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A genetic method for measuring non-disjunction in mice with Robertsonian translocations

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SUMMARY

A high frequency of chromosomal non-disjunction occurs spontaneously in mice heterozygous for some Robertsonian translocations. If animals heterozygous for the translocation and homozygous for different alleles of a marker gene are mated together a few young homozygous for the marker arise through non-disjunction, and their frequency can be used as a measure. This method has been used with the Robertsonian translocation Rb(9.19)163H and the marker ruby *ru* (chr. 19); Rb(4.6)-2Bnr with brown (*b*) and misty (*m*) (chr. 4); and Rb(9.14)6Bnr with hairless (*hr*) and piebald (*s*) (chr. 14) respectively. The frequencies of marked young were: Rb163 0/5260 *ruru*; Rb2 21/1997 *mm bb*; and Rb6 19/1702 *hrhr ss*, and the corresponding calculated non-disjunction frequencies in each arm of the translocation were Rb163, <5 %; Rb2, 15 %; Rb6, 15 %. These figures show reasonably good agreement with values obtained by other methods. A search for genetic or environmental factors affecting the frequency of marked young in Rb2 and Rb6 revealed that in Rb2 the frequency increased with maternal age, whereas in Rb6 the maternal age of the marked young was non-significantly below that of the total progeny. The reasons for this discrepancy are not clear.

1. INTRODUCTION

Chromosomal non-disjunction is clinically of great importance in man, since about 0·3 % of live births are affected by severe congenital defects, such as Down's and Klinefelter's syndromes, due to trisomies resulting from non-disjunction (Jacobs, 1972). However, with the important exception of maternal age, the factors affecting the frequency of non-disjunction are largely unknown. Experimental investigations are hampered by the relative lack of good animal systems. In the mouse, it is possible to study non-disjunction of the sex chromosomes by observing atypical inheritance of *X*-linked marker genes, but in tests with radiation this approach has proved relatively unfruitful (Russell, 1968). Another method involves cytogenetic studies of germ-cells, or early embryos derived from them. This approach has recently given evidence of increased non-disjunction after irradiation (Yamamoto, Endo & Watanabe, 1973*a*, *b*; Uchida & Lee, 1974). However, it might be valuable to have other genetic methods of studying the phenomenon.

A high frequency of non-disjunction occurs spontaneously in mice heterozygous for Robertsonian translocations, particularly the so-called tobacco mouse chromosomes (Gropp, Tettenborn & Lehmann, 1970; Tettenborn & Gropp, 1970). It should be possible to measure this non-disjunction by a method similar to that used for measuring adjacent-2 disjunction in mice with reciprocal translocations. In this method animals heterozygous for the translocation and homozygous for different alleles of a marker gene are mated together. Offspring homozygous (rather than typically heterozygous) for the marker arise through adjacent-2 disjunction (or non-disjunction) and their frequency is noted (Searle, Ford & Beechey, 1971).

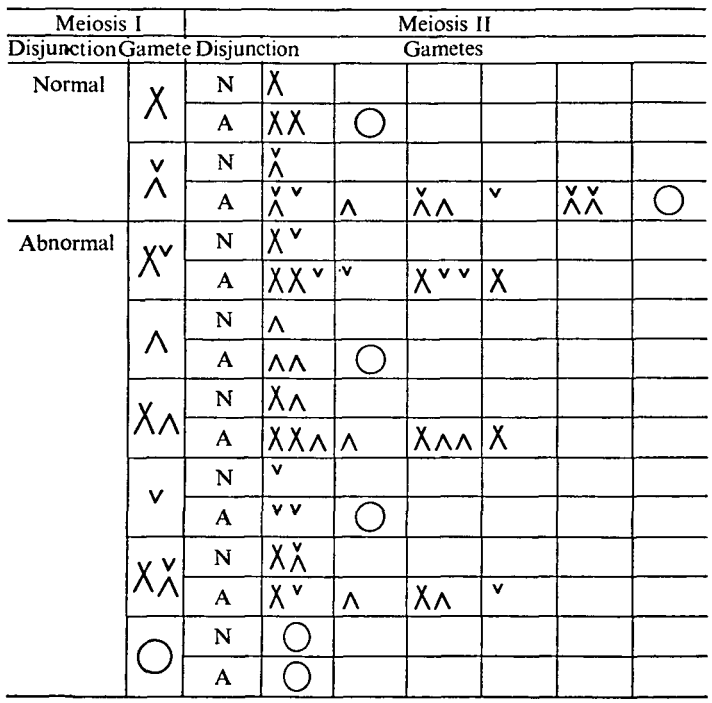

Fig. 1. Types of gametes formed by an animal heterozygous for a Robertsonian translocation after normal (N) disjunction or abnormal (A) disjunction of one or both arms of the translocation at first or second meiotic division.

In animals heterozygous for Robertsonian translocations, abnormal gametes may arise through non-disjunction at the first or second meiotic division, as shown in Fig. 1. The combination of two gametes with complementary aneusomies can give rise to chromosomally balanced viable zygotes. In most cases these zygotes will have received both homologues of one or more chromosome arms from the same parent and these can therefore be detected through the homozygous expression of marker genes. The various chromosomal types derived from non-disjunction occurring at meiosis I or II in the two parents are shown in Fig. 2. Animals homozygous for both the translocation and the marker can only occur as a result

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of non-disjunction at meiosis II in both parents. Similarly, marked chromosomally normal animals would chiefly arise by non-disjunction at meiosis II although abnormality in meiosis I could occur as well, or instead in one parent. Marked animals heterozygous for the translocation, however, would in the main be derived from non-disjunction at meiosis I, with some contribution from meiosis II.

The presence of Robertsonian translocations is thought to increase non-disjunction at meiosis I and there seems no obvious *a priori* reason why meiosis II should be affected. Therefore, since non-disjunction at meiosis I gives rise predominantly to marked animals heterozygous for the translocation, this is the only type that would be expected to occur with a high frequency.

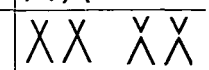
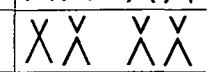
Abnormal disjunction		Viable chromosomal types
Parent 2	Parent 1	
MI	MI	
MII	MII	
MII	MI	
MI and MII	MI	
MI and MII	MII	
MI and MII	MI and MII	

Fig. 2. Viable chromosomal types derived from union of complementary abnormal gametes, formed as shown in Fig. 1.

The present paper describes the measurement by such a method of non-disjunction in mice heterozygous for the Robertsonian translocations Rb(9.19)-163H (Evans, Lyon & Daglish, 1967), Rb(4.6)2Bnr and Rb(9.14)6Bnr (Gropp, Giers & Kolbus 1974; Gropp, Kolbus & Giers 1975) (subsequently referred to as Rb163, Rb2 and Rb6).

In choosing suitable genetic markers, preferably two genes some distance apart on the same arm of the translocation were selected. Aberrant offspring arising by non-disjunction would then be homozygous for both markers and an alternative origin such as mutation or a small deletion would be unlikely. Semi-dominant markers were also deemed valuable, since either type of homozygote could then be recognized, but few suitable markers of this type are known in the mouse. The markers finally chosen were ruby, *ru*, for chromosome 19 of Rb163; brown, *b*, and misty, *m*, for chromosome 4 of Rb2; and hairless, *hr*, and piebald, *s*, for chromosome 14 of Rb6. Although no data are reported in this paper, the markers dilute, *d*, and short-ear, *se*, have also been chosen for chromosome 9 of Rb6, and microphthalmic-white, Mi^{wh}, for chromosome 6 of Rb2.

2. MATERIALS AND METHODS

(i) *Source of animals*

Homozygotes for the translocation were crossed to homozygotes for the chosen genetic marker, and the offspring were intercrossed. The intercross progeny were then tested for the presence of the translocation by biopsy of the testis, spleen or ear skin. By this means a stock homozygous for both the translocation and the marker was constructed. Animals from this stock were crossed to animals homozygous for the marker, but not carrying the translocation. All the offspring from these matings were then heterozygous for the translocation and homozygous for the genetic marker and were used in the experiments. At the beginning of the work a few heterozygotes were used which were derived directly from the intercross F_2, rather than from the later matings.

Animals heterozygous for the translocation but not carrying the marker were obtained by crossing homozygotes for the translocation (Rb2 and Rb6 were kindly provided by Dr B. M. Cattanach) to F_1 hybrids of the type C3H/HeH × 101/H. Crosses in which the female was homozygous for the recessive markers were termed Type I crosses, and the reciprocal type with the male carrying the recessives were termed Type II.

(ii) *Baseline experiment*

Translocation heterozygotes, bred as above, were put together in trios and left to breed as long as they would. The young were observed at birth, 7–14 days, and at weaning for the presence of recessive markers. All such marked young were kept, tested for fertility, and their karyotypes were determined by biopsies of ear skin, or by corneal preparations.

(iii) *Cytological techniques*

For testis biopsies one whole testis was removed under anaesthesia and prepared by Meredith's (1969) method. Similarly, for spleen biopsies, the whole spleen was removed under anaesthesia 5–7 days after a subcutaneous leucocyte-stimulating injection of 0·2 ml of a mixture of equal parts of pertussis vaccine and foetal calf serum. The spleen was minced finely, left for 1 h at room temperature in 199 medium + 0·04 % colchicine, then preparations were made by Ford & Hamerton's (1956) method for bone-marrow. For biopsies of ear skin, 2–3 mm^2 of skin were snipped off and cultured on cover slips for 7–10 days. Then chromosome preparations were made by standard methods.

Corneal preparations were made by Fredga's (1964) method.

(iv) *Calculations*

Since the results (see below) indicated that, as expected, non-disjunction at meiosis II was rare in relation to that at meiosis I, the calculations of non-disjunction frequency were based entirely on meiosis I. Let the frequency of non-disjunction in the two chromosome arms of the Robertsonian translocation be

r_m and s_m in the maternal, and r_p and s_p in the paternal gamete, respectively. Then the frequencies of formation of the various possible types of gamete and zygote considering non-disjunction at first meiosis only, are as shown in Fig. 3.

In practice, since marked young always arise by simultaneous non-disjunction in both parents, it is not possible to measure the frequencies of this process in each sex, and hence for convenience, let $r_m = r_p$ and $s_m = s_p$. However, it is important to bear in mind that this is an artificial assumption. If the presence of the Robertsonian translocation is the only factor affecting non-disjunction then the assumption will be valid. However, if other factors are important then r_m may differ from

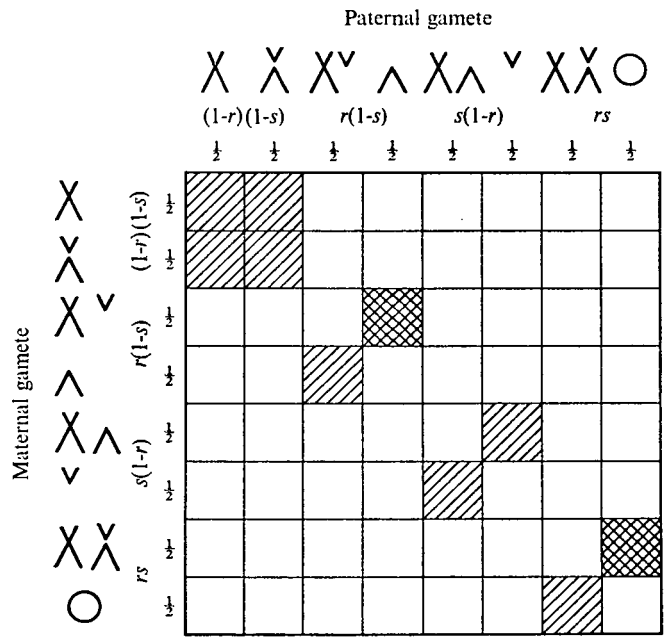


Fig. 3. Frequencies of formation of various types of gamete, if non-disjunction occurs at first meiosis only with frequencies of r and s in the two chromosome arms. Viable zygotes are shown hatched, and viable zygotes expected to show anomalous inheritance of a recessive marker in a particular chromosome arm are double hatched. If simultaneous non-disjunction in both chromosome arms is neglected, the last two rows and columns are omitted, and the frequencies of the remaining three pairs of rows and columns become: $(1-r-s)$, r, and s.

r_p. This may lead to a difference between reciprocal crosses, or a change in observed frequency after some treatment, e.g. irradiation. Similarly, in the work described here, only one chromosome arm of the Robertsonian was marked and hence it was not possible to measure r and s separately. Therefore, again for convenience, let $r = s$. Such evidence as we have suggests that, at least for Rb6, this last assumption is to a first approximation valid.

For relatively low levels of non-disjunction it would also be valid to make another approximation, by omitting from consideration the types of gamete arising by simultaneous non-disjunction in both chromosome arms.

If all zygotes with aneuploid chromosome constitutions die, then the proportion of surviving young, with these approximations is

$$1-4r+5r^2$$

and the proportion of marked young among survivors is

$$\frac{\frac{1}{4}r^2}{1-4r+5r^2}.$$

If simultaneous non-disjunction in both arms is considered then this proportion becomes

$$\frac{\frac{1}{4}r^2-\frac{1}{2}r^3+\frac{1}{2}r^4}{1-4r+7r^2-6r^3+2\frac{1}{2}r^4}.$$

The case in which one parent receives some treatment, such as irradiation, can be dealt with by putting r_m (or r_p) equal to nr. Then the proportion of marked young among survivors becomes

$$\frac{\frac{1}{4}nr^2}{1-2(n+1)r+5nr^2}$$

(if non-disjunction in both arms is discounted).

3. RESULTS

(i) *Baseline experiment*

In the case of Rb163 no genetically marked animals were found although over 5000 young were raised from the experimental crosses (Table 1). We have therefore done no further work with this translocation.

Table 1. *Non-disjunction in mice with Robertsonian translocations*

	Parents		Offspring			
Translocation	♀	♂	Marker	Total	% marker (y)	% non-disjunction (r)
Rb(9.19)163H	*ruru*	× + +	0	2077	0·0	5
	+ +	× *ruru*	0	3183	0·0	
Rb(4.6)2Bnr	*bm/bm*	× + +	6	728	0·82	15
	+ +	× *bm/bm*	15	1269	1·18	
Rb(9.14)6Bnr	*hrs/hrs*	× + +	5	469	1·07	15
	+ +	× *hrs/hrs*	14	1229	1·14	

For both Rb2 and Rb6 there were a number of marked offspring, the frequencies for the two translocations being similar, at about 1 %. In both cases fewer young were obtained from the type I crosses, in which the females were of the mutant type, but the frequencies of marked young were similar for the two types of crosses, suggesting absence of sex differences affecting the measure of non-disjunction.

All the marked offspring on which cytological tests were made proved to be heterozygous for Rb2 or Rb6, consistent with origin by simultaneous non-disjunction at first meiosis. In the case of Rb6, all those animals tested proved to be fertile and in both translocations all the fertile animals transmitted the recessive

marker to all their offspring (Table 2). In Rb2, a few of the marked animals proved to be sterile, but these sterile animals were chromosomally Rb2/+ as expected, and we also found some sterile Rb2/+ animals among the parents of the test crosses. Thus these data suggest that the marked young had indeed arisen by the method assumed.

Others have shown that reduced fertility in heterozygotes for Robertsonian translocations in the mouse can be attributed largely or entirely to non-disjunction (Cattanach & Moseley, 1973; Gropp, Giers & Kolbus, 1974; Gropp, Kolbus & Giers, 1975). It is therefore possible to obtain an independent estimate of non-disjunction in these stocks by measuring the litter size, relative to similar stocks not carrying the translocation. In Table 3 the litter sizes of the two types of cross

Table 2. *Fertility and chromosomal type of marked offspring of test crosses*

		Translocation	
		Rb2	Rb6
Marked young	Total	21	19
	Died	—	4
	Fertile		
	♀	8	8
	♂	11	7
	Sterile		
	♀	1	—
	♂	1	—
Chromosome type	Rb/+	21	15
	Other	—	—
Offspring	Recessive	178	106
	Other	—	—

Table 3. *Reduction in litter-size in test matings of heterozygotes for Robertsonian translocations*

	Rb163		Rb2		Rb6	
	I	II	I	II	I	II
Preparation and stock matings						
No. pairs	11	2	12	5	18	10
Mean	5·25	6·5	4·58	8·0	5·79	8·46
S.E.	0·33	0·67	0·29	0·63	0·28	0·34
Mode	4·5	7·0	4·0	10·0	5·5	9·0
Test matings						
No. pairs	48	42	31	34	26	43
Mean	5·69	7·97	3·34	4·08	3·09	3·65
S.E.	0·14	0·17	0·13	0·14	0·14	0·12
Mode	6·0	7·5	3·0	3·5	3·0	3·5
Ratio test:stock						
Mean	1·1	1·2	0·73	0·51	0·53	0·43
Mode	1·3	1·1	0·75	0·35	0·54	0·39
Calculated non-disjunction	—	—	0·075	0·15	0·14	0·19

S.E. = standard error of mean; I = Type I matings; II = Type II matings.

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in the baseline experiment are shown together with those of the mutant stocks and preparation matings from which the experimental crosses were derived, and which therefore had a similar genetic background. In order to avoid any disturbances due to change in litter size with litter order the first five litters only of all those pairs which had five or more litters are considered. At low litter sizes the mean value may give a poor estimate of the animal's fertility, since litters of zero will not be scored, and some litters of 1 or 2 may be eaten by the mother and so again not scored; therefore, both the mean and the modal litter sizes are given. The frequencies of non-disjunction, calculated according to the formula given in the Materials and Methods for proportion of surviving young agree reasonably well with those obtained from the proportion of marked young.

In Rb163 where no genetically marked young were found, the litter sizes in the test matings were actually higher than in stock matings, indicating the difficulties inherent in the use of litter size as a measure of non-disjunction, since clearly other factors must have been responsible for the difference in this case. Similarly, in all three translocations, the mean and modal litter sizes of the type I matings, with females homozygous for recessive genes, were lower than those of type II, again presumably for reasons unconnected with non-disjunction.

For both types of mating in Rb6 and for type II matings in Rb2, the estimates of non-disjunction obtained from litter size (ranging from 0·14 to 0·19 per arm) were close to those obtained from frequency of marked young. In type I matings for Rb2 the preparation and stock matings had an unusually low litter size of $4{\cdot}58 \pm 0{\cdot}29$, and, here the ratio of test:stock mating litter size was 0·73, giving an estimate of only 7·5 % non-disjunction per arm. Possibly the estimate was disturbed by the factors responsible for the lower litter size of the stock matings. As was obvious in Rb163, the test matings would tend to have hybrid vigour and hence a higher litter size, since all the parents were the products of outcrosses. Hence, litter-size measurements might be expected to give an underestimate of non-disjunction.

(ii) *Factors affecting the frequency of marked young*

(*a*) *Maternal age*

In Tables 4 and 5 the results of the baseline experiment are tabulated according to the age of the mother at birth of each litter.

In crosses involving Rb2 the mean maternal age of the marked young was significantly above that of the total progeny, suggesting that non-disjunction increased with maternal age. Thus, for mothers aged 2–4 months there were 3/740 marked young, corresponding to a non-disjunction frequency of 10 %, at 5–7 months there were 7/779 or 14 % non-disjunction, and at 8+ months 11/475 or 19 % non-disjunction. In order to test whether this apparent change with age was due to any heterogeneity among the pairs, with longer breeding ones having more non-disjunction, the data were recalculated, including only those pairs which bred up to a maternal age of 9 months, and tabulating type I and type II

matings separately (Table 4). Differences in maternal age of marked and total young were still present and of the same general order, for both mating types, but the significance was reduced, so that for type II matings the difference verged on significance and for type I it was not significant. This reduction in significance was probably due to the smaller number of animals per group, rather than to any heterogeneity in the effect, which is therefore considered real.

In Rb6, on the other hand, the effect was reversed, with the marked young having a lower maternal age than the total young, although the difference was not statistically significant. When type I and type II matings were separated, and only pairs breeding to 9 months were included, as with Rb2, the effect seemed to be unchanged. However, the change in frequency of marked young with maternal age was not a smooth one. Rather there was a peak frequency of marked young at medium ages. Mothers aged 5–7 months produced 13/730 marked young or 18 % non-disjunction, whereas those aged 2–4 months gave 4/465 or 14 % and those of 8 months and over gave 2/517 or 10 %. Thus, in Rb6 there is no clear trend in either direction of change in non-disjunction with maternal age.

(*b*) *Variation among families*

The stocks used in this work were not closely inbred, and hence there may have been genetic variation among the parents with respect to factors affecting non-disjunction. Environmental variables persisting throughout an animal's reproductive life are also possible.

Table 4. *Maternal age of total and marked young in Rb2*

Maternal age (*a*) (months)	All data		Mothers breeding to 9 months: Type I		Mothers breeding to 9 months: Type II	
	T*	M†	T	M	T	M
2	200	—	24	—	101	—
3	269	1	56	1	135	—
4	271	2	67	1	131	—
5	271	2	46	—	144	2
6	293	2	66	—	149	2
7	215	3	48	1	143	2
8	167	4	33	1	108	2
9	150	5	42	2	108	3
10	67	1	7	—	60	1
11	42	1	6	—	36	1
12	23	—	—	—	23	—
13	13	—	—	—	13	—
14	13	—	—	—	13	—
Total	1994	21	395	6	1164	13
	$\bar{a}$ 5·69	7·24	5·63	6·67	6·19	7·69
	S.E. $\bar{a}$ 0·058	0·468	0·112	1·05	0·082	0·524
	t 2·72		1·13		1·93	
	$0{\cdot}001 < P < 0{\cdot}01$		$0{\cdot}2 < P < 0{\cdot}3$		$P \sim 0{\cdot}05$	

* T = total young. † M = marked young.

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In order to test for the influence of factors such as these, which are common to sibships, we compared the frequency of marked animals among the sibs of such animals with that in the whole stock. To find the frequency among sibs, one affected animal was subtracted from the total progeny in each family in which any marked young occurred. Since many of the matings were trios, with one male mated to two females, we also studied the frequency among half-sibships, or the progeny of one male.

The only case in which the frequencies of marked young among sibs or half-sibs deviated appreciably from the stock frequency of 1 % was that of type I matings involving Rb6 (Table 6). Here the total number of animals was very low, and the apparently high frequencies were due to a single trio which produced three marked animals, including two in one litter. In view of the small numbers no importance

Table 5. *Maternal age of total and marked young in Rb6*

Maternal age (a) (months)	All data		Mothers breeding to 9 months: Type I		Type II	
	T*	M†	T	M	T	M
2	69	1	33	1	22	—
3	181	2	27	—	121	1
4	215	1	36	—	137	1
5	268	6	63	—	166	5
6	219	4	69	—	122	2
7	233	3	41	—	178	2
8	176	1	60	1	103	—
9	117	1	33	—	84	1
10	99	—	17	—	82	—
11	59	—	17	—	42	—
12	34	—	6	—	28	—
13	32	—	8	—	24	—
Total	1702	19	410	2	1109	12
	$\bar{a}$ 6·32	5·47	6·38	5·0	6·56	5·58
S.E.	$\bar{a}$ 0·063	0·40	0·129	3·0	0·079	0·452
	t 1·41		0·74		1·28	
	$0{\cdot}1 < P < 0{\cdot}2$		$0{\cdot}4 < P < 0{\cdot}5$		$0{\cdot}2 < P < 0{\cdot}3$	

* T = total young. † M = marked young.

Table 6. *Frequency of marked animals among sibships, half-sibships*

Translocation	Mating type	Sibs: T*	Sibs: M†	Sibs: %	Half-sibs: T	Half-sibs: M	Half-sibs: %
Rb2	I	107	1	0·93	150	1	0·67
	II	421	4	0·95	518	7	1·35
Rb6	I	35	2	5·7	49	2	4·1
	II	268	3	1·1	416	4	0·96

* T = total young. † M = marked young.

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is attached to this discrepant result and there is thus no evidence in these stocks for heterogeneity among families in factors affecting non-disjunction.

(c) *Variation among litters*

In addition to factors which persist throughout the reproductive life, various genetic phenomena and congenital defects in the mouse are known to be affected by factors peculiar to individual litters, so that heterogeneity among litters within a family results.

To test for such factors in the present work, we compared the frequency of marked young among litter-mates of an affected animal with the overall frequency. In fact, there was only one instance (in the type I trio in Rb6 already mentioned) in which more than one marked animal occurred in a litter. Hence, there is no evidence of any environmental influence common to litter-mates.

4. DISCUSSION

This work shows that measurement of the frequency of non-disjunction in Robertsonian translocations by the method described here is a practical possibility. In these crosses all the marked young were heterozygous for the translocation, suggesting that as expected they were derived from non-disjunction at the first meiotic division. The estimates of non-disjunction obtained from the frequencies

Table 7. *Comparative estimates of non-disjunction in Robertsonian translocations*

Authors	Method	Rb2	Rb6	Rb163
Evans *et al.* (1967)	MII*	—	—	0·03
Gropp *et al.* (1974)	MII	—	—	0·03
Gropp *et al.* (1975)	MII	—	0·05	0·025
Cattanach & Moseley (1973)	MII	0·07	0·03	—
	Embryonic loss	0·12	0·01	—
Ford & Evans (1973)	MII	0·17	0·17	—
	Embryonic loss	0·15	0·19	—
This paper	Genetic test	0·15	0·15	0·05
	Litter size	0·075–0·15	0·14–0·19	0

* MII = metaphase II counts.
Estimates given are for a single arm.

of marked young were in reasonably good agreement with those from measurements of litter size. Moreover, our estimates from both sources are in accord with the literature (Table 7). In Rb163 we obtained no clear evidence that non-disjunction was occurring, and the upper fiducial limit was thought to be about 5 % in each arm. This agrees with the findings of Evans, Lyon & Daglish (1967) and Gropp *et al.* (1974, 1975) whose observations on metaphase II in spermatocytes of male heterozygotes suggested a total frequency of about 6 % non-disjunction. Similarly, in Rb2 where we found approximately 15 % non-disjunction in each arm corresponding to a total of 30 %, studies of metaphase II (MII) by various authors

have given comparable figures, and in addition Cattanach & Moseley and Ford & Evans obtained similar results from measurements of embryonic death. In Rb6, however, the results of the different authors are more variable. Cattanach & Moseley observed a total of only 6 % non-disjunction at MII, and 0–5 % from embryonic death, and Gropp *et al.* obtained similar figures. Our results of 15 % per arm, however, agree with those of Ford & Evans who observed a total of 29–38 % non-disjunction at MII and at embryonic stages.

In considering discrepancies between our own work and that of other workers in Table 7 an important point is that our observations were based on simultaneous non-disjunction in both sexes, whereas the other work concerned males only, and Gropp *et al.* (1974) reported in other translocations that female heterozygotes gave higher non-disjunction. Moreover, our work was based on one arm only of the translocation and differences between the two arms are possible. However, the observed differences cannot be ascribed entirely to sex or arm differences in non-disjunction since Ford & Evans found markedly higher non-disjunction in Rb6 than Cattanach & Moseley or Gropp *et al.*, although all three groups were working with males and similar methods. This suggests some heterogeneity among stocks with respect to non-disjunction and although in our own work we found no evidence of any genetic or environmental factors tending to cause heterogeneity among families or among litters, this does not mean that such factors do not exist. Such evidence might be forthcoming in other crosses, and indeed we have some indication in work now in progress of a difference between reciprocal crosses involving Rb6.

The observation of an effect of maternal age on the frequency of marked young is not surprising, because this factor is known to be of great importance to the frequency of non-disjunction in man, and has also been claimed to be effective in the mouse (Yamamoto *et al.* 1973*a*, *b*). It is very surprising, however, that the effect should appear to be in opposite directions in Rb2 and Rb6. In Rb2 the frequency of marked young increased steadily as the mothers aged. In Rb6, where the marked young had a lower maternal age than the general population, the highest frequency of marked young did not occur at the lowest maternal ages, but instead the frequency increased as the mothers aged from 2–4 to 5–7 months, and then declined at 8 months, and older. A curve of this kind suggests the action of opposing factors, one tending to increase and the other to decrease the observed frequency of marked young. Further work will be needed to elucidate this problem.

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REFERENCES

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

EVANS, E. P., LYON, M. F. & DAGLISH, M. (1967). A mouse translocation giving a metacentric marker chromosome. *Cytogenetics* **6**, 105–119.

FORD, C. E. & HAMERTON, J. L. (1956). A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. *Stain Technology* **31**, 247–251.

FORD, C. E. & EVANS, E. P. (1973). Non-expression of genome unbalance in haplophase and early diplophase of the mouse and incidence of karyotypic abnormality in post-implantation embryos. In *Les Accidents chromosomiques de la reproduction* (ed. A. Boué and C. Thibault), pp. 271–285. Paris: Institut National de la Santé et de la Recherche Médicale.

FREDGA, K. (1964). A simple technique for demonstration of the chromosomes and mitotic stages in a mammal. *Hereditas* **51**, 268–273.

GROPP, A., TETTENBORN, U. & LEHMANN, E. VON. (1970). Chromosomen variation vom Robertson' schen Typus bei der Tabakmaus *M. poschiavinus*, und ihren Hybriden mit der Laboratoriumsmaus. *Cytogenetics* **9**, 9–23.

GROPP, A., GIERS, D. & KOLBUS, U. (1974). Trisomy in the fetal backcross progeny of male and female metacentric heterozygotes of the mouse: I. *Cytogenetics Cell Genetics* **13**, 511–535.

GROPP, A., KOLBUS, U. & GIERS, D. (1975). Systematic approach to the study of trisomy in the mouse: II. *Cytogenetics Cell Genetics* **14**, 42–62.

JACOBS, P. A. (1972). Human population cytogenetics. *Proceedings of the IVth International Congress on Human Genetics* pp. 232–242. Amsterdam: Excerpta Medica.

MEREDITH, R. (1969). A simple method for preparing meiotic chromosomes from mammalian testis. *Chromosoma* (*Berl.*) **26**, 254–258.

RUSSELL, L. B. (1968). The use of sex-chromosome anomalies for measuring radiation effects in different germ-cell stages of the mouse. In *Effects of Radiation on Meiotic Systems*, pp. 27–41. Vienna: IAEA.

SEARLE, A. G., FORD, C. E. & BEECHEY, C. V. (1971). Meiotic disjunction in mouse translocations and the determination of centromere position. *Genetical Research* **18**, 215–235.

TETTENBORN, U. & GROPP, A. (1970). Meiotic non-disjunction in mice and mouse hybrids. *Cytogenetics* **9**, 272–283.

UCHIDA, I. A. & LEE, C. P. V. (1974). Radiation-induced non-disjunction in mouse oocytes. *Nature* **250**, 601–602.

YAMAMOTO, M., ENDO, A. & WATANABE, G. (1973*a*). Maternal age dependence of chromosome anomalies. *Nature New Biology* **241**, 141–142.

YAMAMOTO, M., SHIMADA, T., ENDO, A. & WATANABE, G. (1973*b*). Effects of low-dose X-irradiation on the chromosomal non-disjunction in aged mice. *Nature New Biology* **244**, 206–208.