/R7H female; breeding tests showed R7H/Y males to be sterile. Heterozygous females were fertile, however, and the translocation was maintained by mating to chromosomally normal males and cytogenetic screening of female progeny.

Table 1. *Numbers of metaphase II spermatocytes with different chromosome constitutions from normal males and males carrying a Robertsonian translocation. Rb = Robertsonian metacentric chromosome; A = autosome*

Genotype	Euploid cells		Aneuploid cells						Total number of cells
	19A+X	19A+Y	19A+X+Y	19A	18A+X	20A+X	18A+Y	20A+Y	
+/Y	241[a]	247	1	1	3	1	4	2[b]	500
	18A+Rb	19A+Y	18A+Rb+Y	19A	17A+Rb	19A+Rb	18A+Y	20A+Y	
R2A/Y	202	216	28[c]	18[d]	19	1	14	2	500
R6H/Y	196[e]	217[f]	25[g]	23[h]	17[i]	1	17	4[j]	500

Totals include: [a] 1 cell, 18A+2 chromatids+Y; [b] 1 cell, 19A+2 chromatids+Y; [c] 2 cells, 17A+Rb+Y; [d] 2 cells, 19A and 1 cell, 19A+1 chromatid; [e] 1 cell, 18A+1 fragment+Rb; [f] 2 cells, 18A+2 chromatids+Y and 1 cell, 19A+1 fragment+Y; [g] 1 cell, 19A+Rb+Y; [h] 1 cell, 18A+1 chromatid and 1 cell, 19A+1 chromatid; [i] 1 cell, 17A+1 chromatid+Rb; [j] 1 cell, 19A+1 chromatid+Y.

(ii) *Cytogenetic analysis of chromosome nondisjunction*

(*a*) *Hemizygous males*. The results of the analyses of metaphase II spermatocytes from 5 chromosomally normal males (3 derived from R2A matings, the remaining 2 from R6H matings) and from 5 males each of the R2A/Y and R6H/Y genotypes are given in Table 1; meiosis in R7H/Y males appeared to be arrested at metaphase I and so they could not be included in this part of the investigation. A low level of nondisjunction involving the sex chromosomes occurred in the normal males. Only one cell was identified to contain both an X and a Y chromosome and one other lacked both sex chromosomes, giving a combined incidence of sex chromosome aneuploidy of 0·4% (Table 1). In contrast, sex chromosome aneuploidy occurred with a greater frequency in cells from Rb/Y males. Approximately 9·2% and 9·6% respectively of the cells from R2A/Y and R6H/Y males had sex chromosome aneuploidy (i.e. chromosome constitutions of 18A+Rb+Y or 19A). The rate of sex chromosome nondisjunction was therefore very similar in both types of Rb.

Autosomal aneuploidy was also found (Table 1). Overall, 1·8% of cells from the wild-type males and 7·2% and 10·8% from R2A/Y and R6H/Y males respectively were numerically abnormal. The principal difference between the wild-type males and the translocation hemizygotes seemed to lie in the proportions of hypohaploid (17A+Rb and 18A+Y) cells which were unaccountably elevated over hyperhaploid (19A+Rb and 20A+Y) cells in the latter to a significant extent. A conservative estimate of the rate of nondisjunction can be obtained from the incidence of hyperhaploidy, which is less influenced than hypohaploidy by cell breakage artefactually increasing its frequency. There is no evidence in the present data that the translocations significantly increased the rate of nondisjunction of the attached autosome, since the rate of autosomal hyperhaploidy in the normal males, 0·6%, was very similar to that of the 2 Rb types, 0·6% and 1·0% for R2A/Y and R6H/Y respectively (Table 1).

As is described in the footnotes to Table 1, a number of cells, particularly from the R6H/Y males, contained separate chromatids. The rate of such anomalies was greater in Rb/Y than chromosomally normal males. The cause of this increase is uncertain, but may be related to the presence of an Rb multivalent increasing the risk of centromere malorientation and premature chromatid separation at anaphase I.

(*b*) *Heterozygous females*. The most reliable means of estimating chromosome nondisjunction at metaphase II in female mice is through use of the frequency of hyperhaploidy ($n+1$), since the rate of hypohaploidy ($n-1$) tends to be artefactually inflated by cell breakage during the preparation of the oocytes. The results from analyses of metaphase II oocytes from chromosomally normal and Rb heterozygous females are given in Table 2. Nondisjunction involving an X-autosome Rb has the potential to produce two types of hyperhaploid cell: the Rb metacentric chromosome plus the acrocentric X; and the Rb metacentric chromosome plus the autosomal acrocentric. Unfortunately, it was not possible to distinguish the acrocentric X from the autosomes at metaphase II, and therefore the hyperhaploidy rates given in Table 2 do not solely represent the rates of X chromosome nondisjunction but include also that affecting the acrocentric homologue of the attached autosome.

A rate of 1·1% hyperhaploidy was found in chromosomally normal females. Two categories of hyperhaploid cell were present in Rb/+ females, namely those with 21 acrocentric chromosomes and those with 19 acrocentric chromosomes plus the Rb metacentric (Table 2). The former presumably arose through segregation errors of the type occurring in chromosomally normal females; the latter type most probably represent nondisjunctional events attributable to the presence of an Rb. Overall, 4·2% of cells from R2A/+ females, 2·1% from R6H/+ females and 2·2% from R7H/+ females were hyperhaploid

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Table 2. *Numbers of metaphase II stage oocytes with different chromosome constitutions from normal females and Robertsonian heterozygous females. Rb = Robertsonian metacentric chromosome; A = acrocentric*

Genotype	Euploid cells		Aneuploid cells				Total number of cells
	20A	18A+Rb	19A	17A+Rb	21A	19A+Rb	
+/+	234[a]	—	29[b]	—	3	—	266
R2A/+	196[c]	122[d]	19[e]	7	2[f]	13	359
R6H/+	177[g]	157[h]	28[i]	16[j]	2	6[k]	386
R7H/+	124[l]	80[m]	11	12	0	5	232

Totals include: [a] 5 cells, 19A+2 chromatids; 1 cell, 19A+3 chromatids; 1 cell, 20A+1 fragment; [b] 2 cells, 19A+1 chromatid; [c] 2 cells, 19A+2 chromatids; [d] 2 cells, 17A+2 chromatids+Rb; [e] 2 cells, 19A+1 chromatid; [f] 1 cell, 20A+2 chromatids; [g] 1 cell, 40 chromatids; 1 cell, 9A+22 chromatids; 1 cell, 15A+10 chromatids; 3 cells, 19A+2 chromatids; [h] 1 cell, 36 chromatids+2Rb chromatids; 2 cells, 17A+2 chromatids+Rb; 1 cell, 18A+1 chromatid+Rb; [i] 1 cell, 19A+1 chromatid; [j] 1 cell, 16A+3 chromatids+Rb; [k] 1 cell, 20A+Rb; [l] 1 cell, 19A+2 chromatids; 1 cell, 40 chromatids; [m] 3 cells, 17A+2 chromatids+Rb.

Table 3. *Karyotypic analyses of post-weaning-age offspring from various crosses involving animals carrying a Robertsonian chromosome*

Cross	Normal females			Aneuploid females		Normal males		Aneuploid males		Total progeny screened
	XX	RbX	RbRb	Rb0	X0	XY	RbY	RbXY	RbRbY	
+/+ × R2A/Y	—	68	—	—	6	46	—	0	—	120
+/+ × R6H/Y	—	50	—	—	3	58	—	9	—	120
R2A/+ × +/Y	28	28	—	—	6[a]	35	25	0	—	122
R6H/+ × +/Y	41	29	—	—	0	34	24	0	—	128
R7H/+ × +/Y	43	26	—	—	1	35	31	0	—	136
R2A/+ × R2A/Y	—	32	15	0	1	12	11	2	1	74
R6H/+ × R6H/Y	—	30	23	0	3	27	25	3	0	111

[a] The presence of marker genes showed two of these animals to have a maternal X chromosome and thus to have arisen due to paternal nondisjunction and therefore independently of the Robertsonian chromosome.

(Table 2). These rates of hyperhaploidy were not significantly different ($\chi^2_{(2)} = 3{\cdot}40$, $P = 0{\cdot}183$). Thus the data do not suggest that the identity of the autosome involved in the rearrangement influenced to any great extent the frequency of Rb-associated nondisjunction. A comparison of the rates of hyperhaploidy in normal females and the combined Rb/+ types showed no significant difference ($P = 0{\cdot}075$, 1-sided Fisher exact test), despite the consistently higher incidences in the latter.

Adler *et al.* (1989) observed fewer than expected offspring carrying a maternally derived Rb in crosses between heterozygous females and hemizygous males. They suggested this was the result of a biased transmission of the Rb chromosome to the polar body during female meiosis. Our analysis of metaphase II oocytes allowed us to look directly for evidence of non-random chromosome segregation. Examination of the data indicate that such a phenomenon did indeed occur. For all 3 Rb/+ types there is a dearth of Rb-bearing oocytes (Table 2), and this deficiency was significant for the R2A ($\chi^2_{(1)} = 15{\cdot}67$, $P < 0{\cdot}001$) and R7H rearrangements ($\chi^2_{(1)} = 6{\cdot}22$, $P = 0{\cdot}013$), although not for the R6H ($\chi^2_{(1)} = 2{\cdot}03$, $P = 0{\cdot}154$).

(iii) *Sex chromosome aneuploidy among liveborn offspring*

The various different combinations of matings involving heterozygous females and carrier males are presented in Table 3. As R7H/Y males are infertile, only two crosses between hemizygous males and chromosomally normal females were possible (Table 3). R2A/Y males produced 4·7% aneuploid offspring, all of which were of the X0 type. Evidence from Adler *et al.* (1989) and that described below from intercrosses indicated that X.2/X/Y males are viable, and their lack here was probably a chance sampling effect. In comparison, R6H/Y males had 10% aneuploid progeny: 2·5% were X0; 7·5% were X.9/X/Y. The difference in rates of aneuploidy between R2A/Y and R6H/Y males was not significant ($\chi^2_{(1)} = 2$, $P = 0{\cdot}16$).

In crosses involving heterozygous females and chromosomally normal males, very few aneuploid offspring were found. From R2A/+ females, approximately 3·3% of the karyotyped offspring were X0 aneuploids; none of the progeny of R6H/+ females and approximately 0·7% from R7H/+ females were of this chromosome constitution. No other type of sex

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chromosome aneuploid offspring was found from these females.

The final type of cross involved heterozygous females and hemizygous males. From the R2A animals, 4·1% aneuploid progeny were identified: 1·4% were X0, and through the presence of marker genes it was found that the chromosome loss event was paternal in origin; 2·7% were X.2/X/Y and again the nondisjunctional events had occurred in the father. Approximately 5·4% of offspring of R6H mice were aneuploid: 2·7% were X0 and 2·7% were X.9/X/Y. No coat marker genes were present in these crosses to permit identification of the origin of the aneuploidies. However, the absence of aneuploid progeny from R6H/+ females mated to chromosomally normal males would suggest that the observed aneuploid animals in the intercrosses were in all likelihood the consequence of paternal nondisjunction.

4. Discussion

We have described here two new X-autosome Robertsonian (Rb) translocations that arose spontaneously among the descendants of a male hemizygous for Rb(X.2)2Ad. It is worth reiterating that both rearrangements appeared to involve the X chromosome derived from a chromosomally normal mother. Given the low expected rate of Rb formation (e.g. Ford, 1970) and the involvement of the X chromosome in both, the occurrence of these two Rbs in successive generations is quite remarkable.

The presence of an X-autosome Rb increases the risk of nondisjunction of the sex chromosomes in hemizygous males. In their original description of R2A, Adler *et al.* (1989) found 10·5% of metaphase II spermatocytes and 4% of progeny to have aneuploidy of the sex chromosomes. Our observations here of 9·2% at metaphase II and 5% of offspring (Tables 1 and 3) are clearly in good agreement with their results. For R6H/Y males, 9·6% of metaphase II spermatocytes and 10% of offspring had sex chromosome aneuploidy (Tables 1 and 3). Although the frequency of aneuploidy was larger in progeny of R6H/Y than R2A/Y males the difference was not significant, and overall it is reasonable to conclude that the rates of sex chromosome nondisjunction were very similar for both types of hemizygote. This suggests that the identity of the autosome involved in the rearrangement had little or no influence on the likelihood of sex chromosome nondisjunction in male meiosis.

While there was a clear-cut increase in the rate of sex chromosome nondisjunction in hemizygous males, the autosomal chromosome was not similarly affected. At metaphase II, the frequencies of the relevant hyperhaploid cells (19A+Rb, 20A+Y; Table 1) in both types of Rb/Y male were not elevated over those of the normal males. Adler *et al.* (1989), however, found evidence of a small increase with 1% of hyperhaploid cells in R2A/Y males compared to 0·2% in normal males. This increment was markedly smaller than for the sex chromosomes. A somewhat similar phenomenon has been found in mice heterozygous for certain autosomal Rbs, where nondisjunction of one of the autosomal chromosomes is over-represented among embryos (see review by Dyban & Baranov, 1987). The mechanism of this effect has yet to be elucidated.

The cytogenetic observations on aneuploidy in secondary spermatocytes and oocytes and the data from the breeding tests showed that although the presence of an X-autosome Rb increased the risk of chromosome nondisjunction, the effect was greater in Rb2Ad/Y and Rb6H/Y males compared to the corresponding heterozygous females. This is contrary to the pattern usually found for autosomal Rbs, where nondisjunction rates tend to be lower in male than female heterozygotes (Gropp & Winking, 1981). However, X-autosome Rbs are not entirely comparable to rearrangements involving autosomes only: in the latter the chromosomes involved in the rearrangement complex are identical in males and females; while in the former the multivalent formed at meiosis I involves an acrocentric X chromosome in females but a Y chromosome in males. The different chromosomal composition of the multivalent in heterozygous females and hemizygous males may underlie the different pattern of nondisjunction in the two sexes.

Shao & Takagi (1990) recently examined the influence of sex chromosome aneuploidy on early embryonic development in the mouse. They made use of maternal heterozygosity for the reciprocal translocation T(X.4)37H to increase the rate of X chromosome nondisjunction in female germ cells. By this means they produced embryos with two maternally derived X chromosomes in addition to the paternally derived sex chromosome. Embryos with an X/X/X or X/X/Y karyotype were found to be severely impaired in development at 5·5–8·5 days postconception, and were presumed to be inviable. In principle, the crosses, described by Adler *et al.* (1989) and here, involving Rb/+ females, should have provided an opportunity to detect X/X/X females and X/X/Y males with two maternally derived X chromosomes. However, as described earlier, the rate of chromosome nondisjunction in heterozygous females was low and the lack of such offspring is perhaps therefore not surprising. The reported deleterious effect of two maternally derived X chromosomes on embryonic development (Shao & Tagaki, 1990) may also be a factor relevant to their absence from these females. Some preliminary observations in this laboratory confirm that this factor contributes to the deficiency of these types of sex chromosome aneuploid progeny. Females heterozygous for both R2A and R6H, and therefore with monobrachial

homology (Gropp & Winking, 1981) for the X chromosome, have been bred. Monobrachial homology has been shown to increase substantially the rate of nondisjunction for the chromosome arm in common in autosomal Rb combinations (Gropp & Winking, 1981). Initial analyses have shown the expected high incidence of sex chromosome aneuploidy among pre-implantation embryos of females with monobrachial homology for the X chromosome. To date, however, among 92 liveborn progeny of monobrachial females none has been found with X.2/X.9/X or X.2/X.9/Y karyotypes, although 13 with an X0 karyotype, as a result of maternal X chromosome loss, have been identified.

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