*Genet. Res., Camb.* (1996), **67**, *pp.* 239–247 *With* 1 *text-figure* Copyright © 1996 Cambridge University Press 

# A study of meiotic pairing, nondisjunction and germ cell death in laboratory mice carrying Robertsonian translocations

C. A. EVERETT[1,2], J. B. SEARLE[1,3] AND B. M. N. WALLACE[4]

[1] *Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS*

[2] *Department of Obstetrics and Gynaecology, Centre for Reproductive Biology, University of Edinburgh, 37 Chalmers Street, Edinburgh EH3 9EW. Tel. 0131 229 2575. Fax. 0131 229 2408. E-mail Clare.Everett@ed.ac.uk*

[3] *Department of Biology, University of York, PO Box 373, York YO1 5YW, UK. Tel. 01904 432947. Fax. 01904 432860. E-mail JBS3@unix.york.ac.uk*

[4] *School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel. 0121 414 5917. Fax. 0121 414 5925 E-mail B.M.N.Wallace@bham.ac.uk*

(*Received 28 August 1995 and in revised form 15 December 1995*)

## Summary

Frequencies of anaphase I nondisjunction, germ cell death and pairing abnormalities at pachytene were assessed in male mice singly heterozygous and homozygous for the Robertsonian (Rb) translocations: Rb (1.3)1Bnr, Rb(11.13)4Bnr and Rb(10.11)8Bnr. Rb homozygotes showed low frequencies of nondisjunction but substantial germ cell death. This germ cell death could not be attributed to problems at pachytene as Rb homozygotes showed no increase in pairing abnormalities over the (C3H/HeH × 101/H)F1 controls. Instead genic factors are involved. Rb heterozygotes showed substantial frequencies of nondisjunction and even greater germ cell death than found in the homozygotes. Pachytene pairing abnormalities were observed and it appears that these, together with genic factors, cause physiological perturbation of meiocytes, thereby promoting germ cell death, with nondisjunction of the trivalent as a sublethal response.

## 1. Introduction

The standard diploid karyotype of the house mouse (*Mus musculus*) consists of 40 acrocentric chromosomes (Committee on Standardised Nomenclature for Mice, 1972). Variation from this standard can occur due to a process known as Robertsonian fusion, where two acrocentrics join at their centromeric ends to form a metacentric or submetacentric chromosome (Gropp & Winking, 1981). The presence of these Robertsonian translocations (Rbs) within a karyotype causes a reduction in the diploid chromosome number not no change in the number of chromosome arms.

Rbs are found in wild populations of house mice in various parts of western Europe and northern Africa and many have also arisen in laboratory stocks (reviews: Searle, 1989; Bauchau, 1990). Rbs in mice cause no known somatic effect but they may disrupt meiosis and gametogenesis (Gropp & Winking, 1981; Redi & Capanna, 1988). This is displayed particularly dramatically in individuals heterozygous for several-to-many Rbs, which may suffer substantial infertility or even sterility (Tettenborn & Gropp, 1970; Capanna *et al.* 1976; Evans, 1976; Gropp *et al.* 1982; Redi *et al.* 1985; Garagna *et al.* 1990; Said *et al.* 1993). Less severe, but still significant, meiotic and gametogenic disruption is exhibited by laboratory strain mice that have been made homozygous or heterozygous for a single wild-derived Rb (Cattanach & Moseley, 1973; Ford & Evans, 1973; Gropp *et al.* 1974; Winking & Gropp, 1976; de Boer, 1986; Winking, 1986).

Infertility in Rb mice manifests itself in two ways (Gropp *et al.* 1982; Redi & Capanna, 1988; Searle, 1993). First, there may be enhanced germ cell death. In males this may result in reduced sperm counts, causing sterility in extreme cases, while females may have a shortened reproductive lifespan. Second, there may be an increased frequency of anaphase I nondisjunction. This leads to the production of aneuploid zygotes which generally die during gestation.

Both forms of infertility can occur in individuals of the same karyotype or even in the same mouse. This is certainly true of laboratory-strain males heterozygous for a single wild-derived Rb (Cattanach & Moseley, 1973; de Boer, 1986). In this paper, we use such mice to consider the extent to which the two forms of infertility co-vary and are attributable to a common cause. In particular, we examine the extent of aberrations in chromosome pairing at pachytene,

https://doi.org/10.1017/S0016672300033723 Published online by Cambridge University Press

which has been implicated as a major contributing factor through inappropriate gene expression and physiological perturbation of the meiocyte (Forejt, 1982; Burgoyne & Baker, 1984).

## 2. Materials and methods

### (i) *Mice*

Three different Rbs were selected for study due to their previously reported high, medium and low frequencies of nondisjunction: Rb(11.13)4Bnr, Rb(1.3)1Bnr and Rb(10.11)8Bnr, respectively (Gropp & Winking, 1981). For brevity, these Rbs will be referred to as Rb4, Rb1 and Rb8. These Rbs are all of feral origin and were introduced into standard laboratory stocks at the MRC Radiobiology Unit, Chilton, Didcot and have since been maintained by homozygous crosses. Rb1 and Rb8 were introduced onto a predominantly (C3H/HeH × 101/H)F1 genetic background while Rb4 was introduced onto a C3H/HeH background. We obtained Rb homozygous (Rb/Rb) and (C3H/HeH × 101/H)F1 stock animals from the MRC Unit and generated experimental individuals (homozygotes and heterozygotes) at the Department of Zoology, Oxford. They were kept under standard laboratory conditions (22–24 °C; 14:10 h light:dark) with food and water provided *ad libitum*. At a later date males from the inbred strains C3H/HeH and 101/H were also obtained from the MRC Unit. The C3H/HeH, 101/H and (C3H/HeH × 101/H)F1 mice will henceforth be known as 'C3H', '101' and '3H1', respectively.

Eight–12-week-old males from each of the following categories were killed by cervical dislocation. These were single Rb homozygotes (Rb/Rb) and single Rb heterozygotes with (*a*) maternal and (*b*) paternal derivation of the Rb (each homozygote or heterozygote could be Rb1, Rb4 or Rb8). Additionally, three control groups were included. These were male 3H1, C3H and 101 individuals. A number of mice were analysed morphologically to determine body, total testes and seminal vesicle weights. A subset of these, consisting of four mice from each category, were used for the study of germ cell death. An assessment of nondisjunction was performed on the same four mice in all groups except those of C3H and 101 males. An analysis of chromosome pairing was performed on a second subset of this group including four males from each of the categories: 3H1, Rb8/Rb8, Rb1/+ Rb4/+ and Rb8/+.

### (ii) *Studies of nondisjunction and germ cell death*

Chromosome preparations were made using the left testis of each mouse, while the right was fixed in Bouin's fluid and wax embedded for histological processing.

Air dried testicular preparations were made according to the method of Evans *et al.* (1964) and conventionally stained. Fifty metaphase II spreads were scored per individual. For each spread, counts of the number of chromosome arms were made. An anaphase I nondisjunction frequency was calculated for each karyotypic group by doubling the hyperploid value (Cattanach & Moseley, 1973).

Seven μm sections were stained by the Periodic acid-Schiff (PAS) reaction and counterstained with haematoxylin (LeBlond & Clermont, 1952) for light microscopic examination. The number of primary spermatocytes and round spermatids were counted in truly transverse cross-sections of seminiferous tubules at stages I, VI and XII of the 12 stage seminiferous epithelium cycle (Oakberg, 1956). Five cross-sections were scored for each stage per individual and the Abercrombie (1946) correction was applied to all the cell counts.

### (iii) *Studies of chromosome pairing*

A surface spreading technique (Hultén *et al.* 1985) was used to examine spermatocytes at pachytene. Two drops of 0·03% 'Joy' detergent (Procter & Gamble) buffered with 0·01 M sodium tetraborate pH 8·5, were placed on either a grease free or a plastic coated slide for subsequent light or electron microscopic examination respectively. One drop of a cell suspension of spermatocytes in Ham's F10 medium was added and the drops gently mixed. The cells were allowed to settle for 6 min and were then fixed by adding five drops of 4% paraformaldehyde buffered with 0·2 M sodium tetraborate pH 8·5 (Moses, 1977), before drying on a hot plate (30–34 °C) for at least 1 h. Following fixation and drying down, slides were washed in distilled water, air dried and stained with 50% silver nitrate in 0·04% formalin at 60 °C for 30–60 min according to the method of Kodama *et al.* (1980). The plastic coated slides were used for EM studies; each coating (together with chromosome spreads) was mounted on a H3 Maxtaform grid and viewed in a Philips 301 electron microscope. EM was used solely for the purpose of illustration. The frequency and type of pairing abnormalities at early/mid pachytene was assessed by analysis under light microscopy.

### (iv) *Statistical analysis*

Statistical tests were performed on an Apple Macintosh computer using the statistical packages 'Multistat' (Biosoft, Cambridge, UK) and 'Statview 4.0' (Abacus Concepts Inc., Berkeley, USA) and routines established on the spreadsheet Microsoft Excel (Microsoft Corporation).

## 3. Results

### (i) *Morphology*

Based on their substantial body and testis weights, large seminal vesicles (an androgen target organ) and

https://doi.org/10.1017/S0016672300033723 Published online by Cambridge University Press

Table 1. *Mean body, testis and relative testis weights for carriers of Robertsonian fusions and all acrocentric controls (3H1, C3H and 101)*

| | | Mean ± S.E. | | | |
|---|---|---|---|---|---|
| Karyotype | Number of mice | Age (days) | Body weight (g) | Weight of combined testes (mg) | Relative testis weight (mg/g body weight) |
| 3H1 | 14 | 63·9 | 23·0 ± 0·6 | 207·6 ± 5·3 | 9·1 ± 0·3 |
| C3H | 5 | 60·4 | 28·1 ± 1·4 | 156·1 ± 5·9 | 5·6 ± 0·2 |
| 101 | 6 | 58·0 | 22·0 ± 0·6 | 175·1 ± 11·6 | 8·0 ± 0·5 |
| Rb1/Rb1 | 10 | 67·3 | 17·2 ± 0·5 | 123·3 ± 3·5 | 7·2 ± 0·2 |
| Rb1/+ | 11 | 63·5 | 20·7 ± 1·0 | 137·3 ± 8·6 | 6·6 ± 0·3 |
| +/Rb1 | 12 | 67·1 | 20·6 ± 0·7 | 150·2 ± 6·9 | 7·3 ± 0·1 |
| Rb4/Rb4 | 7 | 72·6 | 16·8 ± 0·2 | 129·5 ± 2·4 | 7·7 ± 0·2 |
| Rb4/+ | 13 | 66·7 | 18·5 ± 0·4 | 125·3 ± 4·1 | 6·8 ± 0·3 |
| +/Rb4 | 13 | 76·0 | 20·8 ± 0·6 | 141·2 ± 3·6 | 6·8 ± 0·2 |
| Rb8/Rb8 | 8 | 66·1 | 20·6 ± 1·1 | 130·4 ± 6·5 | 6·4 ± 0·3 |
| Rb8/+ | 7 | 60·0 | 20·6 ± 0·8 | 159·8 ± 8·6 | 7·7 ± 0·4 |
| +/Rb8 | 8 | 64·4 | 25·1 ± 0·9 | 194·0 ± 10·6 | 7·8 ± 0·3 |

Rb/+ : Rb = maternal + = paternal.

Table 2. *Mean Abercrombie corrected numbers of spermatocytes and round spermatids/4 for 3H1, C3H, 101 mice and carriers of Robertsonian fusions, at different stages of the seminiferous epithelium cycle*

| | Numbers of spermatocytes | | | Numbers of round spermatids/4 | | % Expected spermatids |
|---|---|---|---|---|---|---|
| Karyotype | I | VI | XI | I | VI | ± S.E.[a] |
| 3H1 | 27·89 | 22·82 | 21·11 | 20·64 | 20·41 | 86·02 ± 2·15 |
| C3H | 26·97 | 27·51 | 27·44 | 19·55 | 18·94 | 70·46 ± 0·24 |
| 101 | 29·97 | 28·91 | 30·08 | 23·18 | 20·95 | 74·37 ± 2·30 |
| Rb1/Rb1 | 24·40 | 23·11 | 21·49 | 15·12 | 14·03 | 63·35 ± 1·45 |
| Rb1/+ | 26·60 | 22·07 | 20·75 | 12·24 | 11·22 | 51·15 ± 2·23 |
| +/Rb1 | 24·10 | 20·50 | 20·42 | 11·63 | 10·88 | 52·01 ± 3·56 |
| Rb4/Rb4 | 26·80 | 22·38 | 22·07 | 13·86 | 12·47 | 55·48 ± 0·84 |
| Rb4/+ | 25·52 | 22·75 | 21·62 | 10·39 | 9·28 | 42·71 ± 1·45 |
| +/Rb4 | 24·10 | 19·42 | 18·02 | 10·67 | 10·38 | 51·19 ± 2·96 |
| Rb8/Rb8 | 21·38 | 16·80 | 16·60 | 11·62 | 11·74 | 63·81 ± 2·30 |
| Rb8/+ | 22·11 | 19·17 | 19·01 | 12·01 | 11·44 | 58·22 ± 2·31 |
| +/Rb8 | 24·27 | 20·46 | 19·31 | 12·11 | 11·03 | 54·15 ± 2·02 |

Rb/+ : Rb = maternal, + = paternal.
[a] [(mean number of spermatids × 0·25)/mean number of spermatocytes] × 100%. This is the number of spermatids compared with the ideal value assuming no germ cell death.

the presence of spermatozoa in the testes, all individuals were considered to be fully mature, as would be expected of 8–12-week-old laboratory mice. Table 1 shows the mean body, testis and relative testis weights (mg/g body weight) for each karyotypic group.

The genetic background of stocks carrying Rb1 and Rb8 is predominantly 3H1 while that carrying Rb4 is predominantly C3H in origin. All Rb stocks have been inbred for many generations. Although the Rb homozygotes showed significantly reduced testis weights ($t$-tests: $P < 0{\cdot}05$) compared with the 3H1, C3H and 101 animals, this was probably, at least in part, a reflection of their reduced body weights ($t$-tests: $P < 0{\cdot}05$) as all of the Rb homozygotes showed relative testis weights between the values of the control mice.

All Rb heterozygotes, with the exception of +/Rb8, showed significantly reduced body weights ($t$-tests: $P < 0{\cdot}05$) and testis weights ($t$-tests: $P < 0{\cdot}001$) compared with 3H1 mice. The reduction in testis weight could not be attributed entirely to differences in body

https://doi.org/10.1017/S0016672300033723 Published online by Cambridge University Press

Table 3. *Metaphase II arm counts for 3H1 mice and carriers of Robertsonian fusions*

| Karyotype | Number of cells scores | Arm counts 17 | 18 | 19 | 20 | 21 | $>20\times2$ (%) |
|---|---|---|---|---|---|---|---|
| 3H1 | 200 | 1 | 6 | 29 | 164 | 0 | 0·0 |
| Rb1/Rb1 | 200 | 8 | 13 | 48 | 128 | 3 | 3·0 |
| Rb1/+ | 200 | 2 | 15 | 39 | 124 | 20 | 20·0 |
| +/Rb1 | 200 | 17 | 33 | 54 | 78 | 18 | 18·0 |
| Rb4/Rb4 | 200 | 9 | 17 | 48 | 123 | 3 | 3·0 |
| Rb4/+ | 200 | 5 | 15 | 45 | 115 | 19 | 19·0 |
| +/Rb4 | 200 | 15 | 17 | 56 | 93 | 19 | 19·0 |
| Rb8/Rb8 | 200 | 6 | 18 | 42 | 134 | 0 | 0·0 |
| Rb8/+ | 200 | 3 | 14 | 39 | 138 | 6 | 6·0 |
| +/Rb8 | 200 | 6 | 18 | 47 | 120 | 9 | 9·0 |

Table 4. *Percentage of pachytene cells in each karyotype showing various types of chromosomal pairing abnormalities. For Rb heterozygotes, the percentage of pachytene cells with trivalents that are fully paired (0 or 1 side arm) or that show unpairing around the centromere (two side arms) is also shown*

| | Univalence | | Side arms on trivalent | | | |
|---|---|---|---|---|---|---|
| Karyotype | XY | Autosome | 0 | 1 | 2 cis | 2 trans |
| 3H1 | 1·7 | 0·8 | Not relevant | | | |
| Rb8/Rb8 | 3·3 | 1·7 | Not relevant | | | |
| Rb1/+ | 5·8 | 0 | 2·5 | 88·3 | 7·5 | 1·7 |
| Rb4/+ | 3·3 | 0 | 2·5 | 80·0 | 16·7 | 0·8 |
| Rb8/+ | 7·5 | 0 | 2·5 | 85·8 | 11·7 | 0·0 |

120 cells scored for each karyotype.

weight as all heterozygotes showed significantly reduced relative testis weights (*t*-tests: $P<0{\cdot}01$).

### (ii) *Germ cell death*

If spermatogenesis had proceeded perfectly, each primary spermatocyte would be expected to produce four spermatids. The ratio of spermatocytes to round spermatids in histological sections should therefore reflect this. However, even in the testes of the 3H1 mice we disclosed loss of germ cells; the number of spermatids was down to 86% of this ideal value (Table 2), close to the 87% previously recorded by Oakberg (1956) for the reciprocal F1. The inbred strains C3H and 101 also showed less than perfect germ cell survival with spermatid values of 70 and 74% of those expected respectively with significantly reduced values compared with their F1 ($U=0$, $n=4$, $P<0{\cdot}05$). For the Rb mice, spermatid numbers ranged from 64% (Rb8/Rb8) to 43% (Rb4/+) of the expected no-loss value (Table 2). There was substantial loss of post-pachytene germ cells between spermatocyte stage XI and round spermatid stage I in all mice except 3H1s.

Both Rb1 and Rb4 homozygotes showed reduced germ cell survival rates compared with all three control groups ($U=0$, $n=4$, $P<0{\cdot}05$). In Rb8 homozygotes, however, germ cell loss was only significantly increased in comparison with 3H1 and 101 males ($U=1$, $n=4$, $P<0{\cdot}05$). The performance of the heterozygotes was poorer than that of the homozygotes (significantly so for Rb1/+, +/Rb1, Rb4/+ and +/Rb8: $U=0$, $n=4$, $P<0{\cdot}05$). All heterozygote groups showed significantly reduced germ cell numbers compared with all three control groups ($U=0$, $n=4$, $P<0{\cdot}05$).

Previous studies have found that Rb heterozygotes have smaller testes and lower relative testis weights than chromosomally normal mice (Cattanach & Moseley, 1973). Care must be taken in the interpretation of such data as the results presented here indicate that testis weights are not necessarily a reflection of germ cell loss. For example, C3H mice showed the lowest relative testis weight (5·6 mg/g body weight) of all groups but a relatively high ratio of observed to expected numbers of spermatids (70·5%).

### (iii) *Reciprocal crosses*

Reciprocal crosses were performed in the production of each type of Rb heterozygote, in order to establish whether or not the parental derivation of the Rb affected the phenotype. Rb4 and Rb8 heterozygotes had lower body weights (Rb4: $t=3{\cdot}01$, D.F. $=24$, $P<0{\cdot}01$; Rb8: $t=3{\cdot}79$, D.F. $=13$, $P<0{\cdot}01$) and smaller testes (Rb4: $t=2{\cdot}92$, D.F. $=24$, $P<0{\cdot}01$; Rb8: $t=2{\cdot}45$, D.F. $=13$, $P<0{\cdot}05$) when the metacentric was transmitted maternally, but there was no significant difference when relative testis weights were considered, despite a significant increase in germ cell

https://doi.org/10.1017/S0016672300033723 Published online by Cambridge University Press

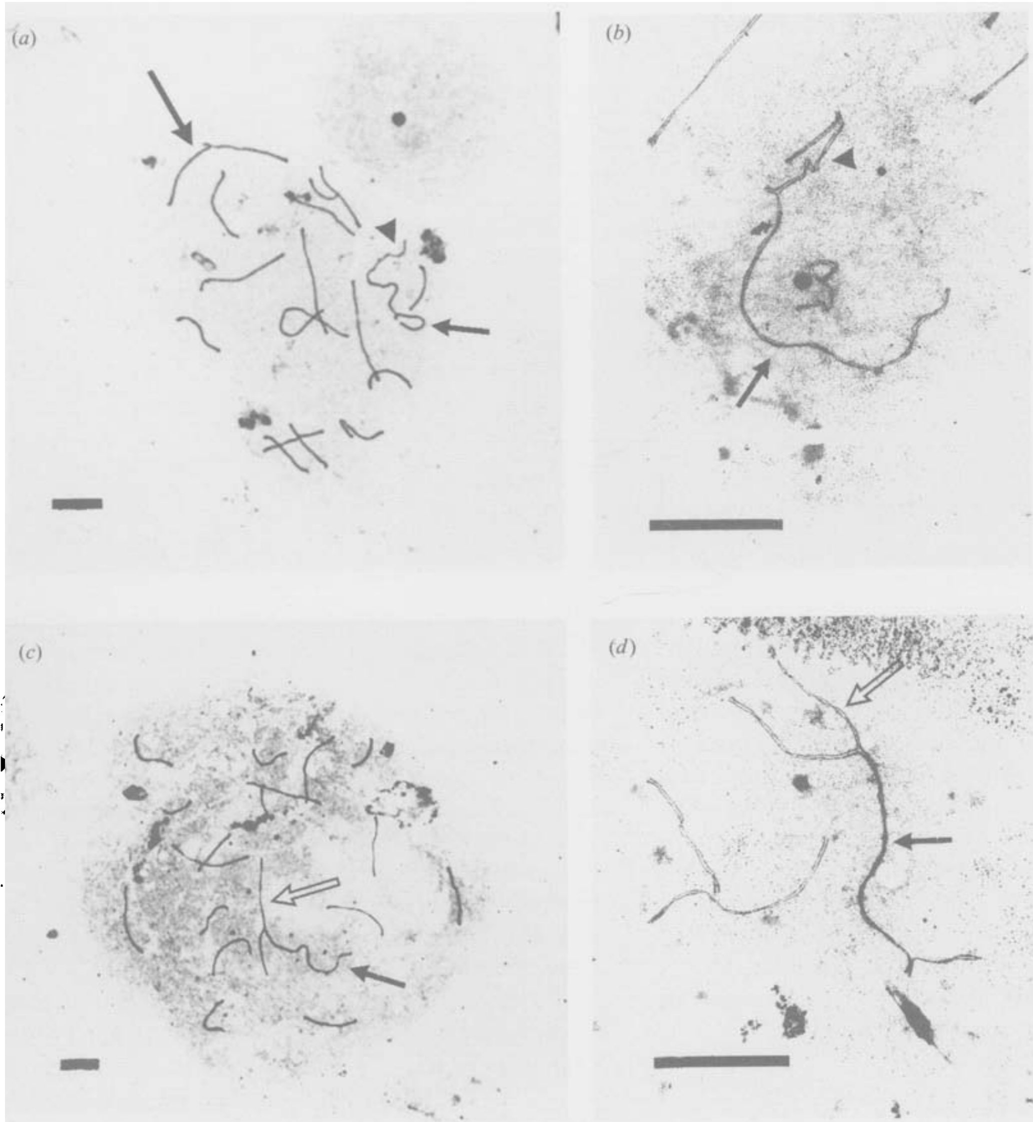

Fig. 1. Light (*a*, *c*) and electron (*b*, *d*) micrographs of surface-spread preparations of pachytene cells from male mice heterozygous for the translocations Rb4 and Rb8 ($2n = 39$). Long arrow: the trivalent configuration, short arrow: XY bivalent, arrowhead: attached bivalent, open arrow: attached trivalent. Scale Bar: 5 $\mu$m. (*a*) Mid pachytene stage cell from an Rb8 heterozygote, showing 17 autosomal configurations and the XY. The trivalent has a distinct side arm and a small bivalent is seen attached to the centromeric end of the X. (*b*) The XY bivalent from an Rb4 heterozygote showing a bivalent attached to the centromeric end of the X. (*c*) Mid pachytene stage spermatocyte from a mouse heterozygous for Rb4 showing 17 autosomal configurations and the XY. The side arm of the trivalent is attached to the centromeric end of the X. (*d*) The XY bivalent from a mouse heterozygous for Rb8 with the side-arm of the trivalent attached to the centromeric end of the X.

death for the maternal Rb4 heterozygote ($U = 0$, $n = 4$, $P < 0{\cdot}05$). In contrast, Rb1 heterozygotes showed no significance for body or testis weights but the relative testis weight was significantly lower when the Rb was transmitted maternally ($t = 2{\cdot}20$, D.F. $= 21$, $P < 0{\cdot}05$). The reason for these reciprocal differences is unclear. However, there are known to be genes influencing growth within the proximal imprinting region of chromosome 11 (Cattanach & Beechey, 1990). So if the structural or regulatory sequences of these genes differ between Rb4, Rb8 and the standard chromosome 11, then a reciprocal difference could occur in growth and hence, final body and organ weight. This still leaves the relative weight for Rb1 and germ cell death for Rb4 unexplained.

(iv) *Anaphase I nondisjunction*

Fifty metaphase II cells were scored per mouse, giving a total data set of 2000 cells (Table 3). All individuals showed substantial hypoploidy, which was assumed to have mostly resulted from cell breakage. The absence of hyperploid spermatocytes from 3H1 mice is consistent with the previous estimate of 0·4% based on studies on various inbred, random bred and F1 mice with a standard 40-chromosome karyotype (Beatty *et al.* 1975). Our nondisjunction data for the three types of Rb homozygote (Table 3: 6 hyperploid cells/600: 2·0% nondisjunction) showed a small increase over this value and also tallied closely with data from Cattanach & Moseley (1973) for seven

https://doi.org/10.1017/S0016672300033723 Published online by Cambridge University Press

Table 5. *Percentage of pachytene cells of each karyotype showing XY–autosome contacts*

| Karyotype | All types XY–autosome contacts | Attachments and interlocks | | | |
|---|---|---|---|---|---|
| | | Total | XY–bivalent | XY–trivalent: Involving side arm | XY–trivalent: Not involving side arm |
| 3H1 | 25·8 | 8·3 | 8·3 (3·3) | Not relevant | |
| Rb8/Rb8 | 32·5 | 8·3 | 8·3 (3·3) | Not relevant | |
| Rb1/+ | 28·3 | 14·2† | 7·5 (3·3) | 5·8 (3·3) | 1·7 (0·0) |
| Rb4/+ | 34·2 | 20·8 | 14·2 (10·0) | 5·0 (4·2) | 1·7 (0·0) |
| Rb8/+ | 32·5 | 16·7† | 12·5 (8·3) | 1·7 (0·8) | 3·3 (0·8) |

120 cells were scored for each karyotype. † Cells with multiple abnormalities. ( ) numbers are cells in which a bivalent or trivalent is attached to the centromeric end of the X.

types (9/700:2·0%) and Gropp *et al.* (1974) for six types (2·7%). The nondisjunction frequencies of Rb8 heterozygotes averaged 7·5% while both Rb1 and Rb4 heterozygotes had nondisjunction frequencies of 19%. There were no differences between the progeny of reciprocal crosses. For Rb1 and Rb4, we generated heterozygotes from the same stocks as Cattanach & Moseley (1973). While we obtained similar nondisjunction frequencies to them for Rb1, the value for Rb4 was lower (19% compared with 30%). In addition, our nondisjunction frequency for Rb8 heterozygotes (6–9%) was rather higher than the 2% reported by Gropp & Winking (1981), but this may be due to the different genetic background.

### (v) *Chromosome pairing at pachytene*

In total 600 cells, 120 for each of the groups: 3H1, Rb8/Rb8, Rb1/+, Rb4/+ and Rb8/+ were examined for sex chromosome–autosome interactions and chromosome unpairing. Most of the configurations showed complete pairing. For the trivalent, the metacentric and two acrocentrics sometimes paired completely so that no side arms were produced (Table 4). However, more typically the trivalent formed a single side-arm, presumably as a result of non-homologous pairing of the centromeric regions of the two acrocentrics (Fig. 1). Trivalent unpairing was represented by the occurrence of two side arms and this was the only source of autosomal unpairing recorded in the Rb heterozygotes (Table 4). The degree of trivalent unpairing ranged between 9 and 18% for the three types of heterozygote with the two side arms usually being in the cis orientation (Table 4). The overall incidence of univalence was low (4·8%). This was largely due to separate X and Y chromosomes (4·3%), with autosomal univalents only present in a total of three cells (0·5%).

For all karyotypic groups the incidence of sex bivalent–autosome contact was determined (Table 5). Little variation between groups could be discerned when all contacts were considered, as these include many random overlaps. When consideration was limited to interlocks and attachments, a much clearer picture emerged. The homozygous mice (3H1 and Rb8/Rb8 taken together) had significantly fewer interlocks and attachments than the heterozygous mice ($\chi^2 = 10{\cdot}2$, D.F. $= 1$, $P < 0{\cdot}01$). Part of this difference can be attributed to sex bivalent–trivalent interactions but for Rb4/+ and Rb8/+ mice there were also intriguing indications of a higher incidence of sex chromosome–bivalent interactions than in the homozygotes, particularly those in which a bivalent was attached to the unpaired (centromeric) end of the X (Table 5; Fig. 1). Although Rb8 heterozygotes showed higher rates of XY-bivalent interactions than Rb8 homozygotes, a comparison could not be made for other Rb carriers due to the lack of data.

## 4. Discussion

Carriers of Rb translocations in a variety of species have been shown to suffer high levels of anaphase I nondisjunction, germ cell death and abnormalities of pachytene pairing (review: Searle, 1993). In this paper, we have used a series of male Rb mice to examine the interrelationship of these three types of disturbance of meiosis and gametogenesis, in particular to determine whether abnormalities at pachytene may promote the two other processes.

Considering Rb homozygous mice first: these showed distinctly increased germ cell death relative to 3H1 controls, but no increase in pairing abnormalities, and negligible nondisjunction frequencies (mean = 3%). It has been suggested that either unpairing or autosome–sex chromosome interactions at pachytene may promote germ cell death (Forejt, 1982; Burgoyne & Baker, 1984). In the present study we found that high levels of germ cell death can occur in homozygous carriers of Rbs *without* substantial pachytene pairing

https://doi.org/10.1017/S0016672300033723 Published online by Cambridge University Press

abnormalities. Thus, we need to look for genic effects (Mahadevaiah, 1990) to explain the enhanced germ cell death in the Rb homozygotes.

After introduction of the wild-derived Rb translocations into a 3H1 or C3H genetic background, the Rb stocks that we used were subject to many generations of inbreeding. The substantial germ cell death that we found in the Rb homozygotes could, therefore be attributed to inbreeding depression arising from homozygosity of alleles derived from the C3H or 101 strains. However, the degree of germ cell death exhibited by the Rb homozygotes in this study was considerably greater than that found in C3H or 101 mice (Table 2) or indeed, in any of the six other laboratory inbred strains reported by Shire & Bartke (1972). Inbreeding depression associated with laboratory strain alleles may explain part but possibly not all the reduction in germ cell numbers exhibited by Rb homozygotes.

Alternatively, or additionally, an Rb effect may be acting. Despite several generations of backcrossing when the wild-derived Rb translocations were introduced into a laboratory genome (Cattanach & Moseley, 1973), some of the genes near the centromeres of the Rb chromosomes may still have 'wild' alleles. Thus, the increase in germ cell death in the Rb homozygotes we analysed may still have been a response of homozygosity of these alleles (an Rb specific inbreeding depression), or due to deleterious epistatic interactions between wild and laboratory strain components of the genome. Wild alleles or Rb translocations, in themselves, are not associated with high levels of germ cell death. In a series of 10 wild-caught homozygotes with 0–4 pairs of Rb translocations, Wallace *et al.* (1992) found, on average, 82% of the expected number of spermatids (compare with Table 2).

Turning now to the Rb heterozygotes: these showed an increase in nondisjunction, germ cell death and pairing abnormalities over their respective homozygotes and 3H1 controls. In this case, abnormalities of pachytene pairing associated with Rb heterozygosity could be a factor involved in germ cell death. In all heterozygotes there was some centromeric unpairing in the trivalent and association of the trivalent and XY bivalent (Tables 4 & 5). These are anomalies which may, according to Forejt (1982) and Burgoyne & Baker (1984), be implicated in germ cell death. However, neither trivalent unpairing (mean: 13% of pachytene cells) nor XY-trivalent association (6% of pachytene cells) seem sufficient in isolation, or in combination, to explain the difference in germ cell survival between 3H1 mice (86% of expected spermatids) and Rb heterozygotes (mean: 52% of expected spermatids).

Genic factors could also be involved in germ cell death in the Rb heterozygotes. Given that the Rb heterozygotes were relatively outbred (they were generated by crosses between inbred Rb homozygous stocks and 3H1 mice), it is unlikely that inbreeding depression can explain the high levels of germ cell death. However, deleterious interaction of the wild and laboratory genome, either at the level of homozygous loci (heterozygous disadvantage) or negative epistatic effects could occur.

It is likely that both pairing abnormalities and genic factors were involved in the high levels of germ cell death that we recorded in Rb heterozygotes. Comparisons with wild mice support this contention. Thus, the wild single Rb heterozygotes studied by Wallace *et al.* (1992) and B. M. N. Wallace (unpublished data) were as prone to pachytene pairing abnormalities as the laboratory Rb single heterozygotes of the present study, despite much higher germ cell survival (means for the wild and laboratory heterozygotes, respectively: 16 and 13% of pachytene cells showed trivalent unpairing, 6% and 6% of pachytene cells showed XY-trivalent attachments and interlocks, and 68 and 52% of expected spermatids were recorded). This discrepancy in germ cell survival between the wild and laboratory Rb heterozygotes implicates genic factors, while that between wild Rb homozygotes and single heterozygotes from the same area (82 and 68% of expected spermatids, respectively), implies that pairing abnormalities can also be important.

Our consideration of germ cell death in mice homozygous and heterozygous for Rb translocations emphasizes the substantial role that genetic factors may have in promoting germ cell death even in individuals with chromosomal rearrangements, where there may have been a presumption that 'chromosomal factors' (i.e. pairing aberrations) are important (King, 1993). This supports previous results with wild mice (Said *et al.* 1993) and lemurs (Ratomponirina *et al.* 1982).

A final consideration is the high frequency of anaphase I nondisjunction in the Rb heterozygotes that we studied (19·0% for Rb1 and Rb4, 7·5% for Rb8). These frequencies were much higher than the 2–3% per heterozygous configuration recorded for wild mice (Winking, 1986; Wallace *et al.* 1992). A substantial part of the nondisjunction in each heterozygote can be attributed to the trivalent configuration (Gropp *et al.* 1974). The segregation from the trivalent will inevitably be more susceptible to error than segregation from bivalents; such error may particularly be expected to occur in physiologically perturbed meiocytes. Thus the high frequency of nondisjunction in Rb heterozygotes may be a reflection of the same process as that which leads to a high degree of germ cell death. Pairing abnormalities and/or genic factors may cause physiological perturbation of a meiocyte, leading either to its death or a heightened chance of nondisjunction as a sublethal response. For example, a physiologically perturbed meiocyte may show an abnormal rate of meiotic progression, which could increase its chances of

https://doi.org/10.1017/S0016672300033723 Published online by Cambridge University Press

Table 6. *A comparison of germ cell survival and nondisjunction for mice singly heterozygous for Rb translocations*

| Rb Translocation | % Expected spermatids[a] | Nondisjunction frequency (%) |
|---|---|---|
| Rb(11.13)4Bnr | 47 | 19 |
| Rb(1.3)1Bnr | 52 | 19 |
| Rb(10.11)8Bnr | 56 | 8 |
| 4.10/9.12/6.13/11.14[b] | 68 | 3 |

[a] See Table 2.
[b] Combined data from individuals singly heterozygous for one of these Rb translocations (see Wallace *et al.* 1992).

nondisjunction (Eichenlaub-Ritter, 1994; Everett & Searle, 1995). More data are required to confirm the link between germ cell death and nondisjunction; however, it is worthy of note that among the four examples of single Rb heterozygotes studied here and by Wallace *et al.* (1992), nondisjunction and germ cell death show a positive relationship (Table 6).

We would like to thank the Genetics Division of the Radiobiology Unit (director: Dr B. M. Cattanach F.R.S.) for mice, Dr S. Garagna, Mrs J. E. Evans and Ms V. Petts for technical advice and Drs E. P. Evans and J. R. Clarke for much encouragement. We are grateful to the Science and Engineering Research Council (C.A.E.), The Royal Society of London (J.B.S.) and The Leverhulme Trust (B.M.N.W.) for financial support.

## References

Abercrombie, M. (1946). Estimation of nuclear population from microtome sections. *Anatomical Record* **94**, 239–247.

Baker, B. S., Carpenter, A. T. C., Esposito, M. S., Esposito, R. E. & Sandler, L. (1976). The genetic control of meiosis. *Annual Reviews of Genetics* **10**, 53–134.

Bauchau, V. (1990). Phylogenetic analysis of the distribution of chromosomal races of *Mus musculus domesticus* Rutty in Europe. *Biological Journal of the Linnean Society* **41**, 171–192.

Beatty, R. A., Lim, M.—C. & Coulter, V. J. (1975). A quantitative study of the second meiotic metaphase in male mice (*Mus musculus*). *Cytogenetics and Cell Genetics* **15**, 256–275.

de Boer, P. (1986). Chromosomal causes of fertility reduction in mammals. In *Chemical Mutagens* (ed. F. J. de Serres), **10**, pp. 427–467. New York: Plenum.

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

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

Cattanach, B. M. & Beechey, C. V. (1990). Chromosome imprinting phenomena in mice and indications in man. *Chromosomes Today* **10**, 135–148.

Cattanach, B. M. & Moseley, H. (1973). Nondisjunction and reduced fertility caused by tobacco mouse metacentric chromosomes. *Cytogenetics and Cell Genetics* **12**, 264–287.

Committee on Standardised Nomenclature for Mice (1972). Standard karyotype of the mouse, *Mus musculus. Journal of Heredity* **63**, 69–72.

Eichenlaub-Ritter, U. (1994). Mechanisms of nondisjunction in mammalian meiosis. *Current Topics in Developmental Biology* **29**, 281–323.

Evans, E. P. (1976). Male sterility and double heterozygosity for Robertsonian translocation in the mouse. *Chromosomes Today* **5**, 75–81.

Evans, E. P., Breckon, G. & Ford, C. E. (1964). An air drying method for meiotic preparations from mammalian testes. *Cytogenetics* **3**, 289–294.

Everett, C. A. & Searle, J. B. (1995). Pattern and frequency of nocodazole induced meiotic nondisjunction in oocytes of mice carrying the 'tobacco mouse' metacentric Rb(16·17)7Bnr. *Genetical Research* **66**, 35–43.

Ford, C. E. & Evans, E. P. (1973). Robertsonian translocations in mice: segregational irregularities in male heterozygotes and zygotic unbalance. *Chromosomes Today* **8**, 117–127.

Forejt, J. (1982). X-Y involvement in male sterility caused by autosome translocations – a hypothesis. In *Genetic Control of Gamete Production and Function* (ed. P. G. Crosignani, B. L. Rubin and M. Fraccaro), pp. 135–151. Academic Press.

Garagna, S., Redi, C. A., Zuccotti, M., Britton-Davidian, J. & Winking, H. (1990). Kinetics of oogenesis in mice heterozygous for Robertsonian translocations. *Differentiation* **42**, 167–171.

Gropp, A., Giers, D. & Kolbus, U. (1974). Trisomy in the fetal backcross progeny of male and female metacentric heterozygotes in the mouse. *Cytogenetics and Cell Genetics* **13**, 511–535.

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

Gropp, A., Winking, H. & Redi, C. A. (1982). Consequences of Robertsonian heterozygosity segregational impairment of fertility versus male limited sterility. In *Genetic Control of Gamete Production and Function* (ed. P. G. Crosignani, B. L. Rubin & M. Fraccaro), pp. 155–134. Academic Press.

Hultén, M. A., Saadallah, N., Wallace, B. M. N. & Creasy, M. R. (1985). Meiotic studies in man. In *Human Cytogenetics: A Practical Approach* (ed. D. E. Rooney and B. H. Czepulkowski), pp. 163–196. Oxford: IRL Press.

King, M. (1993). *Species Evolution: the Role of Chromosome Change*. Cambridge: Cambridge University Press.

Kodama, Y., Yoshida, M. C. & Sasaki, M. (1980). An improved silver-staining technique for nucleolus organizer regions by using nylon cloth. *Japanese Journal of Human Genetics* **25**, 229–233.

LeBlond, C. P. & Clermont, Y. (1952). Spermiogenesis of rat, mouse, hamster and guinea-pig as revealed by the 'periodic acid–fuchsin sulphurous acid' technique. *American Journal of Anatomy* **90**, 167–215.

Mahadevaiah, S. K., Setterfield, L. A. & Mittwoch, U. (1990). Pachytene pairing and sperm counts in mice with single Robertsonian translocations and monobrachial compounds. *Cytogenetics and Cell Genetics* **53**, 26–31.

Miklos, G. L. G. (1974). Sex chromosome pairing and male fertility. *Cytogenetics and Cell Genetics* **13**, 558–577.

Moses, M. J. (1977). Synaptonemal complex karyotyping in spermatocytes of the Chinese hamster (*Cricetulus griseus*). I. Morphology of the autosomal complement in spread preparations. *Chromosoma* **60**, 99–125.

Oakberg, E. F. (1956). A description of spermatogenesis in the mouse and its use in analysis of the cycle of the

seminiferous epithelium and germ cell renewal. *American Journal of Anatomy* **99**, 391–413.

Ratomponirina, C., Andrianivo, J. & Rumpler, Y. (1982). Spermatogenesis in several intra- and interspecific hybrids of the lemur (*Lemur*). *Journal of Reproduction and Fertility* **66**, 717–721.

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

Redi, C. A., Garagna, S., Hilscher, B. & Winking, H. (1985). The effects of some Robertsonian chromosome combinations on the seminiferous epithelium of the mouse. *Journal of Embryology and Experimental Morphology* **85**, 1–19.

Said, K., Saad, A., Auffray, J.-C. & Britton-Davidian, J. (1993). Fertility estimates in the Tunisian acrocentric and Robertsonian populations of the house mouse and their chromosomal hybrids. *Heredity* **71**, 532–538.

Searle, A. G. (1989). Chromosomal variants. Numerical variants and structural rearrangements. In *Genetic Variants and Strains of the Laboratory Mouse*, 2nd edition (ed. M. F. Lyon and A. G. Searle), pp. 582–616. Oxford: Oxford University Press.

Searle, J. B. (1993). Chromosomal hybrid zones in eutherian mammals. In *Hybrid Zones and the Evolutionary Process* (ed. R. G. Harrison), pp. 309–352. New York: Oxford University Press.

Shire, J. G. M. & Bartke, A. (1972). Strain differences in testicular weight and spermatogenesis with special reference to C57BL/10J and DBA/2J mice. *Journal of Endocrinology* **55**, 163–171.

Tettenborn, U. & Gropp, A. (1970). Meiotic nondisjunction in mice and mouse hybrids. *Cytogenetics* **9**, 272–283.

Wallace, B. M. N., Searle, J. B. & Everett, C. A. (1992). Male meiosis and gametogenesis in wild house mice (*Mus musculus domesticus*) from a chromosomal hybrid zone: a comparison between 'simple' Robertsonian heterozygotes and homozygotes. *Cytogenetics and Cell Genetics* **61**, 211–220.

Winking, H. (1986). Some aspects of Robertsonian karyotype variation in European wild mice. *Current Topics in Microbiology and Immunology* **127**, 68–74. Berlin, Heidelberg: Springer Verlag.

Winking, H. & Gropp, A. (1976). Meiotic nondisjunction of metacentric heterozygotes in oocytes versus spermatocytes. In *Ovulation in the Human* (ed. P. G. Crosignani and D. R. Mitchell), pp. 47–56. Academic Press.

https://doi.org/10.1017/S0016672300033723 Published online by Cambridge University Press