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Male meiotic behaviour and male and female litter size in mice with the T(2; 8)26H and T(1; 13)70H reciprocal translocations

By P. de BOER

Department of Genetics, Agricultural University, Wageningen, The Netherlands

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SUMMARY

Two reciprocal mouse translocations T(2; 8)26H and T(1; 13)70H, heterozygous in a Swiss random-bred background, show differences in the spectrum of multivalent configurations and in the segregational behaviour of these multivalent configurations. T26H/+ males mostly contained rings of four (R IV, 53·15%) and T70H/+ males chains of four, missing a chiasma in the shortest interstitial segment (C IV 1_1, 61·55%). The adjacent II frequency, estimated from metaphase II observations, was 8·47% in T26H/+ and 25·22% in T70H/+. Univalents of the shorter translocation chromosome of T70H are able to divide equationally at first anaphase. The hypothesis is advanced that time differences in chiasma terminalization during metaphase I-anaphase are important for explaining the difference in segregation observed between the two translocations. Translocation-caused non-disjunction is probably low in T26H/+ and 4–5% in T70H/+. Univalents involving T70H/+ are usually capable of co-orientation with the other chromosomes of the translocation complex. The summed percentages of adjacent II disjunction and non-disjunction caused by the translocations were estimated from the relative fertility scores of T/+ males and females versus +/+ males and females as 9·8% and 29·0% for T26H/+ and T70H/+ males, respectively, and 9·4% and 27·8% for T26H/+ females and T70H/+ females. For both translocations, the agreement between the various estimates is good. Chiasma frequencies are much higher in telomeric segments than in proximal segments containing centric heterochromatin.

1. INTRODUCTION

Translocation heterozygosity is a widespread phenomenon among plants, animals and humans. It occurs spontaneously and can also be induced by mutagenic chemicals and ionizing radiations. Plant and insect cytogeneticists have studied the meiotic behaviour and consequences of reciprocal translocations for many years, but information about mammalian translocations is scanty. The earlier history of the radio-induction of mouse reciprocal translocations is given by Snell (1946) and Carter, Lyon & Phillips (1955). Apart from earlier unpublished work (C. E. Ford, T. C. Carter & J. L. Hamerton), the first study which attempted to relate the relative fertility of translocation heterozygotes to the behaviour of multivalent configurations was by Searle, Ford & Beechey (1971). The present study

might be regarded as a continuation, because two of the three translocations studied by them, T(2; 8)26H and T(1; 13)70H, are used here.

When the four chromosomes (two normal and two translocation chromosomes) which can be involved in a multivalent configuration at prophase–metaphase of the first meiotic division segregate two by two, three ways of disjunction are classically distinguished:

(*a*) Alternate: alternate centromeres move to the same pole.

(*b*) Adjacent I: adjacent but non-homologous centromeres move to the same pole.

(*c*) Adjacent II: adjacent but homologous centromeres move to the same pole.

When the four chromosomes involved in the translocation segregate three to one (or four to zero), there is numerical non-disjunction. When one chiasma is present in one or both interstitial (between the centromere and the point of exchange) segments, alternate and adjacent I segregations become equivalent, because of the fact that adjacent homologous centromeres have the same genetic material attached to them (see Figs. 1 and 2). It can be understood that the relative frequency of balanced gametes (gametes with either the two translocation chromosomes or the two normal chromosomes) from alternate/adjacent I segregations is 50% (compare Searle *et al.* 1971). Adjacent II segregation and numerical non-disjunction on the contrary produce 100% unbalanced gametes (with deficiencies and duplications, either single or combined). If the fraction of primary spermatocytes displaying adjacent II segregation and numerical non-disjunction is called p, the fraction with alternate/adjacent I disjunction is $1-p$ and thus the fraction of balanced gametes is $\frac{1}{2}(1-p)$ (Searle *et al.* 1971). This is the basis of the formula, describing the relation between the fertility of matings between translocation heterozygotes and normals as compared to normal × normal matings and the summed frequency of adjacent II segregation and numerical non-disjunction.

This paper describes meiotic observations in primary and secondary spermatocytes of T26H/+ and T70H/+ and relates the results to the relative fertility scores of both male and female translocation heterozygotes.

2. MATERIALS AND METHODS

Females heterozygous for the translocations T(2; 8)26H and T(1; 13)70H were obtained through the courtesy of Dr A. G. Searle. Because the two original translocation strains differed with respect to their genetic background, the T/+ females have been outcrossed to a +/+ Swiss random bred stock (Cbp: SE(S)). T/+ animals entered an experiment only when outcrossed for at least five generations. On an average, approximately 97% of the genetic material will be then of Swiss origin. The translocation chromosomes constitute a deviation from this rule, the DNA adjacent to the break-points being involved in genetic recombination only very rarely.

Meiotic preparations of primary and secondary spermatocytes were made accord-

ing to the Evans technique (Evans, Breckon & Ford, 1964). Five males of T26H and seven of T70H were processed. From each male approximately 200 primary spermatocytes and around 100 secondary spermatocytes were scored. Constitutive centric heterochromatin was preferentially stained with a dry-heat Giemsa technique described earlier (de Boer & Groen, 1974) and in some cases the BSG-technique of Sumner (1972) was used. The T70H/+ males were of various ages but not older than 11 months when processed. All T26H/+ males, except one which was approximately 3 months old, were between 27 and 31 days at autopsy. This age group yielded consistently better preparations of this translocation.

Diakinesis-metaphase I's were grouped into three classes of chromosome morphology, as described earlier (de Boer & Groen, 1974). Class 1 contains the least contracted, class 3 the most contracted bivalents. The interstitial and translocated segments of concern with respect to chiasma formation are named according to an earlier proposal (de Boer & van Gijsen, 1974). So, 8_i means the interstitial segment of chromosome 8 and 8_t the translocated segment. Multivalent configurations will be symbolized as usual, thus R IV, C IV, C III + I and II + II stand for a ring of four, a chain of four, a chain of three chromosomes plus an univalent, and for two bivalents, respectively. Any segment with the missing chiasma is added to the configuration symbol. Thus C IV 8_i (occurring in T26H) means a chain of four with a chiasma missing in segment 8_i.

Notations of chromosomes during the second meiotic division give the chromatids which make up the chromosome, because translocation heterozygotes can give rise during the first meiotic division to chromosomes with unequal-sized chromatids. This happens when a chiasma occurs in an interstitial segment provided the translocated segments are of strikingly unequal length. For instance, $(8; 8^2)$ means that a chromatid type 8 and one of type 8^2 are united at their centromeres.

Spermatozoa were counted in the capita epididymes according to a method described by Searle & Beechey (1974). The morphology of the spermatozoa was assessed from uterine smears, prepared shortly after mating and stained according to the method of Bryan (1970). The litter size data refer to the number of live plus dead young out of matings between translocation heterozygotes of each sex and normal mates. For the female translocation carriers their normal sibs were used as controls. All other +/+ animals were Swiss. For the T/+ males and females and the +/+ females, the first four litters were counted, for the +/+ males the first three.

The karyotypes of the females have been established by the use of Fredga's (1964) corneal squash technique. For the males, a low reproductive performance together with the production of at least one semi-sterile son has been taken as evidence for translocation heterozygosity.

The standard deviation of the relative litter size has been calculated with a formula developed by P. Stam:

$$\text{var} = \frac{\bar{y}}{\bar{x}} \frac{nN_1}{n_1^2 N_2},$$

where N_1 = number of litters of the control, N_2 = number of litters of the T/+, n = total number of offspring sired by both T/+ and +/+ males, n_1 = number of offspring sired by control males.

3. RESULTS

(i) *Diakinesis – metaphase I*

The two translocations differ with respect to the spectrum of multivalent configurations at diakinesis–metaphase I (Table 1). This is not surprising in view of the differences between the two translocations in length of the interstitial and translocated segments (de Boer & van Gijsen, 1974). Fig. 1 for T26H and Fig. 2 for T70H give diagrams and photomicrographs of the most frequent types of multivalent configurations seen.

Table 1. *Diakinesis and metaphase I observations on males heterozygous for the T26H and T70H translocations*

T26H (n = 1046)			T70H (n = 1459)		
Configuration	n	%	Configuration	n	%
R IV	556	53·15	R IV	37	2·55
C IV 8_i	385	36·80	C IV 1_i	898	61·55
C IV 2_t	75	7·15	C IV 13_t	16	1·10
C IV 2_i	2	0·20	C III + I ($1_i + 13_t$)	491	33·65
C IV 8_t	1	0·10	II + II ($1_i + 13_i$)	14	0·95
C III + I ($2_t + 8_i$)	17	1·60	II + I + I ($1_i + 13_i + 13_t$)	2	0·15
C III + I ($8_i + 8_t$)	1	1·10	II + I + I?	1	0·05
II + II ($2_t + 8_t$)	8	0·75			
II + II + I?	1	0·10			

T26H/+ males were heterogeneous with respect to the numbers of R IV, C IV 8_i and C IV 2_t configurations ($\chi^2_4 = 22$, $P < 0{\cdot}005$). Quite frequently (5·6 %, $n = 556$) R IV's were seen where a chiasma was slipping off in segment 2_t. Somewhat less frequently and especially in class 3 spermatocytes, R IV's with chiasmata slipping off in 2_t and 8_t resembled two bivalents with the homologous telomeres directed towards each other (1·8%, $n = 556$). Centric association between two centric heterochromatic blocks of chromosome 8 was clearly observed in 2·1% ($n = 385$) of the C IV 8_i configurations. Using a χ^2-test of independence, no relation could be traced between the proportions of R IV, C IV 8_i and C IV 2_t configurations on one side and the chromosome morphology classes on the other side ($\chi^2_4 = 4{\cdot}81$, n.s.). This enables one to relate the chiasma frequencies found to the physical length of the chromosome segments as estimated by Giemsa-banding of mitotic prometaphase chromosomes (Table 2).

T70H/+ males differed highly with respect to the frequencies of C IV 1_i and C III + I ($1_i + 13_t$) configurations ($\chi^2_6 = 72$, $P < 0{\cdot}005$). A χ^2 independence test showed the chromosome morphology class of the cell and the type of configuration to be interdependent. The number of chiasmata scored drops when the chiasmata of the other bivalents in the same cell show terminalization ($\chi^2_2 = 89$, $P < 0{\cdot}005$),

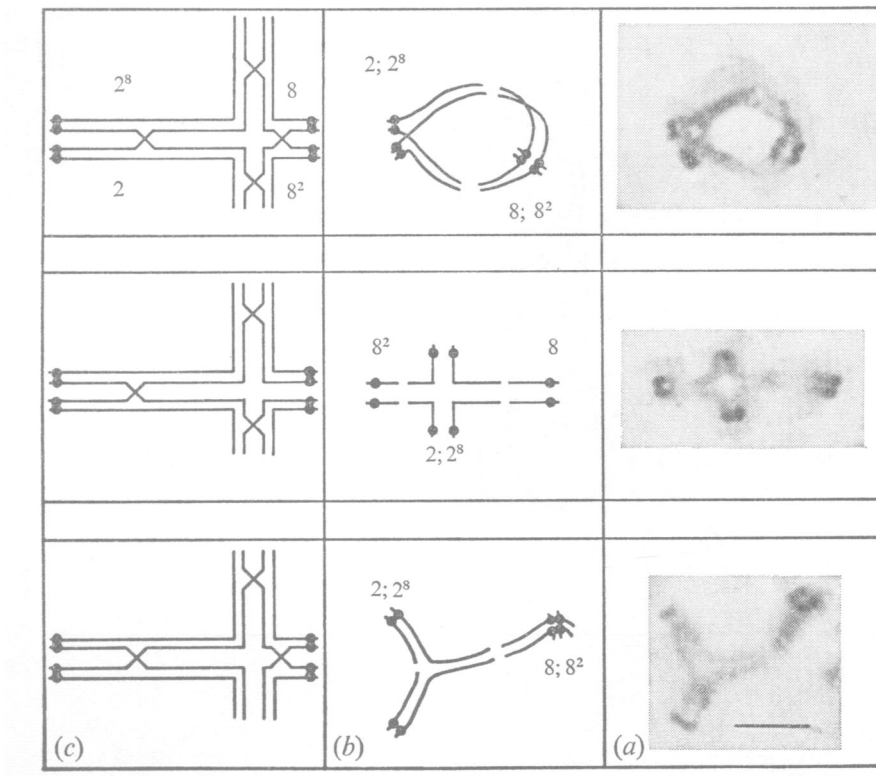

Fig. 1. (*a*) Diakinesis–metaphase I spermatocytes showing a T26H/+ RIV (upper), a C IV 8_i (middle) and C IV 2_t (lower). (*b*) Chromatid diagrams of these multivalent configurations. (*c*) Synapsis with the chiasmata allocated to translocated and interstitial chromosome segments. The bar represents 5 μm.

Table 2. *The length of interstitial and translocated segments of T26H (from de Boer and van Gijsen, 1974) together with the fractions bound by one or more chiasmata*

Segment	Length	Fraction bound	Chiasma frequency per unit length
2_i	5·16	0·997	—
2_t	1·22	0·903	0·740
8_i	1·38	0·614	0·445
8_t	3·21	0·989	0·308

see Table 3. Because of the fact that chiasmata have a tendency to slip off when meiosis proceeds (especially in segment 13_t), a true comparison between chiasma frequency and physical chromosome length during mitotic prometaphase can hardly be made. In 1·2% (n = 491) of the C III+I (1_t+13_t) configurations, chromosome 1^{13} was seen to be situated with its centromere towards the centromere of the *X*-chromosome within the *XY*-bivalent.

XY dissociation amounted to 9·7% (n = 1046) for T26H/+ and to 7·8% (n = 1456) for T70H/+. Autosomal univalence for non-translocation involved

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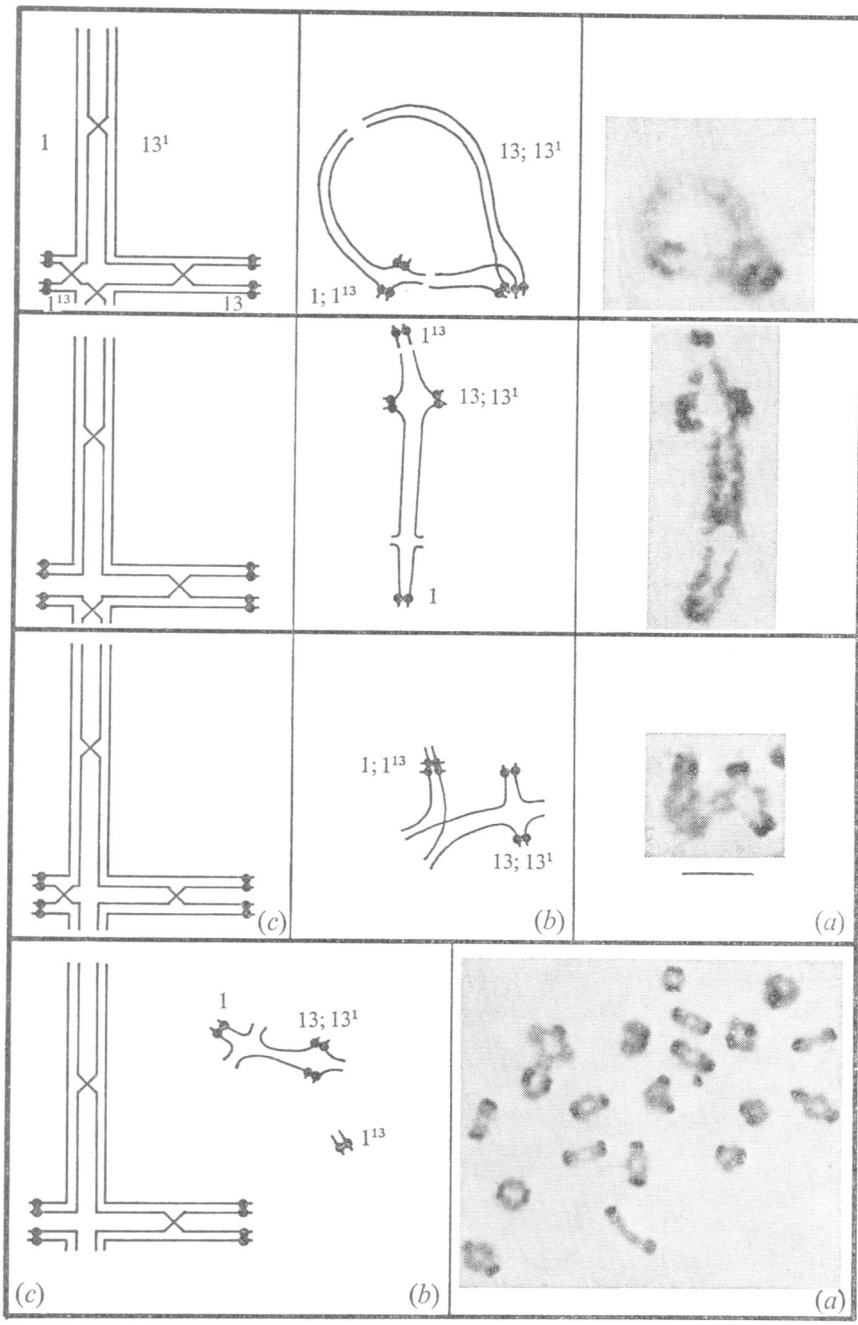

Fig. 2. (*a*) Diakinesis–metaphase I spermatocytes of T70H/+, showing a R IV (upper), a C IV 1_i (middle), a C IV 13_t and a C III + I(1_i + 13_t) (bottom). (*b*) Chromatid diagrams of these multivalent configurations. (*c*) Synapsis with the chiasmata allocated to translocated and interstitial chromosome segments. The bar represents 5 μm.

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bivalents occurred in 2·7 % of the T26H/+ cells and in 1·5 % of the T70H/+ cells. Both differences are not significant, using an χ^2 2 × 2 table. Chromosome morphology class 3 contained significantly more cells with *X* and *Y* univalents ($\chi_1^2 = 25$, $P < 0{\cdot}005$). For autosomal univalents, this relation was not found.

Table 3. *Chiasma terminalization within T70H quadrivalents in relation to the progression of the first meiotic division*

(Class 1 primary spermatocytes contain the least contracted bivalents, class 3 the most contracted ones.)

	Configuration		
Chromosome condensation class	R IV + C IV (*a*)	C III + I and others (*b*)	Ratio *a*/*b*
1	130	25	5·20
2	622	266	2·34
3	199	217	0·92

(ii) *Metaphase II*

Metaphase II observations concerning the segregational behaviour of multivalents and univalents caused by translocation can only be made in the mouse if

(*a*) the translocation chromosomes are sufficiently long or short to be safely recognized in secondary spermatocytes, and/or

(*b*) the normal and translocation chromosome with homologous centromeric ends differ so much in length that a chiasma in the interstitial segment produces two chromosomes with one chromatid clearly longer than the other.

Chromosomes (1^{13}; 1^{13}) and (13^1; 13^1) are examples of the first category, chromosomes (2; 2^8), (8; 8^2), (1; 1^{13}) and (13; 13^1) of the second (see Figs. 1 and 2). For fair judgment within the second category, knowledge of the location of the centromere by C-banding is required. The length difference between the two translocated segments within a reciprocal translocation should be sufficiently large to produce an arm ratio of at least 1·75:1. Differential chromatid contraction is a normal phenomenon within secondary spermatocytes. For this reason, the chromosome (2; 2^8) with an arm-ratio of approximately 1·5:1, originating from T26H, was not a suitable marker, although it can be recognized in favourable cells. Therefore, chromosome (8; 8^2) has been used as a marker in this translocation, despite the fact that an interstitial chiasma in segment 8_1 was present in only 61·4% of all primary spermatocytes.

Observations on marker chromosomes of secondary spermatocytes allow conclusions to be drawn about chiasma frequencies in the interstitial segments assuming that interstitially located chiasmata do not precociously terminalize. In the case of T26H, one can compare the frequency of 42·7 % of secondary spermatocytes without marker chromosomes (8; 8^2) with the frequency of primary spermatocytes not displaying a chiasma in segment 8_1, 38·6 %. This difference was on the borderline of significance ($\chi_1^2 = 2{\cdot}78$, $0{\cdot}05 < P < 0{\cdot}10$).

The frequency of double chiasmata in segment 8_1 has been assumed to be zero. The difference between the two percentages was almost exclusively caused by male no. 1. In the other males, the agreement between the two estimates was very good.

For T70H, the frequency of 3·2% secondary spermatocytes with $(1; 1^{13})$ can be compared with the frequency of primary spermatocytes which show a chiasma in segment 1_1 (3·65%). In the same way, the frequency of secondary spermatocytes with a $(13^1; 13^1)$ marker chromosome (1·4%) closely fits a percentage of 1·2 of diakinesis–metaphase I's with a chiasma lacking in segment 13_1. Again we have assumed that segment 13_1 is too short to accommodate two chiasmata, and in fact two chiasmata have never been observed.

Table 4. *Chromosome numbers and the presence of marker chromosomes* $(8; 8^2)$ *in T26H/+ secondary spermatocytes*

Chromosome number ...	19	20	21	
Marker				
—	10	257	11	
$(8; 8^2)$	12	334	10	
$(8; 8^2)$ $(8; 8^2)$	1	15	1	2·6%
Total	23	606	22	651

From Table 4, the percentage of anaphase I originated non-disjunction for T26H/+ can be calculated to be $6·9 \pm 1\%$. Non-disjunction can take place for normal bivalents and the sex-bivalent as well as for the chromosomes involved in the translocation. For the reasons that:

(*a*) only one marker (chromosome 8; 8^2, see Fig. 3) could safely be used,

(*b*) this marker is representative of 61·4% of the primary spermatocytes, namely the ones which have an interstitial chiasma in segment 8_1, aneuploidy cannot be divided into a translocation-caused category and a non-translocation-caused category. Adjacent II disjunction leads to the presence of two marker chromosomes (8; 8^2) in one secondary spermatocyte. The adjacent II frequency thus measured only applies to 61·4% of the first meiotic divisions. Assuming that C VI 8_1 configurations behave like the R IV, C IV 2_t and II + II($2_t + 8_t$) configurations, the adjacent II estimate yields a figure of

$$(100/61·4) \times 2 \times 2·6 = 8·47 \pm 2·0\%$$

of all primary spermatocytes.

T70H/+ secondary spermatocytes indicative for adjacent II, alternate/adjacent I and non-disjunction, translocation-caused or not have been listed in Table 5 and are illustrated in Figs. 4 and 5. The adjacent II frequency can be calculated to be $A/(A + C + D + E + F) = 25·2 \pm 1·7\%$.

Aneuploid chromosome numbers may have three causes.

(*a*) Numerical non-disjunction of the chromosomes involved in the translocation.

(*b*) Non-disjunction of the non-translocation bivalents.

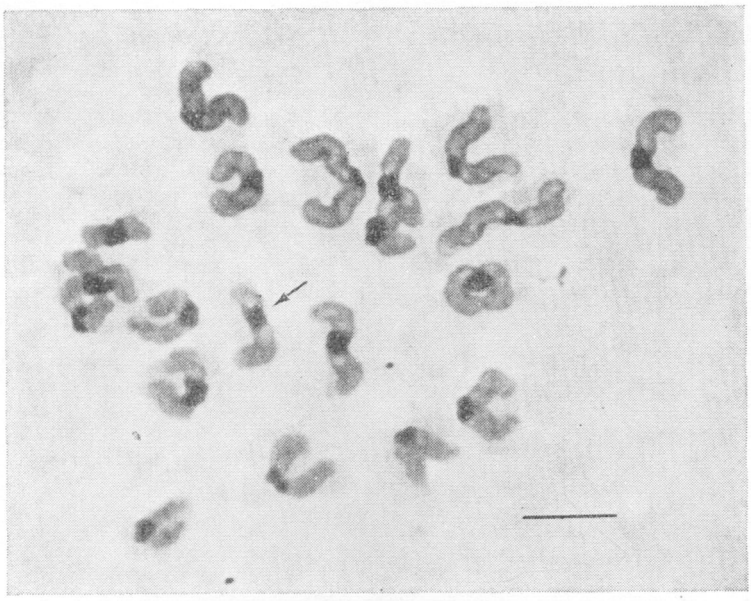

Fig. 3. A T26H/+ secondary oocyte with marker chromosome (8; 8^2) (arrow). Chromosome (2; 2^8) cannot be distinguished with certainty. The bar represent 5 μm.

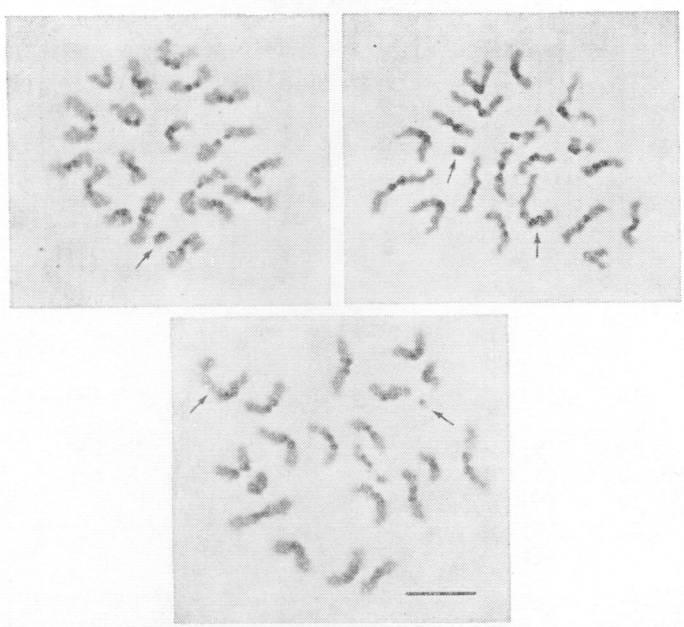

Fig. 4. Some T70H/+ secondary spermatocytes with karyotypes listed in Table 5 and illustrated in Fig. 5. Upper left a result of adjacent II disjunction (A left of Table 5 and Fig. 5), upper right a result of alternate/adjacent I disjunction (C left) and lower a case of numerical non-disjunction caused by equational separation of two 1^{13} chromatids during anaphase I (D right). Arrows point to the marker chromosomes (13; 13^1) and (1^{13}; 1^{13}) and to marker chromatid 1^{13}. The bar represents 10 μm.

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Table 5. *Chromosome numbers (including single chromatids) and the presence of marker chromosomes of T70H/+ secondary spermatocytes*

(Complementary types are grouped opposite each other. A horizontal bar indicates that no first or second marker is present within the cell. The simultaneous occurrence of non-translocation-caused non-disjunction and translocation-caused numerical non-disjunction, leading to a secondary spermatocyte with 20 chromosomes has been considered to be negligible. For further explanation, see the text and Fig. 5.)

Category	Chrom. no.	Chromosal constitution	*n*	*n*	Chromosal constitution	Chrom. no.
A						
Adjacent II	20	$(1^{13}; 1^{13}), -$	82	86	$(13; 13^{1}), (13; 13^{1})$	20
n = 174	19	$(1^{13}; -), -$***	1	1	$(13; 13^{1}), (13; 13^{1}), (1^{13}; -)$*	
	19	$(13; 13^{1}), (13; 13^{1})$**	3			
				1	$(1^{13}; -), -$****	21
B						
Indifferent	20	$(13^{1}; 13^{1}), -$	2			
n = 2						
C						
Alternate/		$(13;13^{1}), (1^{13}; 1^{13})$	192	202	$(13; 13^{1}), -$	
/Adjacent I		$(13^{1}; 13^{1}), (1^{13}; 1^{13})$	4	5	$-$, $-$	
n = 437	20	$(13; 13^{1})$, ?	14			20
		$(13; 13^{1}), (1; 1^{13})$	20			
D						
Translocation-	20	$(13; 13^{1}), (1^{13}; -)$	23	10	$(13; 13^{1}), (1^{13}; -)$	
caused	20	$(13; 13^{1}), (13; 13^{1}), (1^{13}; -)$	1			21
non-disjunction						
n = 36	19	$-$, $-$	1	1	$(13; 13^{1}), (13; 13^{1}), (1^{13}; 1^{13})$	
E						
Presumptive	19	$(13; 13^{1}), -$	14	14	$(13; 13^{1}), (1^{13}; 1^{13})$	21
translocation-	19	$(1^{13}; 1^{13}), -$	5			
caused				1	$(13^{1}; 13^{1}), (1^{13}; 1^{13})$	21
non-disjunction						
n = 34						
F						
Non-translo-	19	$(13; 13^{1}), (13; 13^{1})$**	3	2	$(1^{13}; 1^{13}), -$	21
cation						
caused-	19	$(13; 13^{1}), (1^{13}; 1^{13})$	5	1	$(13; 13^{1}), -$	21
non-disjunction	19	$(13; 13^{1}), (1; 1^{13})$	1			
n = 15	19	$(1^{13}; -), -$ ***	1			
	20	$(13; 13^{1}), (13; 13^{1}), (1^{13}; -)$*	1	1	$(1^{13}; -)$****	21

*, **, ***, **** Karyotypes which were explained by the simultaneous occurrence of adjacent II disjunction and non-translocation-caused non-disjunction are indicated by one or more asterisks.

(*c*) Equational division of the $(1^{13}; 1^{13})$ univalent at metaphase I – anaphase I. Of the cells under D (Table 5) with an euploid chromosome number (when counting the single chromatid as one), half of the second-division products will contain 19 chromosomes if the single chromatid does not get lost during anaphase II. Of

the metaphase II cells with 21 chromosomes containing chromatid 1^{13}, half may give hyperploid spermatozoa, the other half euploid ones. When one compares the classes of complementary metaphase II cells (Table 5), the numbers show a fair agreement except in category D when chromosome (1^{13}; 1^{13}) splits equationally ($\chi^2_1 = 5{\cdot}76$, $P < 0{\cdot}0025$). In our view, the possibility that a chromatid 1^{13} gets

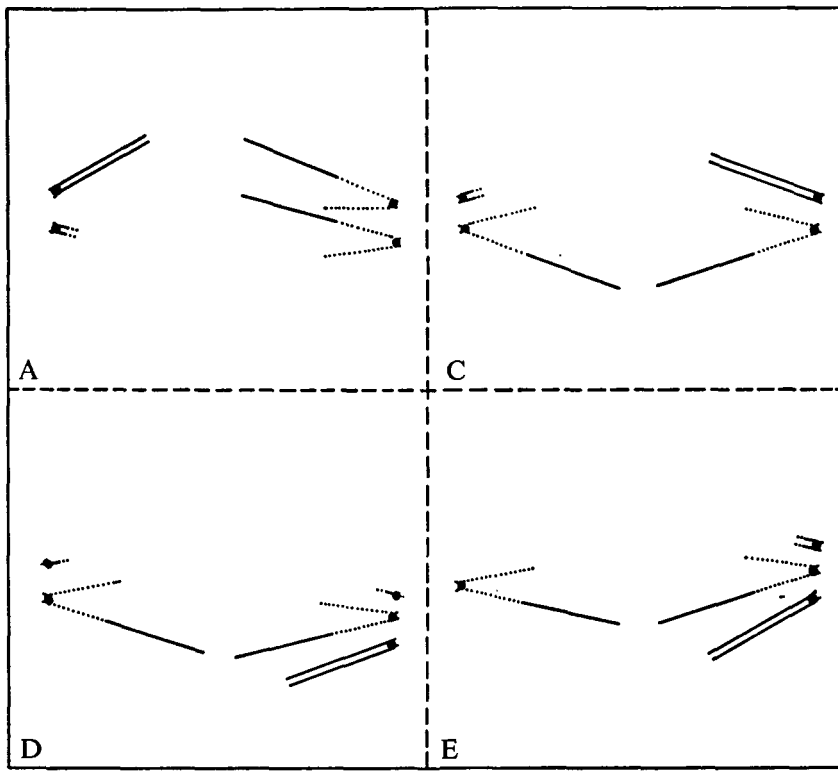

Fig. 5. Schematical representation of segregational events as observed in T70H/+ secondary spermatocytes and given in Table 5. A gives adjacent II segregation, C alternate/adjacent I, D numerical non-disjunction caused by equational separation of two 1^{13} chromatids and E 'normal' numerical non-disjunction. The symbols A, C, D and E follow the division made in Table 5. Chromosome 13 segments are dotted.

lost during anaphase I is greater when equational separation of chromosome 1^{13} occurs. This must be the chromatid which orientates in the same direction as two other chromosomes of the multivalent complex, thus leading to a shortage of cells with 21 'bodies' including chromatid 1^{13}. Therefore, aneuploid cells containing a 1^{13} chromatid have at first been left out when comparing total hyperploid and hypoploid counts of which 19 (with 21 chromosomes) and 29 (with 19 chromosomes) were scored, respectively ($\chi^2_1 = 2{\cdot}08$, n.s.). Because of the fact that not all segregational products of the diakinesis–metaphase I T70H/+ configurations can be cytologically recognized, not all the aneuploid cells can be safely categorized as translocation-caused or non-translocation-caused. Following the causes of

aneuploidy, given in Table 5, a minimal estimate for non-translocation-caused non-disjunction is

$$\frac{F}{A+B+C+D+E+F} = \frac{15}{692} = 2{\cdot}2 \pm 0{\cdot}6\,\%.$$

A maximal estimate is

$$\frac{E+F}{A+B+C+D+E+F} = \frac{49}{692} = 7{\cdot}1 \pm 1{\cdot}0\,\%.$$

If one does assume that single chromatids 1^{13} do not get lost during metaphase II–anaphase II, the minimal estimate for translocation-caused numerical non-disjunction gives a figure of

$$2{\cdot}8 \pm 0{\cdot}6\,\% \quad \left(\frac{D}{A+B+C+D+E+F}\right).$$

Loss of 1^{13} at metaphase II–anaphase II gives a figure of $3{\cdot}8 \pm 0{\cdot}7\,\%$ (there were somewhat more cells with 20 chromosomes including a single chromatid 1^{13} than with 21 chromosomes including a single chromatid 1^{13}). If we consider the cells of category E (of Table 5) as translocation-caused aneuploids, the maximal estimate possible for translocation-caused numerical non-disjunction is

$$8{\cdot}7 \pm 1{\cdot}1\,\% \quad \left(\frac{D+E}{A+B+C+D+E+F} = 4{\cdot}9\,\% + 3{\cdot}8\,\%\right).$$

Table 6. *Litter sizes $\bar{x}$ of T/+ males and females in outcrosses to normals, compared to controls, $\bar{y}$ (see Materials and Methods)*

Cross ♂	Cross ♀	$\bar{x}$	n	$\bar{y}$	n	$\bar{x}_{\text{corrected}}$	$\bar{x}_{\text{corrected}}/\bar{y}$
T26/+	× +/+	4·14	191	9·05	214	4·08	0·451
+/+	× T26/+	4·35	182	9·46	298	4·29	0·453
T70/+	× +/+	3·33	192	9·05	214	3·21	0·355
+/+	× T70/+	3·67	192	9·92	168	3·58	0·361

(iii) *Litter size of male and female translocation heterozygotes*

If there is no intra-uterine selection against small litters in crosses between T/+ and +/+ animals, the relative litter size of T/+ mice can be used as another estimate of the fraction of primary spermatocytes displaying either adjacent II segregation or numerical non-disjunction. When tested against a Poisson distribution with λ estimated by $\bar{x}$ and $\bar{y}$ (see Table 6), the litter sizes of the translocation heterozygotes and Swiss random-bred controls did not deviate significantly. This is evidence for the absence of intra-uterine selection against small litters. Because one cannot observe the class with zero young born (which has a probability, p_0). the average litter sizes have been corrected by multiplying them with the term $1-p_0$.

The value of p_0 can be calculated with the aid of $P_{(k=0)} = e^{-\lambda}(\lambda^k/k!)$ with $\lambda = \bar{x}$. This yields the column $\bar{x}$ corrected in Table 6. From this column, we have calcu-

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lated the summed frequency of adjacent II and numerical non-disjunction using the formula of Searle *et al.* (1971), given in the introduction. Table 7 compares the cytological estimates of these parameters with their summed frequency obtained by Searle's formula.

The first point to note is that in the present large material there is no discrepancy between the relative litter sizes of male and female translocation heterozygotes.

Table 7. *Estimates of frequencies of adjacent II segregation and numerical non-disjunction obtained by two methods of ascertainment in the male translocation heterozygote and one in the female translocation heterozygote*

	T26H/+ ♂		T70H/+ ♂	
	Adjacent II	Numerical non-disjunction	Adjacent II	Numerical non-disjunction
Cytological estimate	8·47 ± 2 %	?	25·22 ± 1·65 %	< 8·67 %
From relative litter size	9·80 ± 3·79 %*		29·00 ± 3·23 %*	
	T26H/+ ♀		T70H/+ ♀	
From relative litter size	9·40 ± 3·63 %*		27·80 ± 3·23 %*	

* The standard deviations (S) of the combined estimates for adjacent II and numerical non-disjunction (p) have been derived from the standard deviations of the relative litter sizes (x/y) as given in the Materials and Methods. Because $\bar{x}/\bar{y} = \frac{1}{2}(1-p)$ (see the Introduction), $S(p) = 2S(x/y)$.

Secondly, numerical non-disjunction must be a rare event in T26H/+ males with a best estimate of 9·80 − 8·47 = 1·33 % (see Table 7). Because of the predominance of R IV and C IV configurations in the first meiotic division (Table 1) and the absence of foetal lethals caused by T26H/+ (de Boer & de Maar, in preparation), this is in accordance with expectation (for discussion, see de Boer, 1973).

A surprising finding is a percentage of 6·9 % aneuploid metaphase II counts in T26H/+ (section (ii) and Table 4). If translocation-caused numerical non-disjunction is low, spontaneous non-disjunction for normal bivalents must be quite high. This is contrary to the literature (Ohno, Kaplan & Kinosita, 1959; Szemere & Chandley, in preparation), where spontaneous non-disjunction has been found to be extremely rare, especially for the sex-chromosomes.

From Table 5 (D + E) one can calculate that the fraction of spermatozoa of T70H/+ origin leading to the production of a tertiary trisomic is 1·37 %. When all survive to birth, they make up 3·87 % of all the young born. The percentage of morphologically recognizable tertiary trisomics at weaning age (18 days p.p.) was 1·21 % (n = 744). In a cytological survey of 97 morphologically normal daughters from matings T70H/+ × +/+, 48 were +/+, 46 T70H/+ and 3 (3·10 %) tertiary trisomic. One can conclude that the data on the recovery of tertiary trisomic offspring from T70H/+ males (3·10 + 1·21 = 4·31 %) are not far from the

expectancy based on the cytological scoring of secondary spermatocytes. From 70H/+ females 4·05 % (n = 642) of the young weaned were classified on the basis of their phenotype as tertiary trisomics. This is significantly more than the same category recovered from the male T70H heterozygotes (χ^2_1 = 11·3, P < 0·0005).

The limits for non-disjunction caused by T70H/+, set in the previous section, were between 2·7 and 8·7 %. The best estimate, derived on the basis of Table 7 and combining cytological and litter size data, is 29·00 − 25·22 = 3·78 %. When we correct for the fact that probably most (1^{13}) tertiary trisomic zygotes survive to birth, a best estimate then obtained is 3·78 + 1·37 = 5·15 %. This is well within the limits set in section (ii). By subtraction, the best estimate derived for non-translocation-caused primary non-disjunction is 8·67 − 5·15 = 3·52 %. Again this is higher than it should be according to the literature.

(iv) *Chiasma frequencies in interstitial versus translocated segments*

When the frequencies of chiasmata in interstitial segments (with centric heterochromatin) and translocated segments (without centric heterochromatin) are compared, there is a preference for chiasma formation in the latter. Although in T26H, 8_i and 2_t are almost equally long (de Boer & van Gijsen, 1974), the frequency of a chiasma in 2_t was approximately 1·5 times as high as the frequency of a chiasma in 8_i (Table 2). Similarly, despite 1_i being twice as long as 13_t in T70H, a chiasma is almost exclusively situated in 13_t (in 98·3 % of the cells where 1^{13} was bound).

Table 8. *Spermatozoal counts of the contents of the capita epididymes*

(The calculations are based on the averages for the two epididymes per animal.)

Karyotype	n	χ^2	S.D.
T26H/+	49	508	156
T70H/+	32	414	111
+/+	55	512	125

(v) *Sperm counts and morphology*

Table 8 gives the sperm count data on the basis of the average for the two capita epididymes per male, counted in a haemocytometer. An analysis of variance indicated a significant F-value ($F_{2.133}$ = 6·22, P < 0·005). This difference must be due to the lower count of T70H/+ males. One difference between T26H/+ and T70H/+ is that the percentage of translocation-caused univalents, observed through all stages of the first meiotic division is 33·85 in T70H/+ and 1·80 in T26H/+ males. In a review, Searle (1974) notes that mouse translocations which are male-steriles have a preponderance of C IV and C III+I configuration during the first meiotic division. In that respect, T70H is almost an exception in that it normally produces offspring.

The two translocations did not differ with respect to the morphology of the

spermatozoa. Around 1000, recovered from 6–7 males, were scored per group. The percentages abnormal are 5·80% for T26H/+, 4·33% for T70H/+ and 9·25% for +/+.

(vi) *The fate of univalents at anaphase I*

Trivalent plus univalent configurations (T70H/+) have an 'overall' frequency of 33·65% (Table 1), irrespective of chromosome morphology class. At metaphase I–anaphase I, it must be higher with a best estimate of close to 50% (Table 3). Random segregation of the univalent 1^{13} at anaphase I should yield at least 17% and probably around 25% aneuploid metaphase II cells. This holds true when there is no selection between the primary and secondary spermatocyte stages. On the basis of the previous section (v) such a selection can take place. The reduction of sperm production in T70H/+ (20% on the basis of the controls), is too small, however, to warrant the statement that all primary spermatocytes with a C III+I configuration simply do not reach metaphase II.

Table 9. *Observations on primary and secondary spermatocytes of T6Ca origin, made by E. P. Evans*

	Metaphase I (n = 1600)			
Configuration	R IV 1·7%	C IV 50·1%	C III+I 47·9%	II+II 0·3%
	Metaphase II (n = 2069)			
Chromosome number	20 49·0%	20(19+15^{14}) 45·0%	19 3·0%	21(20+15^{14}) 3·0%

From Table 5 it can be calculated that 306 cells possess chromosome 1^{13} and 317 do not. As we have see before (section iii), translocation-caused non-disjunction does happen with the low frequency of 4–5%. The conclusion must be that the 1^{13} univalent is capable of co-orientating with the other chromosomes of the translocation complex.

Meiotic studies in T(14; 15)6Ca heterozygote males (Table 9), made by E. P. Evans resemble ours in T70H/+ males in that a high percentage of C III+I configurations is found, together with an 'apparent' lack of aneuploid secondary spermatocytes. The small marker chromosome 15^{14} does not seem to get lost in those cells reaching metaphase II. The difference between the number of metaphase II cells with and without 15^{14} is not significant ($\chi^2_1 = 3{\cdot}17$, $P > 0{\cdot}05$). T6Ca/+ males, however, have an impaired spermatogenesis with death of germ cells occurring from the pachytene stage on (Baranov & Dyban, 1968). This effect hampers a true appraisal of the congruence between the meiotic behaviour of the two types of translocation heterozygous males.

4. DISCUSSION

It is interesting to know whether there exists any relation between the multivalent configurations during diakinesis–metaphase I and the segregation pattern that will follow. Considerable heterogeneity was found for the spectrum of

multivalent configurations among T70H/+ males (see section i). The males also showed heterogeneity with respect to adjacent II segregation ($\chi^2_6 = 15{\cdot}61$, $P < 0{\cdot}025$). No relation could be traced, however, between the spectrum of diakinesis–metaphase I configurations and the frequency of adjacent II segregation. Thus, although both the number of males investigated and the number of metaphase II cells scored per male might be too low to warrant a definitive conclusion, no association between the multivalent configuration spectrum and the adjacent II segregation appears from our data.

Notably the work of Burnham (1950) and Lewis & John (1963) has led to the proposal of a few rules based on translocation work mainly with submetacentric and metacentric chromosomes, which should be helpful to explain the differences in segregational behaviour between translocations.

(*a*) 'The evidence indicates that when chromosomes cross over in the interstitial segment they pass to opposite poles' (Burnham, 1950).

(*b*) Type I chains, with non-homologous centromeres at the ends of the chain show adjacent I segregation. Type II chains show a preponderance of adjacent II segregation (Burnham, 1950; Lewis & John, 1963).

Neither rule seems to be always obeyed by our two reciprocal mouse translocations between acrocentric chromosomes.

The majority (90·3 %) of the chains in T26H/+ are of type II with homologous centromeres at the ends of the chain (see Fig. 1, middle). According to these authors, if alternate/adjacent I segregation occurs in type II chains, it results from an 'unstable' configuration, in this case because of the indirect relation between the centromeres of 8 and 8^2 with at least three chiasmata between these two. Most T26H C IV 8_1 chains undergo alternate/adjacent segregation, however. The almost consistent occurrence of a chiasma in 13_1 of T70H multivalents does not prevent adjacent II segregation. In our view the observation of the precocious slipping off of chiasmata in the segments 2_t and 8_t of T26H R IV's offers a key to explain the segregational behaviour of T26H/+ and T70H/+ males. If the chiasmata of the translocated segments terminalize relatively early at metaphase I and lead to a precocious detachment into two heteromorphic bivalents, then the chiasmata in the interstitial segments are fully operative with respect to co-orientation. If, however, one or both translocated segments are long (as 1_t of T70H), chiasmata in this segment retain the power of leading to co-orientation. Then, adjacent II segregation might depend on the position of the chiasma in 13_1. The more proximal it is, the higher the chance that non-disjunction for the 13-centromeres will follow.

Summarizing, the segregational behaviour of mouse reciprocal translocations between acrocentric chromosomes can be explained on the basis of:

(*a*) The length of the translocated segments and the pattern of chiasma terminalization in these segments.

(*b*) The position of the chiasmata in the interstitial segments at the time of congression. Proximally located chiasmata add to the probability of adjacent II disjunction.

This study presents the first report of cytologically based estimates of adjacent II frequencies for mouse (and for mammalian) reciprocal translocations. More indirect estimates have been obtained in three ways, all of which are described by Searle *et al.* (1971):

(*a*) The frequency of homozygotes for interstitially located gene markers in T/+ intercrosses.

(*b*) The viability of T/+ outcross progeny relative to normal (see Table 7).

(*c*) The viability of T/+ × T/+ intercross progeny relative to normal.

Table 10 summarizes the adjacent II frequency estimates thus reported in the mouse. The estimates based on the frequency of complementation for interstitially located marker genes assume that complementation does not follow numerical non-disjunction, although this is a theoretical possibility. An estimate on the basis of the relative viability of outcross progeny includes embryonic and foetal death caused by numerical non-disjunction.

Table 10. *Adjacent II estimates for reciprocal mouse translocations so far obtained (for further explanation, see the text)*

	Method		
Translocation	(*a*) Frequency of homozygotes for interstitial marker genes	(*b*) Relative viability of outcross progeny	(*c*) Relative viability of intercross progeny
T(2; 8)26H*	0·20	0·12	0·19
T(1; 13)70H*	0·14	0·11§	
T(5; 13)264H*	0·13	0·05§	
T(14; 15)6Ca†	0·29	0·26	
T(9; 17)138Ca‡	0·30		

* From Searle *et al.* (1971).
† From Eicher & Green (1972).
‡ Calculated from Lyon *et al.* (1972).
§ Figures for T/+ males only.

If one compares the results for T26H and T70H in Table 10 with those given in Table 7, the conclusion seems to be warranted that the change of genetic background (to the Swiss stock) has altered the behaviour of T/+ male multivalent configurations: the T26H/+ males behave in a more regular (alternate/adjacent I) way while the frequency of adjacent II segregations has gone up for T70H. For T26H/+ males, Searle *et al.* (1971) found 20% chains of IV and 80% rings of IV (n = 199). T70H/+ males produced 6·9% rings of IV, 80·8% chains of IV and 12·3% chains of III+I (n = 317). When compared to Table 1, the chiasma frequency must have dropped quite strongly during the process of outcrossing T/+ animals to the Swiss random-bred stock.

This cannot account for the change in segregation pattern observed. Differences in the pattern of chiasma terminalization might be a more important factor involved.

In agreement with Eicher (1973) for T(14; 15)6Ca, female T70H/+ mice

produced more tertiary trisomic offspring than males. We did not find a greater overall fertility depression in female translocation heterozygotes than in males as reported by Snell (1946) and Carter *et al.* (1955). Conclusively one could say for T26H and T70H that, although the standard deviations are large (see Table 7), the adjacent II segregation frequencies do not differ much between the sexes.

Previously, we considered preferential chromosome pairing in the telomeric region to be the mechanism by which, in a situation of competitive pairing, more chiasmata were found in the telomeric region (de Boer & Groen, 1974). In both T26H/+ and T70H/+ males, the same phenomenon, i.e. a preference for distally localized chiasmata, was observed, and this may find its origin in preferential telomeric chromosome pairing as well.

However, when pairing is not followed by chiasma formation, a reduction of the latter function in proximal chromosome segments does yield the same cytological picture. In this context, it is remarkable that segment 13_1 of T70H was not bound in 1·15% of the cells. It occupies 4·07 % of the total length of the 'average' haploid complement (de Boer & van Gijsen, 1974), long enough to accommodate at least one chiasma. Disturbed synapsis in the centre of the cross, although it occurs (Eicher & Green, 1972) should not play an important role with segments of this length. The frequency of univalence for all autosomes not involved in T70H was 1·5%. In this translocation, the relative absence of chiasmata in 13_1 is of significance and it might indicate the power of centric heterochromatic segments to reduce chiasma frequencies in proximal chromosome segments.

In agreement with a proposal for a higher density of chiasmata in the distal chromosome segments are the findings with human gene mapping, particularly with chromosome 1 (Cook *et al.* 1974).

With T70H/+ (but not with T26H/+) we found that the chiasma count falls when meiosis proceeds. Forejt (1972) observed the same in two other reciprocal mouse translocations, T(2; 9)138Ca and T(9; 13)190Ca.

Two examples of achiasmate centric association have been observed in this investigation, both with a low frequency (1 – 2%). The first is between the homologous centric heterochromatic blocks of chromosome 8 in C IV 8_1 chains of T26H. An analogous observation has been made by Forejt (1973) for trivalents of T7Bnr/+ origin and for normal bivalents. The non-homologous centric heterochromatin association between 1^{13} and the *X*-chromosome reported earlier for the Ts(1^{13})70H karyotype (de Boer & Groen, 1974), turned up again in the T70H/+ males. The general nature of this phenomenon has been discussed before (de Boer & Groen, 1974).

It is hoped that observations in the T70H/+ and T26H/+ mouse translocations such as the pattern of chiasma formation, chiasma terminalization and segregation of homologous centromeres will serve as points of comparison for the study of human chromosomes.

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