A MEIOTIC STUDY OF TWO TRANSLOCATIONS AND A TERTIARY TRISOMIC IN THE MOUSE

(*MUS MUSCULUS*)

Dit proefschrift met stellingen van

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landbouwkundig ingenieur, geboren te Maarssen op 29 maart 1947, is goedgekeurd
door de promotor, dr.ir. J. Sybenga, lector in de erfelijkheidsleer.

De Rector Magnificus van de Landbouwhogeschool,

H.A. Leniger

Wageningen, 4 december 1974

P. de Boer

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Bij de muis is hyperploïdie een chromosomale conditie, die niet per definitie
leidt tot steriliteit in beide geslachten.

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kleine worpen.

A.G. Searle; C.E. Ford & C.V. Beechey. Genet. Res., Camb.
18: 215-235 (1971)

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Op grond van de "normale" morfologie van blastocysten die chromosomaal
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ouders, mag men bij de muis niet concluderen dat geen enkel aspect van het
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V.S. Baranov & A.P. Dyban. Soviet J. Devel. Biol. 1:
196-205 (1970)

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Aan de wenselijkheid om meer gedeeltelijke banen te creëren in de sfeer van
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Proefschrift van P. de Boer
Wageningen, 27 maart 1975

Voorwoord

Alvorens de lezer of lezeres verder kijkt mag het haar of hem niet ontgaan dat dit proefschrift gelukkig geen éénmansprestatie is.

De heer F.A. van der Hoeven heeft veel bijgedragen tot het resultaat omdat hij bij het hele onderzoek assisteert. Van de dierverzorgers G. van Tintelen, P. van Kleef, W. van Capelleveen en J.W.M. Haas van de centrale proefdieraccommodatie der Landbouwhogeschool is iedere vorm van medewerking ondervonden. De heer A. Arends is speciaal betrokken bij de verzorging van de muizen en met hem wil ik de chef der tuinen van de afdeling erfelijkheidsleer, de heer P.L. Visser memoreren voor zijn de knaagdieren goedgezinde houding.

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I am very grateful to Dr. A.G. Searle for his collaboration and the supply of the heterozygous translocation carriers. Dr. E.P. Evans teached me in most of the cytological techniques used and the discussions with him and Dr. C.E. Ford have added to the results. Thanks to them as well.

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Mijn promotor, Dr.ir. J. Sybenga heeft een grote invloed gehad bij de uiteindelijke vormgeving, publikatie en ideeënproduktie maar vooral heeft hij gezorgd voor de nodige tijd.

Het zal ingewijden niet zijn ontgaan dat dit onderzoek aan de afdeling erfelijkheidsleer der Landbouwhogeschool is uitgevoerd alwaar de schrijver zijn werkzaamheden verricht.

De afdeling tekstverwerking der Landbouwhogeschool heeft de tekst van dit proefschrift tot deze vorm verwerkt en het Pudoc verzorgde het vermenigvuldigen.

Hartelijk dank aan allen die hier genoemd zijn, maar ook aan hen, die op een andere wijze hebben bijgedragen.

General introduction

The cytology of translocation heterozygosity and its consequences has been studied extensively in plants and in some insects. Much less is known about mammalian and human translocations. In the present account, two reciprocal translocations in the mouse (*Mus musculus*) and a tertiary trisomic derived from one of these are considered, with emphasis on the meiotic behavior in and reproductive characteristics of the male.

This thesis consists of four articles presented in a logical order: the second builds forth on the first and the fourth on the third. The conclusions of the second and the fourth complement each other. Because of the fact that the four articles have an introduction each, it is perhaps more appropriate to mention a few general aspects of mammalian and human reciprocal translocations here.

The most efficient method of inducing translocations is by the use of ionizing radiations. There are two ways of detecting the formation of a translocation.

a) The appearance of a typical multivalent association at the first meiotic division.

b) The appearance of "semi-sterile" individuals among the descendants of irradiated animals.

In the mouse, the two frequency estimates for the induction of reciprocal translocations differ considerably, the first one being appr. twice as big as the second one (Ford et al., 1969). This points to the fact that about half of the translocations observed in the primary spermatocytes interfere with normal gametogenesis notably spermatogenesis. This seems to be a more general characteristic of mammalian and human reciprocal translocations. In man, Chandley (1973) reported a frequency of 0.53% of carriers of balanced translocations (mainly reciprocal) among the male patients of a subfertility clinic. The total frequency of balanced reciprocal translocations in the human population is 0.18% (Jacobs, 1972). This is a best estimate and probably still too low. For the mouse, this frequency might be something like 0.4% based on the estimate of the spontaneous mutation rate of 10.4×10^{-4} given by Lüning and Searle (1971).

Reciprocal translocations have been very useful in the classical mouse genetics. They constitute a tool for allocating linkage groups to chromosomes. They can be used for establishing the position of the centromere within the linkage group as well (Searle, 1968). They are very helpful in studies concerning meiotic pairing and chiasma formation in relation to chromosome structure and

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chromosome disjunction. In fact, this is one of the main points in this thesis. Another application is the mapping of biochemical markers to certain chromosomal segments. By the use of a series of translocations with one chromosome in common, this can lead to a rather exact assignment of marker loci to small chromosomal segments. This application finds employment in the field of human gene mapping (W. Burgerhout, personal communication).

All these aspects of translocation heterozygosity make them worth the attention of both plant and animal cytogeneticists and human cytogeneticists.

References

Chandley, A.C.: Karyotypes and phenotypes of infertility. Bull. of the Europ. Soc. Hum. Genet. 4-20 (1973).

Ford, C.E.; Searle, A.G.; Evans, E.P. and West, J.B.: Differential transmission of translocations induced in spermatogonia of mice by irradiation. Cytogenetics 8: 447-470 (1969).

Jacobs, P.A.: Human population cytogenetics. In J. de Grouchy, F.J.G. Ebling and I.W. Henderson eds.: Human genetics (Excerpta Medica, Amsterdam, 1972).

Lüning, K.G. and Searle, A.G.: Estimates of the genetic risk from ionizing irradiation. Mutation Res. 12: 291-304 (1971).

Searle, A.G.: The determination of centromere position in the mouse by translocation intercrosses. Heredity 23: 629-630 (abstr.) (1968).

Fertile tertiary trisomy in the mouse *(Mus muscules)*

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Abstract

A fertile, tertiary trisomic female mouse, daughter of a Harwell-bred T70H female, produced fertile male and female tertiary trisomics in an outcross to a Swiss random-bred male. Morphologically recognizable and non-recognizable 41-chromosome male and female progeny were obtained. In the morphologically recognizable animals, the most obvious abnormality was a malformation of the bones of the skull. Fertility of the tertiary trisomic sons was impaired. Conception rate amounted to 29%, and an average litter size of 3.33 $\pm$ 1.73 (N = 18) was recorded.

Introduction

Individuals heterozygous for a reciprocal translocation can give rise to aneuploid gametes. This holds true for plants, animals, and humans. In mammals and humans this probably occurs when one element of the translocation complex is not bound by a chiasma (or chiasmata) at metaphase of the first meiotic division. These gametes may be capable of fertilization, allowing the subsequent zygote to develop into an adult.

If the extra element is one of the translocation chromosomes and is combined with a normal karotype, the resulting offspring are called tertiary trisomics. Such animals have previously been found in the mouse. Irradiation of spermatids and spermatozoa yielded two translocations (T194H and T158H), giving rise to aneuploid gametes and adult animals with the small translocation product as the extra element (Lyon and Meredith, 1966). Cattanach (1967) produced tertiary trisomic males and females from T6Ca mice, as did Eicher and Green (1972) and Eicher (1973). Beechey (1972, personal communication) reported a tertiary trisomic female descending from T70H mice. All these translocations were of Harwell (M.R.C.) origin. (In man, translocation aneuploidy is not uncommon; Jacobs (1972) estimates the frequency as 0.04% of the liveborn population and as 0.16% of all recognized conceptions.) This paper describes observations on a tertiary trisomic female producing fertile, tertiary trisomic descendants.

Searle et al. (1971) give cytogenetic details about the T70H translocation. A Harwell-bred female (C3H/H-T70H/+) produced a morphologically normal daughter in an outcross to a 3H1 male. The daughter, mated to a Swiss random-bred male (Cpb:SE(S)), produced 41 liveborn young in six litters. In general, translocation heterozygotes are assumed to have reduced fertility. In this case litter size was not conclusive for karyotype, and sons were tested for semisterility by observations of embryonic lethality at day 14 of pregnancy after they were mated to normal females. The first five sons were fully fertile and were discarded, but a deviant sib was then encountered. After cytological examination of the cornea, bone marrow, spermatogonia, and primary spermatocytes, this sib appeared to be a tertiary trisomic male. All animals in the family were saved thereafter, and morphologically deviant or "suspect" males were bred to Swiss random-bred females, all giving rise to morphologically deviant and normal offspring. All members of the family have been examined cytologically except the mother and a severely retarded daughter, who were found dead. Fredga's (1964) corneal squash technique was used universally. The skulls were freed of tissue with the aid of papain, and skull measurements were taken with a vernier.

Observations

Relation between chromosome number and morphology

Observations on chromosome counts and the frequency of morphologically marked animals among the 41-chromosome groups are given in table I. The appearance of the 41-chromosome animals is quite varied. The head seems to be shorter than usual, and some animals make a pugnosed impression (Kidwell et al., 1961). The nasal part may be bent appreciably to the left or to the right (fig. 1), sometimes resulting in a more cranial position of the left molars compared to those on the right. The upper and lower incisors often show abnormal growth, especially the upper ones, which are often underdeveloped. The lower incisors, and sometimes the upper ones as well, have to be cut regularly because of the abnormal anatomy of the skull and the resulting disturbed length regulation of the incisors. Abnormal skull morphology almost always correlates with an underdeveloped animal. In addition, when these animals are handled, muscular tension can be observed to be less than normal. Fig. 2 shows a typical corneal squash preparation from a tertiary trisomic T70H male.

The following measurements of the skull have been taken: condylobasal length, mastoid width, nasal length, palatine length, interorbital width, rostrum height,

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Table I. Karyotype and morphology of the progeny of a presumable tertiary tri-
somic T70H female.

	Chromosome number and sex			
	40♂	40♀	41♂	41♀
Total	1	14	8	6
Recognizable			4	2
Non-recognizable			4	4

Table II. Results of comparisons between skull measurements of individual male
and female tertiary trisomics and their normal sisters (N = 14).

Measure	41 ♀♀ (N = 6)				41 ♂♂ (N = 8)			
	P = NS	0.05	0.005	0.001	NS	0.05	0.005	0.001
Condylobasal Length	4			2	3		1	4
Mastoid width	4		1	1	7	1		
Nasal length	3	3			3	1	1	3
Palatine length	4			2	3			5
Interorbital width	5	1			4			4
Rostrum height	6				8			
Length of mandibles	3		2	1	3	2		3

NS = not significant

and length of the mandibles. Because of a lack of data on 40-chromosome males
(most of them were discarded on the basis of the tests of embryonic lethality),
the only unbiased comparison that can be made is between 40-chromosome females
and tertiary trisomic females. Skull measurements are assumed to show a normal
distribution. The 41-chromosome animals have individually been tested against
the mean of the 40-chromosome females. The probability of significant differences
is given in table II. All differences are skewed to the left, indicating that
the tertiary trisomics are smaller.

Rostrum height is the only measure in which no difference is found. Nasal
length and length of the mandibles are most frequently affected, although the
material is too limited to give a true picture of the abnormalities. The males
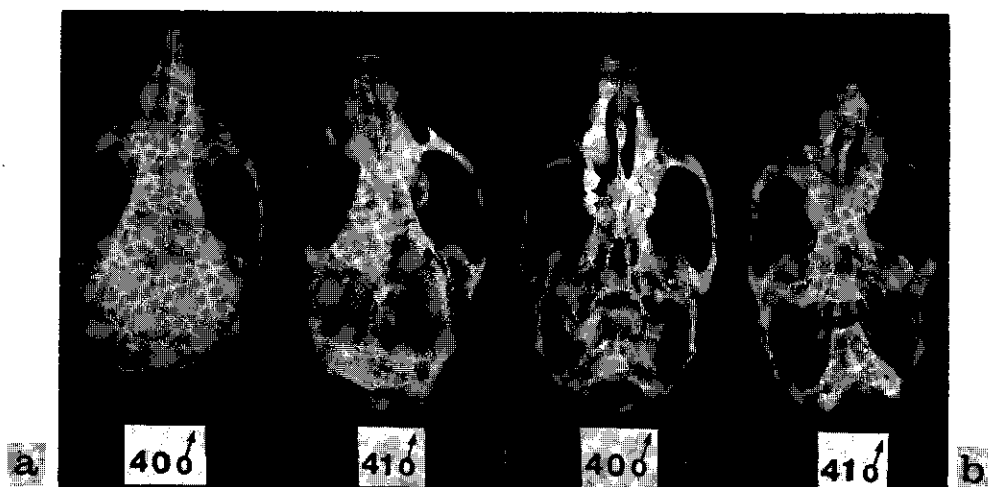reflect the same tendency, but the comparison may be biased by sex differences.

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Fig. 1. a. Dorsal view of the skulls of a normal (left) and a tertiary trisomic (right) T70H male. *b.* The same: ventral view.
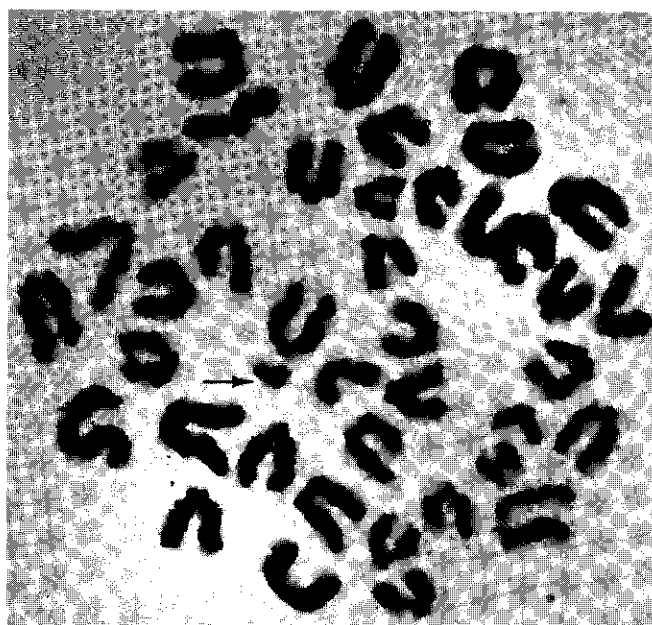

Fig. 2. Corneal squash preparation of a tertiary trisomic T70H male. Arrow indicates the T70H small translocation element.

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Both male and female 41-chromosome animals occur which look perfectly normal, but skull measurements indicate that there are significant differences from the control females.

Fertility

Fertility observations have mainly been made on morphologically recognizable male tertiary trisomics. Females were used for other experiments. Conception rate is definitely lower than among 40-chromosome mice. Three males were followed in this respect. Of the 46 observed vaginal plugs, 14 resulted in litters. Three litters were born without detection of a vaginal plug. The vaginal plug usually is quite small. The conception rate amounts to 29%, whereas the normal conception rate can be assumed to be over 90%. No sterile sons or daughters have been encountered so far. Litter size of the three males for which vaginal plugs have been recorded averages 3.33 $\pm$ 1.73 (N = 18). The range is 1-7. The average litter size (live and dead) of first litters of Swiss random-bred mice (Cpb:SE(S)) has been reported to be 8.45 (Joosten, 1969).

Discussion

The T70H-41 female and sons described in this article definitely showed higher fertility than hitherto was known to occur in tertiary trisomics. Fertility of the mother was quite high (41 young out of six litters). Sterility is common among male primary and tertiary trisomics. All four T6Ca tertiary trisomic males out of Cattanach's (1967) experiment were completely sterile, and observations made by Eicher (1973) on the same chromosomal constitution indicated sterility as well. T194H tertiary trisomic males seem to be sterile because of their low-to-intermediate testis weight, as was the case with the T158H 41-chromosome animals (Lyon and Meredith, 1966). Out of the eight primary trisomic cases known (see below), six were completely sterile and two semisterile, and a translocation trisomic male encountered by Griffin (1967) was also sterile. Sterility seems to be caused by a breakdown of spermatogenesis shortly after or during the first meiotic division (Lyon and Meredith, 1966; Cattanach, 1967).

In tertiary trisomic females, fertility seems to be better. T194H-41 females have been found to have a somewhat lower than normal litter size (average = 4.61), and the same is true of T158-41 females (4.00) (Lyon and Meredith, 1966). T6Ca-41 females have shown signs of fertility as well (Cattanach, 1967, Eicher, 1973). Beechey (1972, personal communication), however, encountered a sterile T70H-41 female with an imperforated vagina and very small ovaries, which contained a few primary oocytes at the germinal vesicle stage. Lyon and Meredith

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(1966) blame the lesser reproductivity of tertiary trisomic females to a reduced ovulation rate. The decreased fertility and lowered conception rate of the sons reported here is blamed on reduced sperm production. This point needs further confirmation.

Our observations on the variation of morphological characteristics of tertiary trisomics, some with abnormalities and some without, confirm those made earlier. Lyon and Meredith (1966) found the same variation in T158H-originated tertiary trisomics. Some, but not all of the T6Ca tertiary trisomics of Cattanach's (1967) experiment showed a nervous, trembling behavior. However, all of the T6Ca tertiary trisomics found by Eicher and Green (1972) and by Eicher (1973) seem to be retarded in development and show trembling behavior. This difference may be due to a difference in genetic background; the piebald (S) gene, for example, was present in homozygous condition in the latter experiment. In the case of T194H 41-chromosome animals, there was also no abnormal phenotype regularly associated with the tertiary trisomic condition (Lyon and Meredith, 1966).

In contrast to the tertiary trisomics, the eight primary trisomics found by Cattanach (1964) and Griffin and Bunker (1964, 1967) showed no sign of external deviation, although, as Lyon and Meredith (1966) point out, there is no certainty as to the origin of the extra chromosome in these cases.

If these animals are indeed primary trisomics, however, this condition seems to cause less developmental harm than a partial trisomic condition for two chromosomes. In contrast to this stand the results of White et al. (1972). They intercrossed the F_1 progeny produced by crossing homozygous T1Wh and T163H animals. Non-disjunction in the F_1 animals yielded 12% primary trisomy for chromosome 19. These animals died on their first day of life because of respiratory distress. Some showed a cleft palate. Primary trisomic embryos generated through a parent with one *poschiavinus* metacentric chromosome usually die between day 12 and day 16 of pregnancy, thus adding to the picture that primary trisomy in the mouse is almost always lethal prenatally (Gropp and Ford, 1973).

All tertiary trisomics known up to now arose out of stocks with high percentages of chain IV and trivalent plus univalent configurations at metaphase of the first meiotic division. T70H diakinesis-metaphase I observations yielded 6.9% rings of IV, 80.8% chains of IV, and 12.3% chains of III + I (N = 317) (Searle et al., 1971). Chains of III + I may well be a prerequisite for the condition to arise. On the other hand, one cannot exclude the possibility of 3:1 segregation of chains of IV. Burnham (1962) mentions a translocation in maize in which chains were able to segregate 3:1. In rye translocations, the

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occurrence of univalents does not seem to raise the chance of recovering tertiary trisomic or translocation trisomic offspring (Sybenga, 1973, personal communication). Hamerton (1971) described 11 familial translocations in man yielding translocation aneuploidy out of a total of 75 (of which 73% was detected through a proband with mental retardation and congenital malformation). Such translocations tend to have short interstitial segments, and one of the unchanged chromosomes is an acrocentric one. This results in a high probability of chains III or IV at metaphase I. These results do not exclude the possibility of chains of IV being able to produce aneuploidy im mammalian (human) translocations. For these species, this point remains open for elucidation.

References

Burnham, C.R.: Discussions in cytogenetics (Burgess Publishing Company, Minneapolis 1962).

Carter, T.C.; Lyon, M.F., and Philips, R.J.S.: Gene-tagged chromosome translocations in eleven stocks of mice. J. Genet. 53: 154-166 (1955).

Cattanach, B.M.: Autosomal trisomy in the mouse. Cytogenetics 3: 159-166 (1964).

Cattanach, B.M.: A test of distributive pairing between two specific non-homologous chromosomes in the mouse. Cytogenetics 6: 67-77 (1967).

Eicher, E.M.: Translocation trisomic mice: production by female but not male translocation carriers. Science 180: 81 (1973).

Eicher, E.M. and Green, M.C.: The T6 translocation in the mouse: its use in trisomy mapping, centromere localization, and cytological identification of linkage groupe III. Genetics 71: 621-632 (1972).

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

Griffin, A.B.: A case of tertiary trisomy in the mouse and its implications for the cytological classification of trisomics in other mammals. Canad. J. Genet. Cytol. 9: 503-510 (1967).

Griffin, A.B. and Bunker, M.C.: Three cases of trisomy in the mouse. Proc. nat. Acad. Sci., Wash. 52: 1194-1198 (1964).

Griffin, A.B. and Bunker, M.C.: Four further cases of autosomal primary trisomy in the mouse. Proc. nat. Acad. Sci., Wash. 58: 1446-1452 (1967).

Gropp, A. and Ford, C.E.: Mouse News Letter, No. 48, pp. 37, 40 (1973).

Hamerton, J.L.: Human cytogenetics, Vol. 1 (Academic Press, London/New York, 1971).

Jacobs, P.A.: Human population cytogenetics. In J. De Grouchy; F.J.G. Ebling and I.W. Henderson, eds.: Human genetics (Excerpta Medica, Amsterdam 1972).

Joosten, W.J.: Een analyse van de vruchtbaarheidskenmerken en een selectieproef op vruchtbaarheid bij Swiss-muizen. Scriptie Veeteelt en Erfelijkheidsleer, Wageningen (1969).

Kidwell, J.F.; Gowen, J.W. and Stadler, J.: Pugnose-a recessive mutation in linkage group 3 of mice. J. Hered. 52: 145-148 (1961).

Lyon, M.F. and Meredith, R.: Autosomal translocations causing male sterility and viable aneuploidy in the mouse. Cytogenetics 5: 335-354 (1966).

Searle, A.G.; Ford, C.E. and Beechey, C.V.: Meiotic disjunction in mouse translocations and the determination of centromere position. Genet. Res. 18: 215-235 (1971).

White, B.J.; Tjio, J.H.; Van de Water, L.C. and Crandall, C.: Trisomy for the smallest autosome of the mouse and identification of the T1Wh translocation chromosome. Cytogenetics 11: 363-378 (1972).

Fertility and meiotic behavior of male T70H tertiary trisomics of the mouse *(Mus musculus)*
A case of preferential telomeric meiotic pairing in a mammal*

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Abstract

Meiotic studies were carried out on fertile male tertiary trisomic mice with the T(1;13)70H small translocation product, carrying the centromere of 1 and the telomere of 13 as the extra element. Appr. 200 primary spermatocytes from five males each were studied. The only configurations found at diakinesis – metaphase I were 19 bivalents and a trivalent (22%) and 20 bivalents and an univalent (78%). Within the cells with a trivalent, the majority (92.7%) appear to be of the type $(13;13;1^{13})$. This indicates that in this case the telomeric region of chromosome 13 has a greater potential to form a chiasma than the proximal region of chromosome 1, containing centric heterochromatin. From the presence of chromosome 1^{13} in appr. 50% (N = 119) of the secondary spermatocytes, it is inferred that the formation of an univalent in primary spermatocytes does not lead to loss of the extra chromosome at anaphase I – telophase I. The impression was gained that the T70H small marker chromosome (1^{13}) can display a positive heteropycnotic behavior in the tertiary trisomic males studied. Seven other T70H tertiary trisomic males were used to generate 301 embryos and fetuses to be karotyped at either 11 days of age or 18 days of age. Of the first age-group 34.6% contained the extra chromosome. Of the second age-group, this figure was 46.3%. Gross differences in litter size of the tertiary trisomic males occur, both within and between males. At day 12 of gestation litter size (live embryos) amounts to 4.44 $\pm$ 2.41 (N = 41). At day 19, the average number of live fetuses is 4.94 $\pm$ 2.75 (N = 36). The low but variable reproductive performance of the tertiary trisomic males is mainly caused by a lowered sperm production.

Introduction

The discovery of fertile tertiary trisomy in female as well as in male mice (de Boer, 1973) offers an opportunity to gain insight into the meiotic

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behavior of the extra chromosome as well as the impact this chromosomal constitution has on the fertility of a carrier. In the case described in this publication, the extra chromosome originates from the T(1;13)70H translocation and constitutes the small translocation product (for cytogenetical details of this translocation, see Searle et al., 1971). Because of their abundancy of diakinesis - metaphase I and metaphase II stages, males have been used in this investigation. Meiotic preparations of male tertiary trisomics have been made previously (Lyon and Meredith, 1966). Both T194-41 as T158-41 males produced analyzable primary spermatocytes. These observations were only qualitative, however.

Three aspects of meiotic behavior have been given attention in this paper:
- Allocation of chiasmata to the interchange segments concerned.
- The fate of the extra chromosome when it remains univalent at diakinesis -
  metaphase I in combination with the gametic types produced.

Although plant cytogeneticists have reported on these points since quite some time (see Burnham, 1962), information from a mammalian source was almost absent until now.

A search has also been undertaken into the karyotypes of 11 days old embryos and 18 days old fetuses produced in outcrosses of tertiary trisomic males to females with the normal karyotype. The fecundity of tertiary trisomic mice, known up to now, has been reviewed by de Boer (1973). It appeared that, except in the case of T70H, the males were always sterile while the females showed a picture of impaired fertility which varied between females with a different extra chromosome. The fertility data, inherent to the recovery of 11 days old embryos and 18 days old fetuses have been used to describe the reproductive potentials of T70H tertiary trisomic males in some more detail.

Material and methods

The males used in the meiotic analysis were full brothers and are part of the material described earlier (de Boer, 1973). The genetic background of the males is made up of a (C3H x 3H1) mother and a Swiss (Cpb:SE(S)) father. The males were between 7 and 9 months of age when killed except one (not included in the main calculations) who was appr. 6 weeks old.

Meiotic preparations were made according to the Evans-technique (Evans et al., 1964). Constitutive centric heterochromatin was preferentially stained (C-banded) by a method suggested to me by Dr. E.P. Evans. Dry slides are heated for appr. 10 min. at a temperature of $120-125^{\circ}$C. Thereafter they are Giemsa-stained in the conventional way (Summer et al., 1971). The temperature range

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was determined with the aid of an incubator. The process is routinely performed on a hot plate with an emperical scale.

Diakinesis - metaphase I's were allocated to three classes of chromosome morphology. In class 1, the chromatids are still thread-like. Class 2 consists of cells with shorter chromosomes but not all chiasmata have yet terminalized. In class 3 all bivalents show terminalized chiasma(ta). The relation between classes and meiotic stage is, at least for class 2 and 3, not necessarily perfect. The position of the vernier on the microscope stage was recorded while screening the slides so that an impression could be gained about cell characteristics relative to the position in the preparation. The sex chromosome condition of secondary spermatocytes has as much as possible been established using the somewhat darker appearance of the Y chromosome, often lacking the C-band. In agreement with Polani (1972), however, the Y does sometimes show centric heterochromatin staining in secondary spermatocytes. These criteria were used in combination with the morphological characteristics of the sex chromosomes in secondary spermatocytes as outlined by Beechey (1973). Homogeneity of distributions was tested against the χ^2-distribution.

Seven male T70H tertiary trisomics were used to generate embryos and fetuses. Two animals (1 and 2) were out of the second outcross to the Swiss random-bred (Cpb:SE(S)) stock, four (4, 5, 6 and 7) out of the third and one (3) out of the fourth. The outcrosses were performed in such a way that the males are as little related as possible. All seven males were morphologically recognizable tertiary trisomics. The males were caged once a week for nine successive weeks with two appr. 3 months old virgin Swiss random-bred females from the stock mentioned above. All females were inspected for vaginal plugs on each of the seven days after the day of caging. The day a vaginal plug was recorded was designated as day 1. Autopsy was carried out on the 12th and 19th day of pregnancy thus yielding embryos of 11 days old and fetuses of 18 days old. At autopsy the numbers of big and small moles were counted as well as the numbers of live embryos. The 11 days pregnant females were used for estimation of the number of corpora lutea. According to Falconer et al. (1961), counts of the number of corpora lutea approach the true number of ovulated eggs reasonably well. The embryos and the remnants of an embryo and/or embryonic membranes of some big moles were karyotyped using a technique described by Evans et al. (1972). At the 12th day of pregnancy, the embryonic membranes were usually taken, at the 19th day, the fetal livers proved to be best for obtaining sufficient mitoses for karyotyping. The embryos and fetuses were sexed with the aid of C-bands produced by dry heat as decribed earlier (see fig. 6). When

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C-banded, the heterochromatic Y chromosome is somewhat less bleached than the euchromatic parts of the autosomes and the X chromosome. It usually lacks stainable centric heterochromatin (Hsu et al., 1971). These two criteria, used in combination with the morphological features of the Y chromosome as described by Ford (1966) always sufficed to determine the sex of a specimen.

During the 10th week of the experiment, after separation of the last females, sperm counts were made of the seven tertiary trisomic males using a method described by Searle and Beechey (1974). The number of sperm heads of one caput epididymi, both normally and abnormally shaped, were counted in all 16 squares of a Thoma bright-line hemocytometer. The epididymes of one male were treated separately. The figure given for one animal is the average for the two epididymes.

Results

Diakinesis – metaphase I

Theoretically, in addition to several types of univalents and bivalents, the tertiary trisomic condition can give rise to six different types of multivalents including a pentavalent, two types of quadrivalents and three types of trivalents. The first three types have never been found in our material presumably due to the shortness of the T70H interchange segments concerned. Figures 1-4 present the observed associations between homologous chromosome segments. Attention has been focused on the morphological difference between a trivalent $(1;1;1^{13})$ and a trivalent $(13;13;1^{13})$.

The distinction between the two types of trivalents has been based on both the relative positions of the C-bands as well as the relative size (as judged by eye sight) of the trivalent among the bivalents. It should be remembered that chromosome 1 is the longest chromosome of the mouse complement and chromosome 13 is medium sized. So, a trivalent $(1;1;1^{13})$ will be larger than a trivalent $(13;13;1^{13})$. In the case of a $(13;13;1^{13})$ trivalent, the centric heterochromatin of chromosome 1^{13} is always found in the proximity of the telomeric region of chromosome 13. The criterion, that in case of a trivalent $(1;1;1^{13})$ one should find the centric heterochromatin of chromosome 1^{13} in the proximity of the proximal region of chromosome 1, was not always correct. Trivalents with a $(13;13;1^{13})$ C-band composition were found of which the non-segmented part was too big to be composed of two 13 chromosomes. These were regarded as $(1;1;1^{13})$ trivalents. This indicates that a chiasma between 1 and 1^{13} can terminalize over the non-homologous segments of 1 and 13. The possibility of such a chiasma

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terminalization was already known in plant cytogenetics (J. Sybenga, 1973). Non-homologous chiasma terminalization may have inflated the distinction between tri-valents $(1;1;1^{13})$ and trivalents $(13;13;1^{13})$ to some extent. The few cells in which there was any doubt between twenty bivalents and an univalent (20II+I) and nineteen bivalents and a trivalent (19II+III), have been excluded from further inspection.

Table I gives the result of the chromosome associations, found at diakinesis metaphase I. Significant differences do occur between males, $\chi_4^2 = 14.36$ (P < 0.01).

Table I. Differences between males in respect to chromosome configurations found at diakinesis - metaphase I.

Male	1	2	3	4	5	Total
Configuration						
Univalent (1^{13})	150	167	144	164	162	787
Trivalent $(13, 13; 1^{13})$	43	24	44	34	34	179
Trivalent $(1; 1; 1^{13})$	2	2	2	4	4	14
Trivalent of either type	9	3	5	2	10	29
Total	204	196	195	204	210	1009
% of trivalents	26.5%	14.8%	26.2%	19.5%	22.9%	22%

Total no. of trivalents (k) = 222, standard deviation = 13.2

For an estimate of the frequency of trivalents $(1;1;1^{13})$ among all identifiable trivalents, the animals have been pooled because of the low number of observed $(1;1;1^{13})$ configurations. The best estimate of this frequency equals P = 0.073 (N = 193). The coefficient of variation of this estimate is 25.8%, which is rather high, because of the low number of trivalents $(1;1;1^{13})$ actually observed. It is clear that the majority of the trivalents appear to be of the type $(13;13;1^{13})$.

Observations on one male T70H tertiary trisomic (not included in table I), killed at appr. 6 weeks of age, indicated a percentage of 18.9% (N = 169) primary spermatocytes in which an unusual arrangement was seen. In these cells, the cen-tric heterochromatin of the extra translocation chromosome was in close proximity of the centric heterochromatin of the X chromosome (see fig. 4b), yielding a $(1^{13};X;Y)$ trivalent. The extended material presented here revealed only 13 (1.3%) such cases and it is uncertain whether these were chance associations or cytolo-gically meaningful associations.
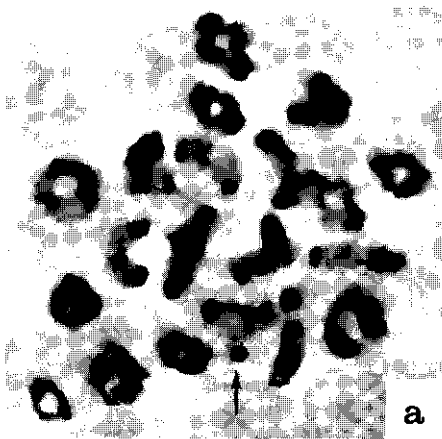
Fig. 1.a. Diakinesis - metaphase I spermatocyte, showing a (13; 13; 1^{13}) trivalent. *b.* Schematical representation of chiasma position. *c.* Chromatid diagram of a (13; 13; 1^{13}) trivalent with both chiasmata terminalizing distally. Dots reflect centric heterochromatin.
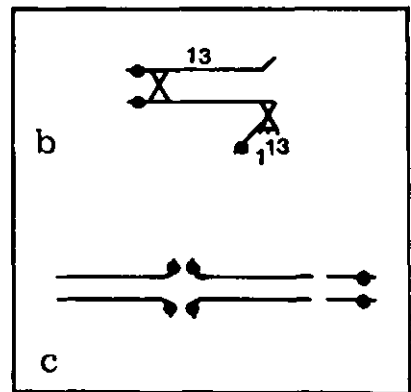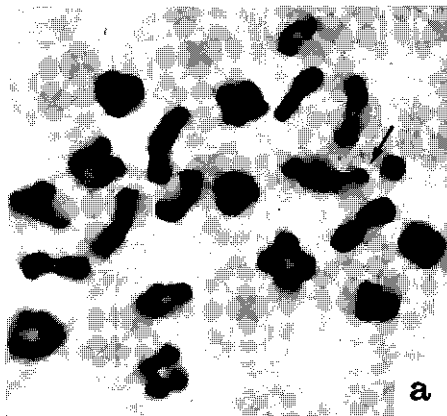
Fig. 2.a. Diakinesis - metaphase I spermatocyte with a (13; 13; 1^{13}) trivalent. Note X-Y dissociation. *b.* Schematical representation of chiasma positions. *c.* Chromatid diagram of a (13; 13; 1^{13}) trivalent with one chiasma in proximal position and the other terminalizing distally.
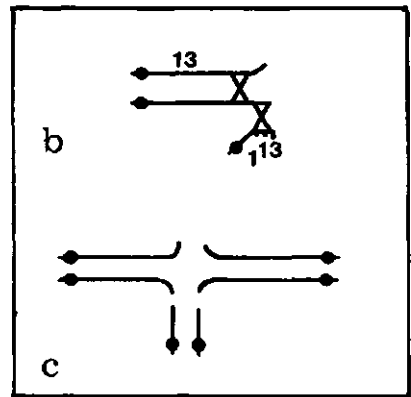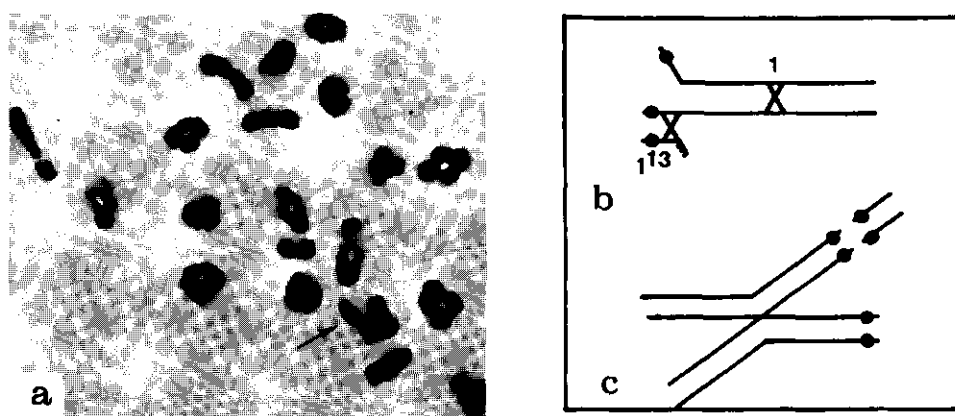
Fig. 3.a. Diakinesis - metaphase I spermatocyte showing a (1; 1; 1^{13}) trivalent. *b.* Schematical representation of chiasma position. *c.* Chromatid diagram of a (1; 1; 1^{13}) trivalent with one chiasma interstitial and the other proximal.

Fig. 4.a. Diakinesis - metaphase I spermatocyte with chromosome 1^{13} as an univalent. *b.* Two examples of non-homologous association between chromosome 1^{13} and the proximal end of the X chromosome.

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One striking observation made during this project was the positive hetero-
pycnotic nature the T70H small translocation product sometimes displays in a
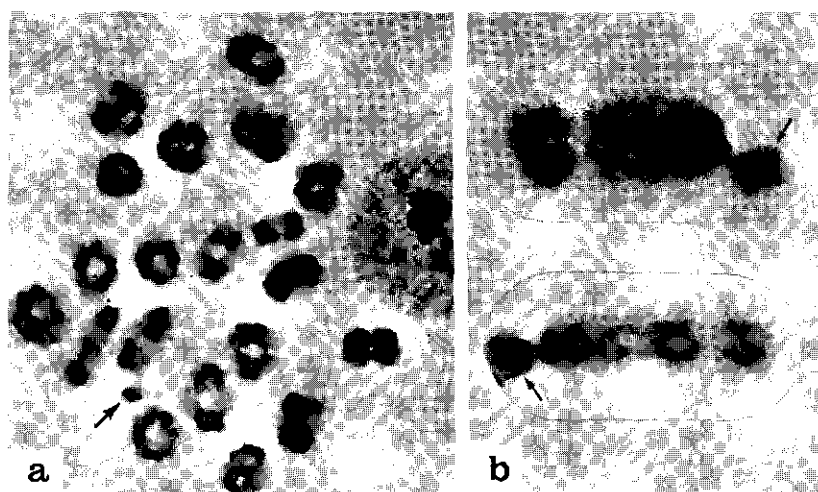tertiary trisomic karyotype. Fig. 5 shows a primary spermatocyte in diplotene
with the extra element close to the centromere of the X chromosome and equally
heteropycnotic as the sex chromosomes at this stage.

We have made an attempt to find indications of an influence of the technical
procedures inherent to the production of air-dried preparations and the consis-
tency of chiasmata. Data about diakenesis - metaphase I configurations are of im-
portance only when such an overall or preferentially operating influence is ab-
sent. Therefore, for the males 1-5 the distribution of primary spermatocytes
20II+I and 19II+III has been plotted against the position on the slide and
against the classes of chromosome morphology as defined in the Methods. The
position of the cell on the slide as indicated by the value of the vernier did
not influence the percentage of trivalents. Table II gives the distribution of
spermatocytes 20II+I and 19II+III among chromosome morphology classes.

Table II. Distribution of spermatocytes 20II+I and 19II+III among chromosome
morphology stages (see Methods).

Class	1	2	3
20II+I	32	332	424
	73%	75%	81%
19II+III	12	113	79
	27%	25%	19%
Total	44	445	521

Class 3 contains less trivalents and the overall distributions are dependent
(χ^2_2 = 14.84; P < 0.005). X-Y chromosome dissociation and the occurrence of auto-
somal univalents other than the small translocation product have as well been
taken as indicators for vigorous cell handling. The incidence of X-Y dissocia-
tion was remarkably constant, amounting to 4.5%, 5.5%, 5.6%, 5.5% and 4.3% (av.
5.1%, N = 1423) for the five males concerned. The frequency of autosomal uni-
valents other than the small translocation product was very low. They appeared
in 1.1% (N = 1009) of all cells examined. The actual numbers found of the two
classes are too low to permit investigation of the relation between these cri-
teria and the incidence of 20II+I against 19III+I primary spermatocytes.

In our hands, the air-dry technique exerts an influence on the morphology
of the chromosomes produced. Differences in chromosome morphology between the
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males do occur as indicated by a χ^2_4 value of 139.22 (P < 0.005). Table III gives the numbers for each male.

Table III. Distribution of primary spermatocytes at diakinesis - metaphase I of different chromosome morphology stages (see Methods) between males.

Males	1	2	3	4	5
Class 1	14	15	9	3	3
Class 2	88	105	126	94	32
Class 3	102	76	60	108	175
Percentage Class 3	50%	39%	31%	53%	83%
Total	204	196	195	205	210

These differences probably do not reflect differences between males but between subsequent copies of the technique. As indicated by table II, the % of cells with a trivalent is somewhat less in class 3 spermatocytes. The rank correlation coefficient of Spearman between the percentage of trivalents and the percentage of class 3 spermatocytes within males is very low and not significant, however $(r_s = - 0.1)$.

Metaphase II

Cytological observations on metaphase II secondary spermatocytes allow conclusions to be drawn about the fate of an (1^{13}) univalent at anaphase - telophase of the first meiotic division. They also give information about the gametic types formed at anaphase-telophase of the second meiotic division.

Metaphase II observations (N = 141) of the five males have been pooled because too few analyzable cells were found in each male separately. Besides the expected classes of 20 chromosomes without the small translocation chromosome and 21 chromosomes including chromosome 1^{13}, two additional classes were found: 20 chromosomes including 1^{13} and 21 chromosomes without 1^{13}. The actual numbers are given in table IV. If one neglects the small classes of secondary spermatocytes where $19+1^{13}$ or 21 normal chromosomes are present, segregation of the T70H small translocation product follows a 1:1 pattern. This indicates that in those cases where it is present as an univalent at metaphase I (see table I) it does not get lost and always moves to one pole or another. So far as the data permit one can say that no special affinity exists between the small marker chromosome and either of the two sex chromosomes during metaphase I - anaphase I. The class

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■ Number of live embryos plus fetuses
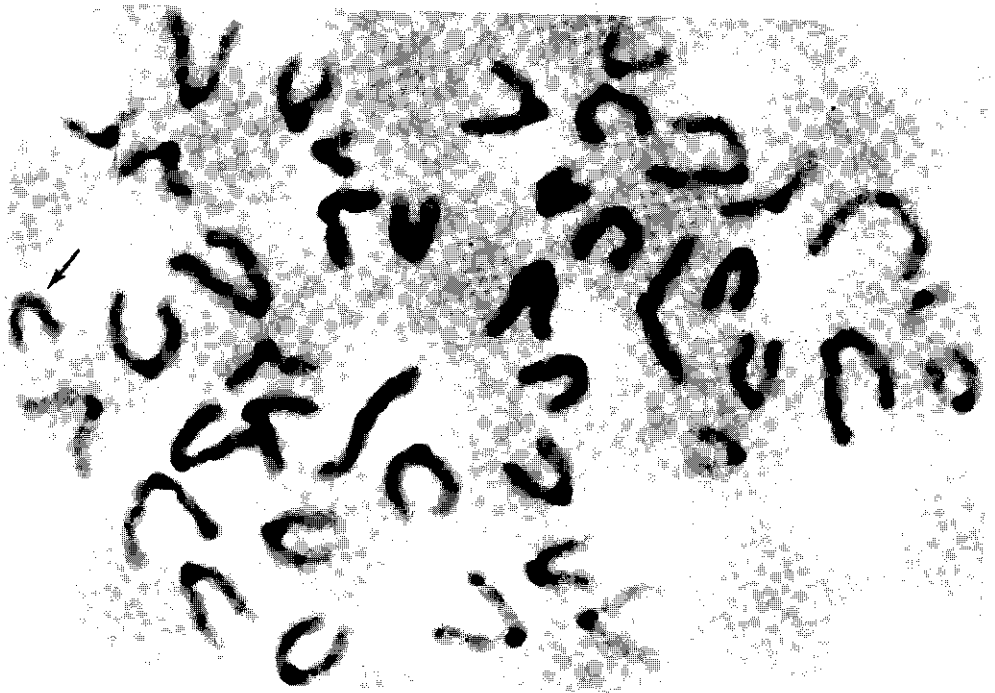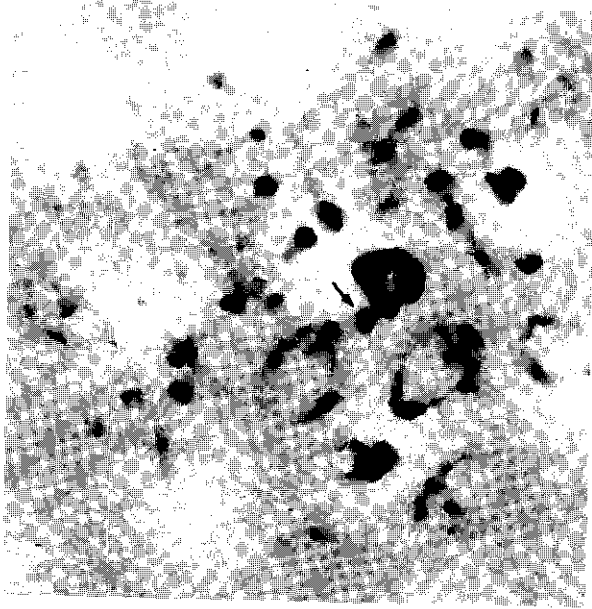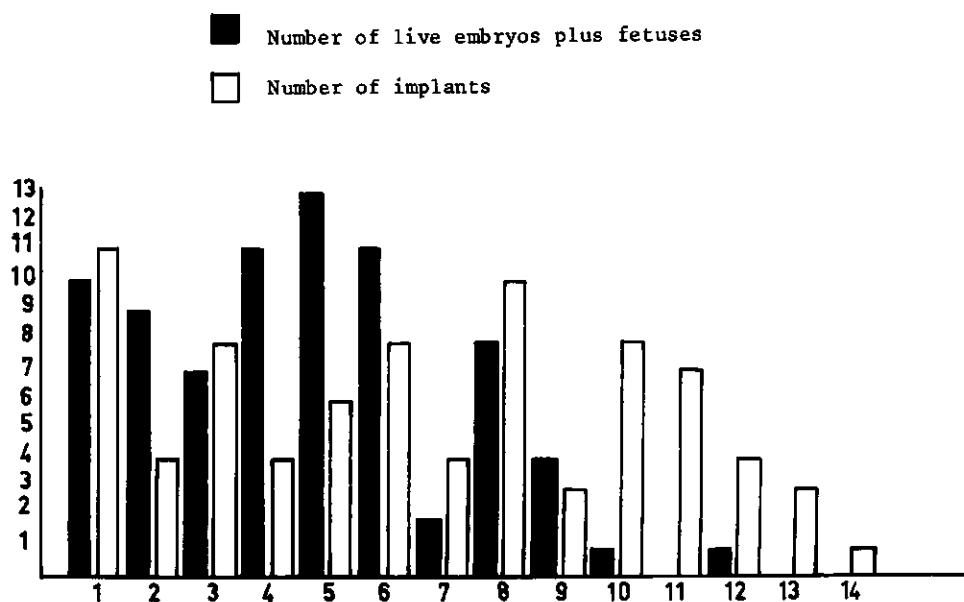
□ Number of implants

Fig. 7. Histogram showing the distribution of live embryos and fetuses
and total number of implants sired by tertiary trisomic males in normal
females. The data of two stages of pregnancy (day 12 and day 19) have been
treated together.

Fig. 5 (left upper). Diplotene spermatocyte showing a positive heteropycnotic
sex-bivalent and a positive heteropycnotic chromosome 1^{13} adjacent to the
proximal part of the X chromosome.

Fig. 6 (left lower). Mitotic metaphase of a male tertiary trisomic specimen
which has been C-banded. Arrow indicates the Y chromosome.

of 19 chromosomes plus chromosome 1^{13} may be exaggerated because of loss of a normal chromosome due to the technique.

Table IV. Gametic types found at metaphase II - anaphase II.

Chromosome number	20	$20+1^{13}$	$19+1^{13}$	21
Sex				
X	19	20	4	1
Y	24	25	8	3
No judgment	17	14	4	2
Total	60	59	16	6

The karyotypes of embryos and fetuses

The karyotypes encountered and their numbers are shown in table V. Of a total of 301 sexed embryos and fetuses, 155 (51.5%) appeared to be females and 146 (48.5%) males. The sex-ratio (number of males for every 100 females) is 87.8 for 11 days old embryos and 100 for 18 days old fetuses. The sex-ratio of 11 days old embryos does not differ significantly from a 1:1 ratio ($\chi^2_1 = 0.60$). The distribution of tertiary trisomic embryos and fetuses among the sexes appeared to be homogeneous ($\chi^2_1 = 0.37$).

We have observed a significant difference between 11 days old embryos and 18 days old fetuses in the frequency of tertiary trisomic specimens. ($\chi^2_1 = 4.014$; $P < 0.05$). Among the 11 days old embryos, the frequency of tertiary trisomics (excluding the primary trisomics) was 34.6% $\pm$ 5.4%. Among the fetuses this percentage was 46.3% + 6.3%. In agreement with this, the segregation of the T70H small marker chromosome is deviant from a 1:1 ratio in the 11 days old embryos ($\chi^2_1 = 12.31$; $P < 0.005$; N = 130). This in contrast to the situation at 18 days of prenatal age when the observed segregation does not disagree with a hypothetical 1:1 ratio ($\chi^2_1 = 0.9$; N = 160). An explanation of this apparent discrepancy will be put forward in the Discussion.

Table VI shows the numbers of embryos either normal or tertiary trisomic for each of the males used. There is a significant heterogeneity of tertiary trisomic embryos and fetuses between males ($\chi^2_4 = 21.45$; $P < 0.005$). This effect is mainly caused by the males 3 and 4 and the cause may be meiotic drive. The other five males show a rather good agreement.

One special category consists of specimens with 41 chromosomes but without the T70H small marker chromosome. Nine of them were recovered among 11 days old embryos and two among 18 days old fetuses. They seem to correspond with the

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Table V. Number of litters karyotyped, average litter size and the numbers for each karyotype found. Percentages are given for the pooled (11 plus 18 days) numbers.

Embryo age	Number of litters	Number of embryos	Average litter size	2n ♂	2n ♀	Tert. tri-somic ♂	Tert. tri-somic ♀	Prim. tri-somic ♂	Prim. tri-somic ♀
11 days	33	139	3.21	41	44	20	25	4	5
18 days	34	162	4.76	39	47	40	34	2	
Percent				26.5%	30.5%	19.8%	19.5%	2%	1.7%
				57%		39.3%		3.7%	

Table VI. Fertility scores for each male, the average number of sperm for each male and the numbers of normal and tertiary trisomic embryos/fetuses for both stages of pregnancy.

Male	1	2	3	4	5	6	7
Number of litters	14	14	10	13	9	10	12
Average number of implants (12 + 19 days)	8.36	4.31	2.60	7.62	5.22	8.00	8.00
Average number of live embryos and fetuses (12 + 19 days)	5.79	4.14	2.60	4.61	4.89	3.90	5.25
Sperm count x 10^6	1.45	0.95	0.47	1.17	0.52	1.59	1.34
Specimens with 2n chromosomes	30	25	7	31	24	18	36
Tert. trisomic specimens	21	13	18	11	19	12	25
Percent	41.2%	34.2%	72%	26.2%	44.2%	40%	41%

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class of secondary spermatocytes with 21 normal chromosomes (see table IV) and are considered to be primary trisomics. From the two primary trisomics found at 18 days, one had already died and the other was alive but much smaller than its litter mates. Two big moles, karyotyped at day 12 of gestation both appeared to be tertiary trisomics.

The frequency of morphologically recognizable tertiary trisomic young at weaning age (18 days pp) has been compared with the frequency of tertiary trisomic fetuses found at day 19 of gestation. For this purpose, the litters produced by second and third outcrosses to Swiss females of other tertiary trisomic males of our tertiary trisomic stock have been analyzed. Table VII gives the results. When we assume the percentage of morphologically recognizable tertiary trisomics to be 30.1% at the time of birth (table VII), there is still a significant difference (χ_1^2 = 12.15, P < 0.005) with the share of tertiary trisomic fetuses at 18 days of prenatal age (46.3%, N = 160).

Table VII. The percentages of morphologically recognizable tertiary trisomic young of 18 days of age out of matings between tertiary trisomic males and normal females.

	Young born alive	Young born dead	Young weaned	Tert. tri-somics	As a % of total number born*	As a % of total number weaned
Second outcross males (N = 5)	165	4	156	38	30.2%	24.4%
Third outcross males (N = 6)	147	2	135	32	29.9%	23.7%
Average					30.1%	24.1%

* The assumption has been made that young which were dead around the time of parturition or died before weaning age (18 days) were tertiary trisomics. Young with a balanced genotype seldom die before weaning, especially in small litters.

Fertility of tertiary trisomic males

Table VIII presents the data of matings between the same seven tertiary trisomic males and normal females. The actual numbers are too low for statistical tests but the data do not suggest gross differences between males with respect to their mating behavior as indicated by the production of vaginal plugs, to conception rate and to the percentage of females they got pregnant. The percentage of plugged females seems well within normal limits. Conception rate and the percentage of females actually pregnant under this mating scheme seem to be below

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Table VIII. Fertility records of matings between the 7 T70H tertiary trisomic males and normal females at two stages of pregnancy.

	12 days			19 days			significancies
	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.	
Number of females used	61			61			
Number of females plugged	54 (88.5%)			52 (85.2%)			$\chi^2_1 = 0.29$ n.s.
Number of pregnant plugged females	42			32			
Conception rate	77.8%			61.5%			$\chi^2_1 = 3.37$ n.s.
Percentage of females pregnant	73.8%			60.7%			$\chi^2_1 = 2.38$ n.s.
Average number of corpora lutea	45*	10.78	1.91	**			
Average number of implants	45*	5.93	3.45	37	7.30	4.00	W = 1.59 n.s.
Average number of live embryos and fetuses	41	4.44	2.41	36	4.94	2.75	W = 0.66 n.s.

* some females appeared to be pregnant in which no vaginal plug was detected.

** at this stage of pregnancy, corpora lutea are difficult to count.

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normal. No differences in the three criteria exist between 12 and 19 days in-
dicated by non-significant χ^2-values. The percentage of pregnant animals in
which no plug was detected amounts to 9.8% (N = 82). Altogether 7.4% (N = 106)
of the females got plugged more than once. In these cases, the first mating has
been taken as the successful one.

All data on litter size (total number of implants at 12, 19 and (12 plus
19) days of gestation) as well as the number of live embryos or fetuses at 12,
19 and (12 plus 19) days have been treated with the aim to test for the normal-
cy in distribution. All distributions were approximately symmetrical but
showed significant negative kurtosis, indicating that they are flat-topped. For
this reason, differences between 12 and 19 days concerning the total number of
implants and the total number of live young have been tested with the aid of the
Wilcoxon signed rank test. Both differences (W) appeared non-significant. Fig. 7
gives histograms of the pooled (12 plus 19 days) data of the total number of
implants and of the total number of live embryos and fetuses. The broad spectrum
of litter sizes encountered is also demonstrated by the large standard deviation
values for these characteristics (see table VIII).

The mean difference between the number of corpora lutea (N = 45) and the
numbers of implants and live embryos/fetuses is substantial. On the basis of the
mean number of corpora lutea as an indicator of the number of secondary oocytes
shed, the post-implantational losses (averaging 12 and 19 days) amount to 17.3%,
whereas the sum of unfertilized eggs and pre-implantational losses amounts to
39.2%. As control values, the results obtained with a fully comparable Swiss
random-bred (Cpb:SE(S)) stock have been taken (Schreuder, 1972); see table IX.

Table IX. Average number of implants and live embryos and fetuses at (12 + 19)
days of gestation and the partitioning of losses of eggs as a percen-
tage of the number of corpora lutea between those giving rise to a
decidual reaction and those which do not (N = number of females).

	Tert. trisomic ♂ x normal ♀			Control		
	N	x̄	s.d.	N	x̄	s.d.
Number of corpora lutea	45	10.78	1.91	15	9.13	1.23
Number of implants	82	6.55	3.75	41	8.55	1.92
Number of embryos or fetuses	77	4.68	2.56	41	7.71	2.06
Percentage of losses causing a decidual reaction		17.3%			9.2%	
Without causing a decidual reaction		39.2			6.4%	
Total		56.6%			15.6%	

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Fertility differences between males clearly exist (see table VI). The average sperm count for each male is also given in this table. We gained the impression that the share of abnormally shaped heads increases when the total number drops but we have not distinguished between these two categories. The actual differences in the number of sperm which are capable of fertilizing might well be more pronounced than expressed in our figures. The average count of sperm heads, normal and abnormal, amounts to 1.07×10^6. For Swiss random-bred males, this figure is $3.75 \pm 0.82 \times 10^6$ (N = 12). We attempted to study the relations between the number of sperm thus counted and the % of females plugged, the % of females used that got pregnant, the average number of implants at 12 plus 19 days and the average number of live embryos/fetuses at 12 plus 19 days. Of the Spearman rank correlation coefficients thus computed, a significant one (r_s = 0.9, P < 0.025) was obtained for the relation between the sperm count and the average (12 plus 19 days of gestation) number of implants.

Discussion

A discussion of the chromosome association pattern found at diakinesis - metaphase I is only worthwhile if the hypotonic treatment and subsequent fixation and air-drying of cells does not disrupt chromosome associations in general or preferentially. Possibilities to investigate this are limited because only one technique was used. Within this technique, not much variation in association was found due to the position of the cell in the preparation. The impression was, however, that the share of cells with a trivalent in class 3 of chromosome morphology was somewhat less. This effect is not so pronounced that it causes males with a high percentage of class 3 spermatocytes to have a low percentage of 19II+III configurations at diakinesis - metaphase I. In our view, the technique may cause an overestimate of 20II+I spermatocytes. An appraisal of this excess cannot be made. Remembering the absence of a negative correlation between class 3 spermatocytes and 19II+III spermatocytes, it must be small, however.

Other indications of crude handling of cells seem to be infrequent as well in this investigation. Beechey (1973) found a higher percentage X-Y dissociation in his controls (P = 0.1; N = 150), while 1.5% of his cells (N = 200) (ours 1.1%; N = 1009) possessed autosomal univalents.

Variation in the frequency of trivalents between males exists. To a small extent, this may be due to the technical procedure. The main part of the differences are believed to be related to intrinsic differences between males.

One striking observation is the low frequency of trivalents of type $(1;1;1^{13})$, (P = 0.073), among all trivalents classified. If one assumes randomness of pairing between homologous segments in zygotene-pachytene and an equal likelihood for the occurrence of a chiasma in every unit of chromosome length, one would expect a much higer frequency of $(1;1;1^{13})$ trivalents. This expectation is based on the length of the interchange segments as estimated by G-banding of chromosome 1^{13} (de Boer and van Gijsen, 1974), with the chromosome 1 part twice as long as the chromosome 13 part. The assumption has to be made that chromosome length estimated at mitotic prophase-prometaphase reflects chromosome length at the time of synapsis during meiotic prophase.

The preferential chiasma localization thus observed poses questions upon the role of centric heterochromatin in tying homologous chromosomes together (Yunis and Yasmineh, 1971). If one describes the function of the synaptonemal complex (SC) as greatly enhancing the chance of chiasma formation (Brown, 1972) and pairing proceeds in a "zipper-like" action from a certain point along the chromosome (Sybenga, 1966, Comings and Okada, 1970), one likes to know where the formation of the SC starts. Comings and Okada (1970) review evidence that pairing starts at the chromosome ends where the homologs are close to each other near to the nuclear membrane. This view is in agreement with the observation of Woollam et al. (1966) that, for the mouse, field vole and golden hamster, the number of attachment sites of the SC on the nuclear membrane coincides with that predicted on basis of the number of chromosome ends. For the mouse and field vole, the centric heterochromatin and centromere roughly coincide with one chromosome end. For the golden hamster with 17 metacentric chromosomes on a total of 21, chromosome ends are equivalent to telomeric regions and the latter apparently have a greater affinity to their homolog on the nuclear membrane than the centric regions. Both Henderson (1963) and Fox (1973) postulate a model of sequential chiasma formation with the first chiasma close to the telomere, on basis of diplotene analysis in *Schistocerca gregaria*. According to Henderson (1963), chiasma formation sometimes starts at both ends of the chromosome. For the acrocentric *Schistocerca gregaria* chromosomes, this means that centric heterochromatin as well serves a function in the initiation of meiotic pairing. There are also other observations, for instance Darlington's on *Fritilaria* and *Mecostethus* (see Sybenga, 1966), which show chiasmata in the region of the centromere only. Both Sybenga (1966) and Maguire (1972) however, lay more emphasis on the telomere as a point from which homolog pairing proceeds than the region of the centromere.

The mouse seems to fit this general preference for chiasmata to be formed from the telomeric regions on. This paper points towards a preference of telo-

meric meiotic pairing. Initiation of meiotic pairing at the centromeric ends
of mouse chromosomes is possible, however. This fact is indicated as well by
the numerous multivalents found in mouse reciprocal translations as in T70H
(Searle et al., 1971). Inferences concerning meiotic pairing initiation can
only be drawn from the study of chiasmata when meiotic pairing and the occur-
rence of a chiasma are interdependent. It is known that the chiasma frequency
is reduced in heterochromatic regions. If centric meiotic pairing more often
fails to produce a chiasma than telomeric meiotic pairing, this category is
underestimated by the study of chiasma(ta).

Centric heterochromatin can play a role in less intimate pairing between
homologs(Yunis and Yasmineh, 1971). Telomeres seem to play such roles as well,
especially in flowering plants, as stated by Brown (1972). The high frequency
of cells with the T70H small translocation product in close proximity of the
centric heterochromatin of the X chromosome in one tertiary trisomic male re-
ported in this investigation may well be an example of non-homologous attraction
caused by centric heterochromatin. Because of the difficulty of believing this
phenomenon to be an artefact in this particular male, its absence in the five
full brothers studied in more detail is somewhat surprising. It is quite likely
that the air-dry technique is not suitable for the appraisal of non-homologous
association during diakinesis - metaphase I. Anyway, no signs have been found
of coorientation between the X chromosome and chromosome 1^{13}, neither in the
secondary spermatocytes, nor in the embryos and fetuses. One would expect this
if the non-homologous association represents something like distributive pairing
(Grell, 1971). Earlier attempts (Cattanach, 1967) have also failed to demonstrate
this phenomenon in the mouse.

The production of gametes with 19 chromosomes plus chromosome 1^{13}, and those
with 21 "normal" chromosomes by the tertiary trisomic males probably results
partly from non-disjunction for chromosome 13 in a $(13;13;1^{13})$ trivalent. This
observation, the production of primary trisomics by tertiary trisomics has been
known for a long time in plant cytogenetics (see Burnham, 1962). A connecting
finding presented here is a percentage of 6.5% (N = 139) primary trisomics among
11 days old embryos. If these are also caused by non-disjunction for chromo-
some 13 in a $(13;13;1^{13})$ trivalent, this figure corresponds with a frequency of
13% for primary spermatocytes exhibiting this phenomenon, whereas the metaphase II
observations lead to an estimate of appr. 9%. The percentage of primary sperma-
tocytes with a trivalent approaches 22% and the majority (92.7%) of trivalents
are of the $(13;13;1^{13})$ chromosomal make-up. If all the primary trisomics are due
to non-disjunction for the chromosome 13, then the fraction of spermatocytes with

this type of trivalent exhibiting non-disjunction for chromosome 13 amounts to 50% or more. If the behavior of the centromeres, present in this type of trivalent, is random with respect to orientation at anaphase I - telophase I, one would expect 33% of chromosome 13 non-disjunction. Although the actual numbers of primary disomic secondary spermatocytes and primary trisomic embryos found are low, the conclusion seems to be warranted that the association of chromosome 1^{13} on to a (13;13) bivalent causes a considerable non-disjunction for chromosome 13.

The encounter of heteropycnotic behavior of the extra chromosome 1^{13} in diplotene of the first meiotic division in combination with indications of heteropycnosis in somatic cells in division suggest a relation between genetic inactiviation of the extra element and the variety of phenotypes found in T70H tertiary trisomics (de Boer, 1973). It will be interesting to check if there exists a correlation between the phenotype of the T70H tertiary trisomics and the condition of its extra chromosome during prophase.

Although mating behavior of T70H tertiary trisomic males as judged by the percentage of females they plug within one week after caging appears normal, other fertility characteristics show clearly that they are inferior to males with a normal karyotype. The percentage of females actually pregnant at 19 days (60.7%) is below the control figure given by Bakker (1974) (92.2%, N = 64) and obtained for Swiss random-bred mice of the same origin. When the discrepancy between the number of corpora lutea as an indication for the number of secondary oocytes shed and the number of embryos and fetuses is partitioned in losses causing a decidual reaction and losses which do not, the tertiary trisomic sired litters show a preponderance of the latter category. This picture resembles the one found by Léonard et al. (1971) for 8 inbred strains of mice. Under the same mating scheme as used here, these strains also showed a low percentage of pregnant females. The results of our control stock, losses accompanied by a decidual reaction exceed those which are not, are in line with results obtained in an outbred stock of rats (Harper, 1964) and with Bateman's (1966) H(igh) fertility line (selected on an outbred basis). We blame this difference to the variable but low production of spermatozoa of the tertiary trisomic males, causing a high percentage of unfertilized eggs. The high fraction of abnormally shaped sperm heads, we got the impression of, will intensify this effect.

Searle and Beechey (1974) found that when the count of spermatozoa drops below 10% of normal (after a dose of 200 rads acute X-irradiation) a reduced fertility is likely to occur. We observe this reduction much earlier. In fact, none of the males exhibits a normal pattern of fertility (see table VI).

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Surprising is the fact that we found a significant lower share of tertiary trisomic embryos of 11 days old (34.6%) than of 18 days old fetuses (46.3%). In combination with this, the average number of implants and live fetuses at day 19 of gestation were somewhat higher than at day 12 of gestation while the percentage of pregnant females was lower. We do not assume that these results conflict with the observed 1:1 segregation between normal spermatozoa and spermatozoa with the T70H small marker chromosome as the extra element observed in second metaphases. A phenomenon like certation is highly unlikely in the mouse (and mammals in general) as well (Ford, 1972). The explanation might be the following; the data indicate that small litters have a higher chance of getting lost during pregnancy. Causes for small litters from tertiary trisomic males are, besides the low production of functional spermatozoa, embryonic and fetal death due to the occurrence of primary trisomic embryos for chromosome 13, deficiency-duplication embryos with 40 chromosomes, including the T70H small translocation product and tertiary trisomic embryos which fail to survive. When death of embryos of the latter category before day 12 of gestation endangers the survival of the whole litter up to day 19 of gestation, the fraction of tertiary trisomic embryos of 11 days of age will be smaller than the fraction of tertiary trisomic fetuses of 18 days of age. This explanation is supported by the finding of two tertiary trisomic big moles of 11 days of age. We conclude that in the genetic background concerned, the tertiary trisomic karyotype shows a considerable variation in viability. Some probably die before day 12 of gestation, some between the day of birth and weaning age and some in the weeks of rapid growth thereafter. Table VII indicates that part of them are morphologically normal and escape detection at weaning age, which we have found earlier (de Boer, 1973). The fact that one chromosomal constitution i.e. the T70H tertiary trisomic is represented by a range of phenotypes with a varying potential of survival is not at all new. Some fetuses with G(21) trisomy and to a lesser extent trisomy for group D(13) and E(18) in man are known to survive to birth, but most of the fertilized eggs of these chromosomal constitutions give rise to abortions before the 120th day of post-menstrual age, however (Carr, 1972). The same holds true for sex-chromosome abnormalities in man (Jacobs, 1972).

Acknowledgements

References

Bakker, H.: Pers. communication, 1974.

Bateman, N.: Ovulation and post-ovulational losses in strains of mice selected from large and small litters. Genet. Res., Camb. 8: 229-241 (1966).

Beechey, C.V.: X-Y chromosome dissociation and sterility in the mouse. Cytogenet. Cell Genet. 12: 60-67 (1973).

Boer, P. de: Fertile tertiary trisomy in the mouse (*Mus musculus*). Cytogenet. Cell Genet. 12: 435-442 (1973).

Boer, P. de and Gijsen, M. van: The location of the positions of the break-points involved in the T26H and T70H mouse translocations with the aid of Giemsa-banding. Can. J. Genet. Cytol. In press.

Brown, W.V.: Textbook of cytogenetics. (The C.V. Mosby Company, Saint Louis, 1972).

Burnham, C.R.: Discussions in cytogenetics (Burgess Publishing Company, Minneapolis, Minnesota, 1962).

Carr, D.H.: Chromosomal anomalies in human fetuses. Res. Repr. 4: 3-4 (1972).

Cattanach, B.M.: A test of distributive pairing between two specific non-homologous chromosomes in the mouse. Cytogenetics 6: 67-77 (1967).

Comings, D.E. and Okada T.A.: Mechanism of chromosome pairing during meiosis. Nature 227: 451-456 (1970).

Evans, E.P.; Breckon, C. and Ford, C.E.: An air-drying method for meiotic preparations from mammalian testes. Cytogenetics, 3: 289-294 (1964).

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

Falconer, D.S.; Edwards, R.G.; Fowler, R.E. and Roberts, R.C.: Analysis of differences in the numbers of eggs shed by the two ovaries of mice during natural oestrus or after super-ovulation. J. Reprod. Fert. 2: 418-438 (1961).

Ford, C.E.: The murine Y-chromosome as a marker. Transplantation 4: 333-334 (1966).

Ford, C.E.: Gross genome unbalance in mouse spermatozoa: does it influence the capacity to fertilize? (Proceedings of an International Symposium, University of Edinburgh, Scotland, 1971. The Organizers, Edinburgh, New York, 1972).

Fox, D.P.: The control of chiasma distribution in the locust, *Schistocerca gregaria* (Forskål). Chromosoma 43: 289-328 (1973).

Grell, R.F.: Distributive pairing in man? Ann. Génét. 14: 165-171 (1971).

Harper, M.J.K.: Observations on amount and distribution of prenatal mortality in a strain of albino rats. J. Reprod. Fert. 7: 185-209 (1964).

Henderson, S.A.: Chiasma distribution at diplotene in a locust. Heredity 18: 173-190 (1963).

Hsu, T.C.; Cooper, J.E.K.; Mace, M.L. Jr. and Brinkley, B.R.: Arrangement of centromeres in mouse cells. Chromosoma 34: 73-87 (1971).

Jacobs, P.A.: Human population cytogenetics (Human genetics, J. de Grouchy, F.J.G. Ebling, I.W. Henderson, Excerpta Medica, Amsterdam, 1972).

Léonard, A.; Deknudt, Gh. and Linden, G.: Ovulation and prenatal losses in different strains of mice. Exp. Anim. 4: 1-6 (1971).

Lyon, M.F. and Meredith, R.: Autosomal translocations causing male sterility and viable aneuploidy in the mouse. Cytogenetics 5: 335-354 (1966).

Maguire, M.P.: Role of heterochromatin in homologous chromosome pairing: Evaluation of evidence. Science 176: 543-544 (1972).

Polani, P.E.: Centromere localization at meiosis and the position of chiasmata in the male and female mouse. Chromosoma 36: 343-374 (1972).

Schreuder, I.: Een vergelijking van pré- en post-implantoire verliezen in op vruchtbaarheid geselecteerde muizen en een controle populatie. Scriptie Veeteelt, Wageningen 1972.

Searle, A.G. and Beechey, C.V.: Sperm-count, egg-fertilization and dominant lethality after X-irradiation of mice. Mutation Res. 22: 63-72 (1974).

Searle, A.G.; Ford, C.E. and Beechey, C.V.: Meiotic disjunction in mouse translocations and the determination of centromere position. Genet. Res., Camb. 18: 215-235 (1971).

Sybenga, J.: The zygomere as hypothetical unit of chromosome pairing initiation. Genetica 37: 186-198 (1966).

Sybenga, J.: Pers. communication (1973).

Sumner, A.T.; Evans, H.J. and Buckland, R.: New technique for distinguishing between human chromosomes. Nature New Biology 232: 31-32 (1971).

Woollam, D.H.M.; Ford, E.H.R. and Millen, J.W.: The attachment of pachytene chromosomes to the nuclear membrane in mammalian spermatocytes. Exptl. Cell Res. 42: 657-661 (1966).

Yunis, J.J. and Yasmineh, W.G.: Heterochromatin, satellite DNA and cell function. Science 174: 1200-1209 (1971).

The location of the positions of the breakpoints involved in the T26H and T70H mouse translocations with the aid of Giemsa-banding*

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The positions of the breakpoints involved in the T(2;8)26H and T(1;13)70H mouse translocations have been located to specific minor bands using a trypsin-Giemsa banding method and a nomenclature system for band patterns as developed by Nesbitt and Francke (1973). The breakpoint positions are 2H1 and 8A4 for T26H and 1A4 and 13D1 for T70H. The interstitial segments occupy 80.9% of chromosome 2, 30.1% of chromosome 8, 14.4% of chromosome 1 and 88.0% of chromosome 13. It is concluded that the variation of the location of the breakpoint positions is mainly caused by differential chromosome contraction and measuring errors and only to a small extent by the resolving power of the G-banding technique.

Introduction

The analysis of the meiotic behavior of translocation heterozygotes and derivates like tertiary trisomics and translocation trisomics is greatly facilitated by a precise knowledge of the length of the translocated or interchanged segments and of the interstitial segments. This enables one to relate the frequency of the occurrence of chiasmata in a known translocated or interstitial segment with its physical length during prometaphase-metaphase.

The many mutated loci known in the mouse permit the location of translocation breakpoints relative to the marker genes on the linkage map. This approach can be considered to be the first approach of translocation breakpoint location and it provides estimates of the genetic length of the translocated and interstitial segments. The linkage map cannot be related directly to the physical chromosome, and in addition often does not give accurate information for the following reasons, (1) the non-availability of recombination percentages between the telomere and the most distal marker, (2) difficulties in obtaining an unbiased recombination percentage between the most proximal marker and the centromere (Cattanach and Moseley, 1973), and (3) lack of markers in certain parts of the genome and a reduced number of chiasmata in heterochromatic regions.

A second approach uses techniques to differentiate between varying segments

of prometaphase-metaphase chromosomes of which Q- and G-banding methods are the most widely used. They allow estimation of the physical prometaphase-metaphase length. However, they have their limits too. We have estimated the physical length at mitosis of the translocated and interstitial segments for two mouse translocations, T(2;8)26H and T(1;13)70H, using G-banding. Nesbitt and Francke (1973) have inferred the positions of the breakpoints involved in these translocations from G-banding results. The G-banding method should yield more accurate information about the length of the translocated and interstitial segments, however. For cytogenetical information concerning these translocations, the reader is referred to Searle et al. (1971).

Materials and methods

The heterozygous translocation carriers used for bleeding and for siring embryos in this investigation originate from the Harwell T26H/T26H and T70H/T70H strains and were in the fourth outcross to a Swiss random-bred stock (Cpb: SE(S)).

Suitable prometaphase-metaphase spreads were obtained from two sources. Peripheral leucocytes, obtained by a tail cut, were grown according to the method of Bryan and Hybertson (1972) and harvested in the usual way. There was no abundant cell proliferation but the quality of the spreads was satisfactory for banding. Embryonic livers were processed according to the method given by Evans et al. (1972). The embryos were 14-15 days old and were from matings between T/+ males and +/+ females. KCl was used as a hypotonic. Preparations were made on slides heated on a hot plate. The temperature of the hot plate ranged from 44-51°C. Embryonic livers with elongated chromosomes and the chromatids adjacent to each other are regularly although not always obtained. The chromosomes were banded within one week after the preparation of the slides. The trypsin-Giemsa banding method by Wurster (1972) was used throughout. Using a Zeiss photomicroscope, five suitable cells for each translocation were slected and photographed on Agfa duplo ortho film and developed in Kodak D76 finegrain developer. Prints were made, giving a final magnification of appr. 3600 X.

The positions of the breakpoints are expressed in terms of the nomenclature for mouse-bands as proposed by Nesbitt and Francke (1973). Measurements have been taken from the photographs using a vernier. The chromosomes of male cells were measured. The total length of all chromosomes of one diploid cell (including the X- and Y-chromosomes) has been equated to 200%. The length of segments and normal and translocation chromosomes are thus expressed as a percentage of an "average" haploid set of chromosomes. The segments have also been expressed as

a percentage of the normal chromosomes they belong to. The following symbols for segments are used in this investigation: i means interstitial segment and t translocated segment. N indicates position in a normal chromosome, T in a translocation chromosome. These symbols are used in combination with the number of the chromosome the segment originates from. Thus, $1_{i,N}$ means the interstitial segment of chromosome 1 situated in the normal chromosome and $13_{t,T}$ means the translocated segment of chromosome 13, situated in the translocation chromosome. Chromosome 13 consists of $13_{i,N}$ and $13_{t,N}$. The translocation chromosomes are denoted as 1^{13} and 13^1 for T70H and 2^8 and 8^2 for T26H. Chromosome 2^8 is composed of $2_{i,T}$ and $8_{t,T}$. Generally speaking a chromosome or segment will be represented by the symbol a. The average length of the normal chromosomes involved in the translocations has been calculated using the total length of the segments present in the normal and in the translocation chromosomes.

Results

The banding patterns obtained (see fig. 1) follow those schematisized by Nesbitt and Francke (1973) although not all their bands were visible in every chromosome of any cell. Chromosome length has been assumed to show a distribution which is deviant from normal, because of the contraction process, and the fact that the use of colchicine leads to an accumulation of artificially contracted chromosomes. The interstitial (i) and translocated (t) segments have been measured in the normal (N) and translocation (T) chromosomes both. Differences between any segment $a_{i,t}$, measured in the normal and translocation chromosome proved to be non-significant using the Wilcoxon signed rank test. Therefore the averages for the segments a_i and a_t are based on measurements in the normal and translocation chromosomes both. Table I gives the results for T26H and T70H.

The position of the breakpoints in terms of the nomenclature system proposed by Nesbitt and Francke (1973) are 1A4 and 13D1 for T70H and 2H1 and 8A4 for T26H. All four minor bands are Giemsa-negative regions.

The variance of the length of a segment, interstitial or translocated, can be separated into two components. One due to the resolving power of the technique (the amount of differentiation the bands produce along a chromosome) and the other the sum of the effects of differential chromosome contraction and measuring errors. Theoretically, when both breakpoints of a translocation are situated either in a Giemsa-positive or -negative band, the variance component intrinsic to the resolving power of the banding technique can be calculated from the width of the smallest band involved in the position of the breakpoint. It does not matter

Table I. The relative length of the chromosomes 2, 8, 1 and 13 and the inter-
stitial and translocated segments as present in T26H and T70H prometa-
phase-metaphase cells.

T26H

chromosome or segment	2	8	2_i	2_t	8_i	8_t
% of total haploid set	6.37%	4.59%	5.16%	1.22%	1.38%	3.21%
% of the chromosome from which the segment originates			80.9%	19.1%	30.1%	69.9%

T70H

chromosome or segment	1	13	1_i	1_t	13_i	13_t
% of total haploid set	7.64%	4.62%	1.10%	6.55%	4.07%	0.55%
% of the chromosome from which the segment originates			14.4%	85.6%	88.0%	12.0%

Table II. Variance of breakpoint position of T26H and T70H due to the resolving
power of the technique and due to differential chromosome condensation
and measuring errors. The variance and standard deviation are expressed
in percentages of the length of the chromosome in which the breakpoint
is situated. The positions of the breakpoints are of course coincident
with the distal ends of the interstitial segments.

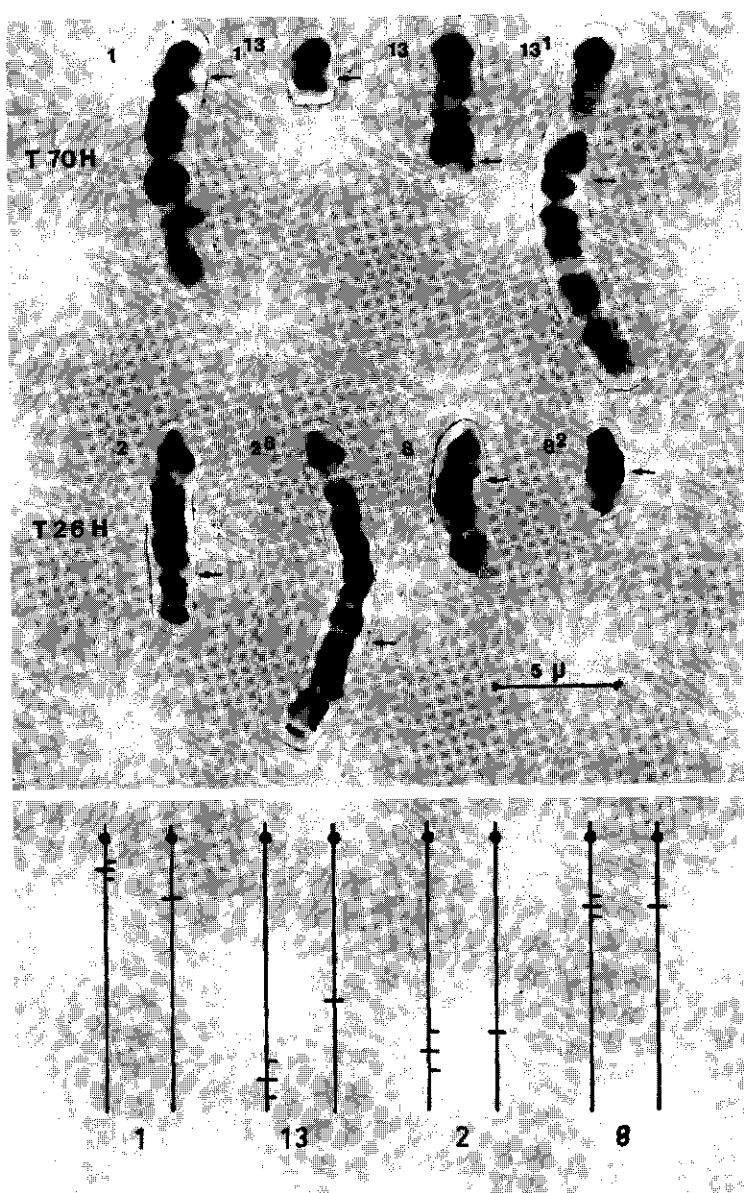| source	chromosome segments and numbers measured			
	2_i 10	8_i 10	1_i 10	13_i 10
variance due to the width of the smallest interband	2.16%	3.00%	0.57%	1.34%
variance due to differential chromosome contraction	36.18%	8.90%	4.71%	43.62%
total variance	38.34%	11.90%	5.28%	44.96%
total standard deviation	6.19%	3.45%	2.30%	6.71%

Fig. 1. Upper. Breakpoint location (arrows) in G-banded chromosomes involved in T26H and T70H.

Lower. Schematical representation of the total standard deviation (see table II) and breakpoint location (left chromosomes) compared with the breakpoint positions according to Searle and Beechey (1973) (right chromosomes).

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whether this band is an original band or one caused by the translocation. This
is because of the fact that, when one interstitial or translocated segment is
known, the others can be found by subtraction. Within the smallest Giemsa-nega-
tive band, as it occurs in T26H and T70H, the real breakpoint is unknown and
shows an uniform distribution along the width of this particular band. The va-
riance of this type of distribution can be described with the formula $\frac{w^2}{12}$. The
width of the band is represented by w. For T70H the smallest band involved in
the translocation is band 1A4 and for T26H it can be concluded to be 2H1 although
the composite band in chromosome 8^2 is quite small as well (see fig. 1). The
width of these two bands have been taken from Nesbitt and Francke (1973). Table
II gives the variances due to either source. Fig. 1 gives the breakpoints in the
banded normal and translocation chromosome.

Discussion

The resolving power of the G-banding technique is very good. The contribution
to the total variance in breakpoint location of the width of the smallest minor
band is minimal. In terms of minor bands, breakpoint positions can be accurately
determined.

The question is how the length measurements of the interstitial and trans-
located segments as measured in mitotic prometaphase-metaphase cells relate to
the length of these segments in pachytene primary oocytes and spermatocytes.
As table II shows, variation due to differential chromosome contraction is con-
siderable, even when the chromosomes are corrected on the basis of the total
length of all the chromosomes in a diploid cell. This phenomenon is well known
in the mouse. Considerable differences in length can occur between homologs
within a cell (Francke and Nesbitt, 1971) and the ranking on the basis of length
of a certain chromosome is by no means fixed (Buckland et al., 1971, Francke and
Nesbitt, 1971). It is not clear if individual chromosomes have individual constant
chromosome condensation patterns nor if the relative size reduction from pachytene
chromosomes to mitotic prometaphase-metaphase chromosomes is constant for all
chromosomes and segments. Although our material is limited (n = 10) we have com-
puted rank correlation coefficients (Spearman) between the relative contributions
of chromosomes 1 and 2 and the absolute length of all chromosomes in the diploid
cell. This correlation (r_s) amounts to 0.50 (0.05 < P < 0.10) for chromosome 1
and 0.14 (n.s.) for chromosome 2. It is quite possible that among the elongated
chromosomes selected for the analysis of G-bands, there are some with an exagger-
ated relative length due to a reduced rate of contraction or due to stretching by

preparation. There exists no information about the relation between the length of mouse chromosomes at pachytene of the first meiotic division and their length in a mitotic colchicine-metaphase as far as we know. The description of pachytene chromosome length as a linear function of prometaphase-metaphase length with a constant factor for all chromosomes is only an approximation. Especially for short chromosome segments containing centric heterochromatin the relation between chiasma frequency and physicical "pachytene" length may be specific (de Boer and Groen, 1974).

Another question is whether the two parts of a translocation chromosome do influence each other's spiralization behavior as has been shown to occur in X-autosome translocations (Eicher, 1970). However, comparisons between segments $a_{(i,t),N}$ and segments $a_{(i,t),T}$ never showed a significant difference. These comparisons only involved the segments of five cells.

All four breakpoints of T26H and T70H are situated in Giemsa-negative regions. This is in agreement with the observations of Seabright (1973) who irradiated human lymphocytes at G_1. Findings of San Roman and Bobrow (1973), who, using the same cell source as Seabright (1973), had the impression that breaks occur predominantly in quinacrine-dull regions point in this direction as well.

Work on the location of translocation breakpoints has until now in the mouse mainly been done with the aid of Q-banding. As Nesbitt and Francke (1973) point out G-banding should permit a finer breakpoint location. They summarize the positions of the breakpoints for T26H and T70H as inferred from Q-banded cells published by O.J. Miller et al. (1971) and D.A. Miller et al. (1971). There is always agreement between one of their proposals and our findings except in the case of the T70H breakpoint in chromosome 13 which we think is in D1 and not in D2. Comparisons of our breakpoint estimates with those most recently given by Searle and Beechey (1973) and based on chromosome length drawn to scale and on linkage between the translocation chromosomes and mutant alleles (assuming that the overall genetic length of the mouse genome is 1250cM) shows that the agreement is rather good. The major discrepancy is the position of the T70H breakpoint in chromosome 13 which should be more distal than suggested by Searle and Beechey (1973). Fig. 1 compares their breakpoint estimates and ours.

References

Boer, P. de and Groen, A. 1974. Fertility and meiotic behavior of male T70H tertiary trisomics of the mouse (*Mus musculus*). A case of preferential telomeric meiotic pairing in a mammal. Cytogenet. Cell Genet. In press.

Buckland, R.A., Evans, H.J. and Sumner, A.T. 1971. Identifying mouse chromosomes with the ASG technique. Exptl. Cell Res. 69: 231-236.

Bryan, J.H.D. and Hybertson, R.L. 1972. The in vitro stimulation of lymphocytes from peripheral blood and lymph nodes of the laboratory mouse. Cytogenetics 11: 25-34.

Cattanach, B.M. and Moseley, H. 1973. Nondisjunction and reduced fertility caused by the tobacco mouse metacentric chromosomes. Cytogenet. Cell Genet. 12: 264-287.

Eicher, E.M. 1970. X-autosome translocations in the mouse: total inactivation versus partial inactivation of the X chromosome. Adv. Genet. 15: 176-259.

Evans, E.P., Burtenshaw, M.D. and Ford, C.E. 1972. Chromosomes of mouse embryos and newborn young: preparations from membranes and tail tips. Stain Techn. 47: 229-234.

Francke, U. and Nesbitt, M. 1971. Identification of the mouse chromosomes by quinacrine mustard staining. Cytogenetics 10: 356-366.

Miller, O.J., Millder, D.A., Kouri, R.E., Dev, V.G., Grewal, M.S. and Hutton, J.J. 1971. Assignment of linkage groups VIII and X to chromosomes in *Mus musculus* and identification of the centromeric end of linkage group I. Cytogenetics 10: 452-464.

Miller, D.A., Kouri, R.E., Dev. V.G., Grewal, M.S., Hutton, J.J. and Miller, O.J. 1971. Assignment of four linkage groups to chromosomes in *Mus musculus* and a cytogenetic method for locating their centromeric end. Proc. Nat. Acad. Sci. U.S. 68: 2699-2702.

Nesbitt, M.N. and Francke, U. 1973. A system of nomenclature for band patterns of mouse chromosomes. Chromosoma 41: 145-158.

San Roman, C. and Bobrow, M. 1973. The sites of radiation induced breakage in human lymphocyte chromosomes, determined by quinacrine fluorescence. Mutation Res. 18: 325-331.

Seabright, M. 1973. High resolution studies on the pattern of induced exchanges
in the human karyotype. Chromosoma 40: 333-346.

Searle, A.G. and Beechey, C.V. 1973. Map of structural changes affecting the
standard karyotype. Mouse News Letter 49: 18.

Searle, A.G., Ford, C.E. and Beechey, C.V. 1971. Meiotic disjunction in mouse
translocations and the determination of centromere position. Genet. Res. 18:
215-235.

Wurster, D.H. 1972. Mouse chromosomes identified by trypsin-Giemsa (T-G) banding.
Cytogenetics 11: 379-387.

Male meiotic behavior and litter size of the T(2;8)26H and T(1;13)70H mouse reciprocal translocations*

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Abstract

Two reciprocal mouse translocations T(2;8)26H and T(1;13)70H, heterozygous in a Swiss random-bred background, show differences with respect to the spectrum of multivalent configurations formed in primary spermatocytes and with respect to the segregational behavior of these multivalent configurations. The most numerous diakinesis – metaphase I configurations found in T26H/+ were RIV (53.1%) and $CIV8_i$ (36.8%). In T70H/+, $CIV1_i$ was found most frequently (60.6%) with $CIII+I(1_i+13_t)$ as the second frequent configuration (33.8%). The adjacent II frequency was estimated from metaphase II observations. Adjacent II segregation was almost absent in T26H/+ (4.25%) but it was relatively common in T70H/+ (22.4%). Although there was heterogeneity among males with respect to the multivalent configuration frequency, this heterogeneity was absent in the segregation products observed in the secondary spermatocytes. The hypothesis is advanced that time differences in chiasma terminalization during metaphase I – anaphase I are important for explaining the difference in segregation observed between the two translocations.

It is concluded that the frequency of numerical non-disjunction must be very low in T26H/+ and around 4% with a maximum estimate of about 9% in T70H/+.

The summed frequency of adjacent II disjunction and numerical non-disjunction can be estimated from the relative fertility scores of T/+ males versus +/+ males as well. These estimates, with relative litter size as the criterion, agree satisfactorily with the estimates made on the basis of cytological observations. They amount to 5% for T26H/+ and 22.4% for T70H/+. Chiasma frequencies were much higher in telomeric segments than in centric heterochromatin containing proximal segments. It is suggested that the centric heterochromatin exerts an influence on the adjacent chromosome segments with respect to chiasma formation.

Introduction

Translocation heterozygosity is a widespread phenomenon among plants, animals and humans. It occurs both spontaneously and can be induced by mutagenic chemicals and ionizing radiations. Plant and insect cytogeneticists have devoted

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* submitted for publication

attention to the meiotic behavior and consequences of reciprocal translocations for many years. The generally used squash technique however, is not very suitable for the study of mammalian meiosis. A convenient air-dry technique was not available before 1964 (Evans et al., 1964). This technique offers the advantage of producing numerous well-spread primary and secondary spermatocytes and the quantitative meiotic study of males, heterozygous for a reciprocal translocation, became a possibility.

The induction and use of mouse reciprocal translocations between acrocentric chromosomes has been a side-branch of mutation studies involving ionizing radiations. The earlier history of the radio-induction of mouse reciprocal translocations is given by Snell (1946) and Carter et al. (1955). Apart from earlier unpublished work (Ford et al.), the first study which attempted to relate the relative fertility of translocation heterozygotes to the behavior of multivalent configurations was by Searle et al. (1971). The present study might be regarded as a continuation, because of the three translocations studied by them, two, 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 (McClintock, 1945).
a) Alternate   : alternate centromeres move to the same pole.
b) Adjacent I   : adjacent situated but non-homologous centromeres move to the same pole.
c) Adjacent II : adjacent situated 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 become equivalent with respect to the genetic material attached to them (see figs. 1 and 2). It can be understood that the relative frequency of balanced (with either the two translocation chromosomes or the two normal chromosomes) gametes 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 (with deficiencies and duplications, either single or combined) gametes. 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 $\frac{1-p}{2}$ (Searle et al., 1971). This
can be a description of a relation between the fertility of matings between trans-
location heterozygotes and normals versus normals times normals and the summed
frequency of adjacent II segregation and numerical non-disjunction (see the re-
sults).

Observations on the characteristics of translocations between acrocentric
chromosomes have been made earlier (for instance Kayano and Nakamura, 1960;
Sarkar, 1955 and Sannomiya, 1968) but not on such an extensive scale as reported
here. This type of translocation does occur among human acrocentric D- and G-
group chromosomes and these cases have been reported as well (see Hamerton, 1971).

It is worthy to consider to what extent knowledge of mouse reciprocal
translocations could add to the understanding of human reciprocal translocations.
The spontaneous mutation frequency of reciprocal translocations is probably un-
derestimated by the figure of 0.18% given by Jacobs (1972). Thus, reciprocal
translocations are quite frequent in man. Most of them are exchanges between
metacentric chromosomes and meta- and acrocentric chromosomes, however. So far,
the comparison of mouse and human reciprocal translocations is not very useful.
One has to remember as well that each translocation is unique and able to
express unique properties. Knowledge about the behavior of univalents and about
the chiasma frequency in certain chromosome segments as gained in the present
study might be more apt to extrapolation. It is hoped that the findings presented
here parallel those occasionally gathered in human meiotic studies.

Materials and methods

Translocation heterozygous females of T(2;8)26H and T(1;13)70H origin were
obtained through the courtesy of Dr. A.G. Searle. Heterozygotes of both translo-
cations were obtained by outcrossing homozygous T/T animals to +/+ ones. Because
of the fact that the two homozygous translocation strains differed with respect
to their genetic background, the T/+ females have been crossed out to a third
(+/+) stock. For this purpose, a Swiss random-bred stock (Cpb:SE(S)), known for
its selection-potential (Bakker, 1974) has been chosen. This stock is kept in a
breeding-nucleus of 26 pairs and propagated according to a system of minimal in-
breeding, described by Falconer (1967) and shown in table I. One characteristic
of this system is that one descendant of a family keeps the family number. This
supplies the experimentator with a framework for combining mutations with the
Swiss genetic background in a regular manner. The scheme, used for outcrossing
the translocation heterozygotes is also shown in table I. The aim is to super-

Table I. A regular mating system with minimal inbreeding as devised by Falconer (1967) left and the outcrossing scheme for T/+ mice used here (right). Family numbers are indicated by n for outbred Swiss mice and by n^* for translocation heterozygotes.

generation number	Swiss ♂ x Swiss ♀		T/+♂ x Swiss ♀	
	x	x-1	x	x-1
1	n = n x n+1		$n^* = n^*$ x n	
2	n = n x n+2		$n^* = n^*$ x n+1	
3	n = n x n+3		$n^* = n^*$ x n+2	
etc.				

impose the translocation cytological marker chromosomes upon the variation of genotypes found in the Swiss random-bred stock. The originally obtained T/+ females (partially full-sibs) were each given a family number and entered into the outcrossing scheme given in table I. After the first generation, the number of families has been fixed at between 15-20 for each translocation. The translocation chromosomes were usually passed on to the next generation by the males. In the type of cross employed (T/+ x +/+), the offspring segregates for the translocation in heterozygous condition. The classification of male descendants was based on the criteria of Carter et al. (1955), taking a minimal number of dead implantations, presumably caused by unbalanced and/or aneuploid translocation products as indicative for the T/+ genotype of the male tested. The +/+ females used for testing purposes mainly originated from a Swiss (Cpb:SE(S)) line selected for high litter size ($\bar{x}$ = 12.31, Schreuder, 1972). This was done in order to minimize the number of inconclusive tests. T/+ animals only entered an experiment when outcrossed for at least 5 generations. On an average appr. 97% of the genetic material will be then of Swiss-origin. The translocation chromosomes constitute a deviation from this rule. In fact, the DNA-stretches adjacent to the breakpoints will never, or with a very low probability get involved in genetic recombination.

Meiotic preparations of primary and secondary spermatocytes were made according to the Evans-technique (Evans et al., 1964). Five males of each translocation were processed. No more than one male per family was chosen. From each male a number of appr. 200 primary spermatocytes and around 100 secondary spermatocytes were scored. The number of analyzable secondary spermatocytes is always less than the number of analyzable primary spermatocytes. To some degree, this can be

explained by the fact that secondary spermatocytes, especially the ones with long and elongated chromosomes, yield a lower percentage of analyzable cells. We got the impression however, that in T70H/+ males the ratio of secondary to primary spermatocytes was lower throughout than in T26H/+ males. Constitutive centric heterochromatin was preferentially stained with a dry-heat Giemsa technique described earlier (de Boer and Groen, 1974) and in some cases the BSG-technique of Sumner (1972), performed according to Chandley and Fletcher (1973) was used. The T70H/+ males were of various ages but not older than 11 months when processed. All T26H/+ males but one which was appr. 3 months old were between 27 and 31 days at the moment of autopsy. This age group yielded consistently better preparations in this translocation.

Diakinesis - metaphase I's were grouped into three classes of chromosome morphology, as described earlier (de Boer and Groen, 1974). Class 1 contains the least contracted, class 3 the most contracted bivalents. The criterion for class 3 primary spermatocytes, i.e. the occurrence of (a) terminalized chiasma(ta) in every bivalent of the cell has not been taken too literally. This division into classes has been done on a subjective basis. The correlation between chromosome morphology and meiotic stage is not necessarily perfect and artefacts because of the technique can interfere with it.

In this article, the interstitial and translocated segments of concern with respect to chiasma formation are named according to an earlier proposal (de Boer and 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 RIV, CIV, CIII+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. To distinguish between the possible types of chains of four, chains of three and an univalent, and two bivalents, the segment(s) with the missing chiasma(ta) is (are) added to the configuration symbol. Thus $CIV8_i$ (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. The reason for this is that translocation heterozygotes can give rise to chromosomes with unequal sized chromatids during the first meiotic division. 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.

The litter size data presented here refer to the litter size of heterozygous males of both translocations in comparisons with control Swiss males. All females

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were Swiss. Young born alive and dead are presented in one figure. For the T/+
males the first four litters have been counted, for the +/+ males the first three.

Differences between fractions are when possible treated with the aid of the
expression

$$\sqrt{\frac{(n_1+n_2) \ \{k_1(n_2-k_2) \ - \ k_2(n_1-k_1)\}^2}{(k_1+k_2) \ (n_1+n_2-k_1-k_2) \ n_1 n_2}} = \chi$$

in order to test for significance.

Results and discussion

Diakinesis – metaphase I

The two translocations differ with respect to the spectrum of multivalent
configurations at diakinesis - metaphase I. This is not a surprising finding in
view of the differences in length of the interstitial and translocated segments
between the two translocations (de Boer and van Gijsen, 1974). The configurations
found and their frequencies are given in table II. Fig. 1 for T26H and fig. 2
for T70H give diagrams and photomicrographs of the most frequent types of multi-
valent configurations seen.

For T26H, rings were most prominent (53.1%) with chains, lacking a chiasma
in segment 8_i as the next one (36.8%). Males were heterogeneous with respect to
the numbers of RIV, $CIV8_i$ and $CIV2_t$ configurations (χ^2_4 = 22.26, P < 0.005). The
percentage of rings per male ranges from 46.2% to 64.4% (n $\approx$ 210). Within the
chains however, the males did not differ significantly with respect to the
shares of $CIV8_i$ and $CIV2_t$, the former one being appr. 5 times as frequent as
the latter. When two bivalents were formed, 3 could be judged to have missing
chiasmata in the two translocated segments, in case of the other 5 this has been
assumed. Quite frequently (5.6%, n=556) RIV's were seen where a chiasma was slip-
ping off in segment 2_t. Somewhat less frequently and especially in class 3 sperma-
tocytes, RIV'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 8 centric heterochromatic blocks was clearly observed in
2.1% (n=385) of the $CIV8_i$ configurations. In our opinion, non-homologous chiasma
terminalization can occur in T26H RIV's. Two clear cases have been encountered
altogether, resembling a cross with the Giemsa-positive dots on the ends of each
arm. The T26H/+ males used differed with respect of the chromosome morphology with-
in the cells scored (χ^2_4 = 15.11, P < 0.005). Using a χ^2-test of independence,
no relation could be traced between the shares of RIV, $CIV8_i$ and $CIV2_t$ configu-

Table II. Diakinesis and metaphase I observations of translocation heterozygous males of the T26H and T70H types.

T26H n=1046

	RIV	$CIV8_i$	CIV_2t	$CIV2_i$	$CIV8_t$	$CIII+I(2_t+8_i)$	$CIII+I(8_i+8_t)$	$II+II(2_t+8_t)$	$II+I+I?$
n	556	385	75	2	1	17	1	8	1
%	53.1	36.8	7.2	0.2	0.1	1.6	0.1	0.8	0.1

T70H n=1043

	RIV	$CIV1_i$	$CIV13_t$	$CIII+I(1_i+13_t)$	$II+II(1_i+13_i)$	$II+I+I(1_i+13_i+13_t)$	$II+I+I?$
n	30	632	13	353	12	2	2
%	2.9	60.6	1.2	33.8	1.2	0.2	0.1

Fig. 1.a. Diakinesis-metaphase I spermatocytes showing a T26H/+ RIV (upper), a $CIV8_i$ (middle) and $CIV2_t$ (lower). *b.* Chromatid diagrams of these multivalent configurations. *c.* Synapsis with the chiasmata allocated to translocated and interstitial chromosome segments.

Fig. 2 (right) *a.* Diakinesis-metaphase I spermatocytes of T70H/+, showing a RIV (upper), a $CIV1_i$ (middle), a $CIV13_t$ and a $CIII+I(1_i+13_t)$ (bottom). *b.* Chromatid diagrams of these multivalent configurations. *c.* Synapsis with the chiasmata allocated to translocated and interstitial chromosome segments.

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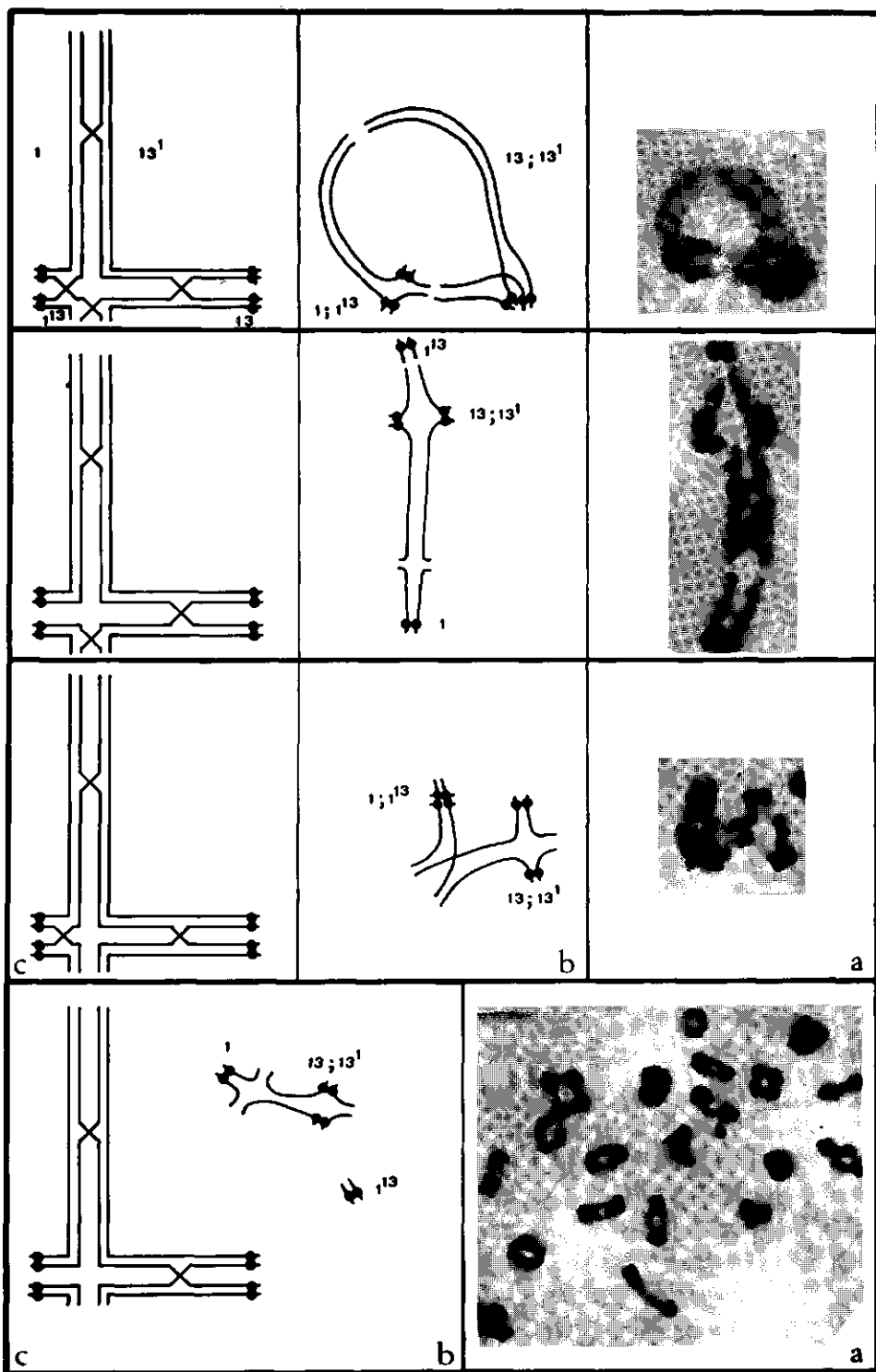
rations on one side and the chromosome morphology classes on the other side (χ_4^2 = 4.81, n.s.). This enables one to relate the chiasma frequencies found to the physical length of the chromosome segments as estimated by Giemsa-banding of mitotic prometaphase chromosomes. Table III gives the frequencies of the interstitial and translocated segments bound by one or more chiasma(ta). Only segment 2_i was occasionally bound by more than 1 chiasma. For the other segments, the number of chiasmata per unit of chromosome length (one percent of the "average" haploid genome) has been given as well. It is apparent that the chiasma frequency in 2_t is much higher than in 8_i, despite the fact that both are of approximately the same size.

Table III. The length of T26H interstitial and translocated segments (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

Unlike T26H, the majority (60.6%, see table II) of the T70H spermatocytes displays a chain quadrivalent with a chiasma missing in segment 1_i. The second frequent type is $CIII+I(1_i+13_t)$ and makes up 33.8% of all the cells scored. Males differed highly with respect to the frequencies of $CIV1_i$ and $CIII+I(1_i+13_t)$ configurations (χ_4^2 = 45.27, P < 0.005). The percentage of $CIV1_i$'s among all cells scored within a male varied between 44.3% and 69.7%. As with T26H, the T70H males were heterogeneous for chromosome morphology as defined in the methods (χ_4^2 = 86.94, P < 0.005). A χ^2 independency 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 (χ_2^2 = 46.44, P < 0.005). The actual numbers are shown in table IV. Because of the fact that chiasmata have a tendency of slipping 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. For segment 13_t for instance, this will lead to a serious underestimate. In 1.7% (n=353) of the $CIII+I(1_i+13_t)$ configurations, chromosome 1^{13} was seen to be situated with its centromere towards the centromere of the X-chromosome within the XY-bivalent. No case of centric heterochromatin association has been found among the $CIV1_i$ configurations.

Table IV. 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.

chromosome morphology class	configuration		
	RIV+CIV	CIII+I and others	ratio
1	75	17	4.41
n = 92			
2	435	188	2.31
n = 623			
3	165	163	1.01
n = 328			

When the T26H/+ and T70H/+ observations were made, XY dissociation and the occurrence of autosomal univalents have been scored as well. XY dissociation was more common here than in a previous experiment (de Boer and Groen, 1974) and amounted to 9.7% (n=1046) for T26H/+ and to 8.7% (n=1040) for T70H/+. Autosomal univalence for non-translocation involved bivalents occurred in 2.7% of the T26H/+ cells and in 1.3% of the T70H/+ cells. This difference, treated with the formula for the difference between fractions, given in the materials and methods section was significant (χ = 2.17 P < 0.025). Chromosome morphology class 3 contained significantly more cells with X and Y univalents (χ^2_1 = 14.50, P < 0.005). For autosomal univalents, this relation was absent.

Metaphase II

Metaphase II observations concerning the segregational behavior of multivalents and univalents, formed as a consequence of translocation heterozygosity, 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.

Chromosome $(1^{13};1^{13})$ and $(13^1;13^1)$ are examples of the first category, chromosome $(2;2^8)$, $(8;8^2)$, $(1;1^{13})$ and $(13;13^1)$ of the second (see figs. 1 and 2). For

fair judgment within the second category, knowledge about the location of the centromere in second meiotic division chromosomes in required. Therefore, the differential staining of centric heterochromatin within secondary spermatocytes is very helpful. The length difference between the two translocated segments within a reciprocal translocation, producing the chromosomes with the unequal sized chromatids (when a chiasma occurs in an interstitial segment) should be sufficiently large to produce an arm-ratio of at least 1.75. Differential chromatid contraction is a normal phenomenon within secondary spermatocytes. Even secondary spermatocytes from chromosomally normal mice produce chromosomes with chromatids of unequal size. We feel that an arm-ratio of at least 1.75 suffices to distinguish translocation produced marker chromosomes with chromatids of unequal length from "normal" chromosomes. For this reason, the T26H originated chromosome $(2;2^8)$ with an arm-ratio of appr. 1.5 was no help in studying the segregational behavior of T26H/+ multivalent configurations, although it can be recognized in favorable cells. Therefore, chromosome $(8;8^2)$ has been used as a marker chromosome in this translocation, despite the fact that an interstitial chiasma in segment 8_i 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 during first meiotic prophase and metaphase. It is assumed then 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 (a) marker chromosome(s) $(8;8^2)$ with the frequency of primary spermatocytes not displaying a chiasma in segment 8_i of 38.6%. This difference, tested with the aid of the formula given in the materials and methods section, was on the borderline of significance ($\chi = 1.67$ P = 0.0475). The frequency of double chiasmata in segment 8_i has been assumed to be zero. The difference between the two percentages was almost exclusively to blame to male no. 1, the first male scored in this series. In the other males, the agreement between the two estimates was very good. This adds to the reliability of adjacent II estimates in T26H.

For T70H, the frequency of secondary spermatocytes with $(1;1^{13})$ can be compared with the frequency of primary spermatocytes which show a chiasma in segment 1_i. Both percentages are 4.1. In the same way, the frequency of secondary spermatocytes with a $(13^1;13^1)$ marker chromosome (1.8%) closely fits a percentage of 1.5 of diakinesis - metaphase I's with a chiasma lacking in segment 13_i. Again we have assumed that segment 13_i is too short to accommodate two chiasmata and in fact, two chiasmata have never been observed.

Table V gives the types of metaphase II cells observed with their chromosome

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

	chromosome number			
marker	19	20	21	
-	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

numbers and frequencies for T26H. The almost equal numbers of cells with 19 and 21 chromosomes suggest that the analysis was not seriously handicapped by broken cells. The percentage of anaphase I originated non-disjunction can be calculated to be $6.9 \pm 1\%$. Non-disjunction can take place for normal bivalents and the sex-bivalent and results in aneuploidy. An unequal distribution of the four translocation involved chromosomes over the two anaphase I products leads to aneuploidy as well. For the reasons that

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

b) this marker is representative for 61.4% of the primary spermatocytes, namely the ones which have had an interstitial chiasma in segment 8_i, aneuploidy cannot be divided in a translocation caused category and a non-translocation caused category.

The 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 $CIV8_i$ configurations behave alike the RIV, $CIV2_t$ and $II+II(2_t+8_t)$ configurations, the adjacent II estimate yields a figure of $\frac{100}{61.4}$ x 2.6 = $4.25 \pm 1.0\%$ of all primary spermatocytes.

Table VI gives the metaphase II observations of heterozygotes for T70H. Marker chromosome $(1^{13};1^{13})$ can usually be recognized in T70H secondary spermatocytes. In only 14 cells there was some doubt. The complementary types have as much as possible been placed in the same category. Fig. 5 gives schematical illustrations of the categories A, C, D and E. Of the marker chromosomes and chromatid used, most are present in fig. 4. Two types of segregation, adjacent II and translocation caused numerical non-disjunction, are of special interest: both segregation types yield 100% unbalanced gametes. Thus, if these frequencies are known, the expected fertility of T/+ males can be compared with the realized fertility scores. Because of the fact that there is almost always a chiasma in
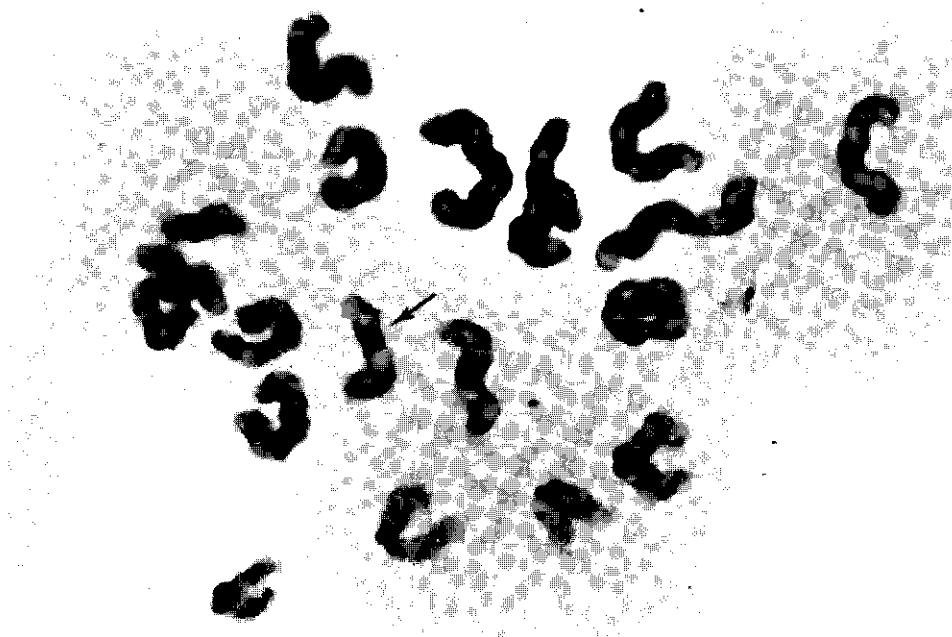
Fig. 3. A T26H/+ secondary oocyte with marker chromosome $(8;8^2)$ (arrow). Chromosome $(2;2^8)$ cannot be distinguished with certainty.

13_i, secondary spermatocytes with either none or two marker chromosomes $(13;13^1)$ are indicative for adjacent II segregation. This holds true when numerical non-disjunction was not taking place during the preceeding anaphase I. From table VI, the adjacent II frequency can be calculated to be $\frac{A}{A+C+D+E+F} = \frac{110}{491} = 22.4 \pm 1.9\%$.

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.

c) Equational division of the $(1^{13};1^{13})$ univalent at metaphase I - anaphase I. The latter possibility is a reality because of the recurrent finding of a 1^{13} chromatid in secondary spermatocytes. Of the cells with an euploid chromosome number (when counting a single chromatid for one), half of the second division products will contain 19 chromosomes if a 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. It is not known whether single chromatids have a chance of getting lost during the second meiotic division. When one compares the classes of complementary metaphase II cells (table VI), the numbers show a fair agreement except in category D when chromosome $(1^{13};1^{13})$ splits equationally ($\chi^2_1 = 5.26$, $P < 0.0025$). In our view, the possibility that one of both chromatids 1^{13} gets lost during anaphase I might be bigger. This will then 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 13 (with 21 chromosomes) and 24 (with 19 chromosomes) were scored respectively. This difference is not significant ($\chi^2_1 = 3.27$, $0.05 < P < 0.10$). 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 in translocation caused and non-translocation caused. Following the causes of aneuploidy, given in table VI, a minimal estimate for non-translocation caused non-disjunction is $\frac{F}{A+B+C+D+E+F} = \frac{12}{492} = 2.4 \pm 0.6\%$. A maximal estimate is $\frac{E+F}{A+B+C+D+E+F} = \frac{38}{492} = 7.7 \pm 1.2\%$. Consequently, estimates for numerical non-disjunction of translocation involved chromosomes range from practically zero to $5.7 \pm 1\%$. If one does assume that single chromatids 1^{13} do not get lost during either anaphase I or metaphase II - anaphase II, the latter estimate is increased by $\frac{(D)}{A+B+C+D+E+F} = \frac{22}{984} = 2.2 \pm 0.7\%$. Loss of 1^{13} at metaphase II - anaphase II does mean an increase by $3.3 \pm 1\%$ of aneuploid spermatozoa. So the highest estimate possible for numerical

Table VI. Chromosome numbers (including single chromatids) and the presence of marker chromosomes of T70H/+ secondary spermatocytes. Complementary types are grouped opposite to 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. Karyotypes indicated with an asterix occur in two rows. For further explanation, see the text and fig. 5.

category	chrom. number	chromosomal constitution	n	n	chromosomal constitution	chrom. number
A	20	$(1^{13};1^{13})$, -	49	56	$(13;13^1)$, $(13;13^1)$	20
adjacent II	19	$(1^{13}; -)$, - ***	1	1	$(13;13^1)$, $(13;13^1)$,$(1^{13}; -)$*	
n=110	19	$(13;13^1)$,$(13;13^1)$**	3			
B	20	$(13^1,13^1)$, -	1			
indifferent						
n=1						
C	20	$(13;13^1)$,$(1^{13};1^{13})$	138	148	$(13;13^1)$, -	20
alternate/		$(13^1;13^1)$,$(1^{13};1^{13})$	4	3	- , -	
/adjacent I		$(13;13^1)$, ?	14		- , -	
n=325		$(13;13^1)$,$(1,1^{13})$	18			
D	20	$(13;13^1)$,$(1^{13}; -)$	16	6	$(13;13^1)$,$(1^{13}; -)$	21
translocation	20	$(13;13^1)$,$(13;13^1)$,$(1^{13}; -)$*	1			
caused	19	- , -	1			
non-disjunction						
n=24						

continuation table VI.

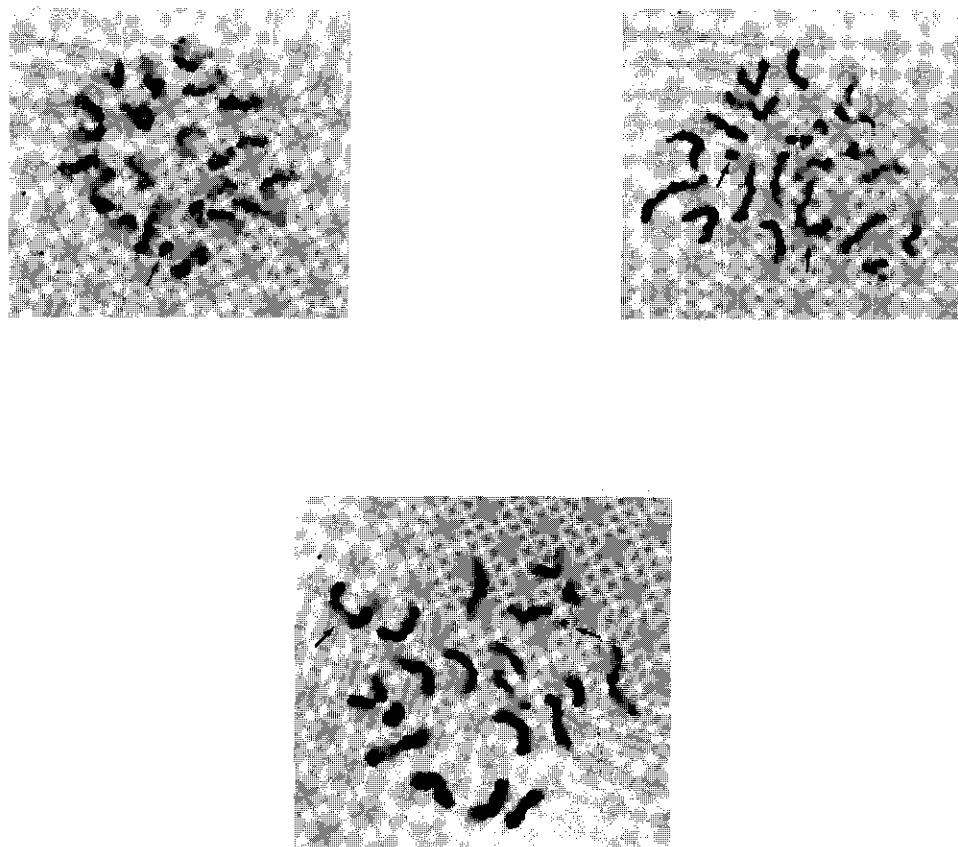| category	chrom. number	chromosomal constitution	n	n	chromosomal constitution	chrom. number
E	19	$(13;13^1)$, -	13	9	$(13;13^1),(1^{13};1^{13})$	21
presumable	19	$(1^{13};1^{13})$, -	3	1	$(13^1;13^1),(1^{13};1^{13})$	21
translocation						
caused						
non-disjunction						
n=26						
F	19	$(13;13^1),(13;13^1)$**	3	2	$(1^{13};1^{13})$, -	21
non-translocation	19	$(13;13^1),1^{13};1^{13})$	3	1	$(13;13^1)$, -	21
caused	19	$(13;13^1),(1;1^{13})$	1			
non-disjunction	19	$(1^{13};-)$, - ***	1			
n=12	20	$(13;13^1),(13;13^1)$ $(1^{13}; -)$*	1			

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Fig. 4. Some T70H/+ secondary spermatocytes with karyotypes listed in table VI and illustrated in fig. 5. Upper left a result of adjacent II disjunction (A left of table VI 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}.
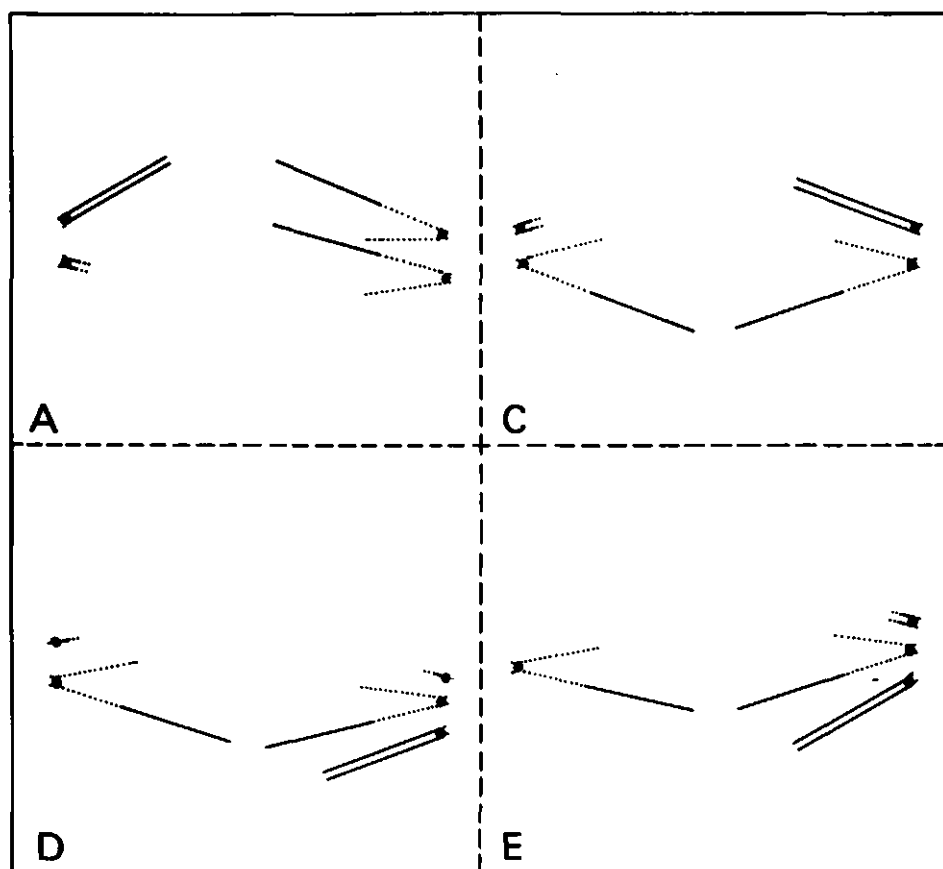
Fig. 5. Schematical representation of segregational events as observed in
T70H/+ secondary spermatocytes and given in tabel VI. A gives adjacent II
segregation, B alternate/adjacent I, D numerical non-disjunction caused by
equational separation of two 1^{13} chromatids and E "normal" numerical non-
disjunction. The symbols A, C, D and E follow the division made in table VI.
Chromosome 13 segments are dotted.

non-disjunction is 5.7 + 3.3 = 9.0%. In the next section this will be shown to
be an overestimate.

Litter size of translocation heterozygous males

Table VII gives the data concerning the litter sizes of crosses between the
two types of T/+ males and Swiss +/+ females and of the control (Swiss +/+ males
x Swiss +/+ females). The difference between T26H/+ and T70H/+ males was signif-
icant, using Student's t (t = 2.64 P < 0.005). If one assumes that the spectrum
of litter sizes produced by T/+ males in crosses to normals is not seriously
biased by intra-uterine selection against small litters and thus represents the
gametes, capable of development into young (live or dead) carried to term, the
relative litter size of T/+ males can be used as another estimate of the fraction
of primary spermatocytes displaying either adjacent II segregation or numerical
non-disjunction. Searle et al. (1971) have divised the expression $\frac{\bar{x}}{\bar{y}} = \frac{1-p}{2}$, where
$\bar{x}$ and $\bar{y}$ are the average litter sizes for T/+ and control animals respectively.

Table VII. Litter sizes $\bar{x}$ of T/+ males compared with +/+ males ($\bar{y}$) in crosses to
+/+ females.

cross $\male$	$\female$	$\bar{x},\bar{y}$	s.d.	n	$\bar{x}/\bar{y}$
T26H/+	x +/+	4.25	1.90	126	0.475
T70H/+	x +/+	3.47	2.96	125	0.388
+/+	x +/+	8.95	3.38	132	

The fraction of adjacent II and numerical non-disjunctional events at anaphase I
is p. Other disturbances of the reproductive capacity are assumed to be the
same for the mutant and for the control. The standard deviation of the relative
litter size has been calculated with a formula developed by Dr. P. Stam.

$$\text{Var}\frac{\bar{x}}{\bar{y}} = \frac{\frac{\bar{x}}{\bar{y}} n N_1}{n_1^2 N_2} \quad \text{where}$$

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

Table VIII summarises the estimates for the adjacent II percentages and

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Table VIII. Estimates of frequencies of adjacent II segregation and numerical
non-disjunction obtained by two methods of ascertainment.

	T26H/+		T70H/+	
	adjacent II	numerical non-disjunction	adjacent II	numerical non-disjunction
cytological estimate	4.25 ± 1%	?	22.4% ± 1.9%	< 9.0%
from relative litter size	5.0 ± 5%		22.4 ± 4%	

percentages numerical non-disjunction derived by cytological methods and by the
comparison of litter sizes. In general, the agreement between the two methods is
good.

Still under the assumption that no selection against small litters occurred,
numerical non-disjunction must be a rare event in both translocations. If one
reviews the knowledge about the numerical non-disjunctional behavior of mouse
translocations (de Boer, 1973), those with a high frequency of CIV and CIII+I
configurations at diakinesis - metaphase I have a predisposition for numerical
non-disjunction leading to aneuploidy. Therefore, the frequency of T26/+ primary
spermatocytes displaying numerical non-disjunction must be small indeed. T70H
heterozygotes are more liable to it. This has been demonstrated by the production
of tertiary trisomic offspring as well (de Boer, 1973). The frequency of numerical
non-disjunction in male T70H carriers must be small for the following reasons.
a) The total amount of aneuploidy found in T26H/+ (6.9%) and T70H/+ (± 9.9%) does
   not differ much.
b) According to table VIII, the frequency of numerical non-disjunction must be
   very small in T26H/+ (5%-4.25% = 0.75%) and this is in agreement with the
   expectation derived from diakinesis - metaphase I observations.
c) The comparison of the cytological and litter size estimates in T70H/+ is
   therefore more meaningful. Both the cytological adjacent II frequency as the
   summed frequency of adjacent II segregation and numerical non-disjunction
   from the litter size data amount to 22.4%.
The discrepancy is minimal and does not leave much room for numerical non-dis-
junction. About 4% may be a realistic estimate on the basis of the available
data.

Trivalent plus univalent configurations (T70H/+) have an "overall" frequency

of 33.8% (table II) irrespective of chromosome morphology class. At metaphase I -
anaphase I, it must be higher with a best estimate of close to 50% (table IV).
Random segregation of the univalent 1^{13} at anaphase I should yield at least 17%
and probably 25% aneuploid metaphase II cells. This situation is clearly not
met in the present study. The conclusion must be that the 1^{13} univalent coorien-
tates in one or another way with the other chromosomes of the translocation com-
plex. The equational division of chromosome 1^{13} in two 1^{13} chromatids at ana-
phase I may be one of the causes for tertiary trisomic offspring in this trans-
location.

*The relation between multivalent configuration at diakinesis - metaphase I and
anaphase I segregation*

When, as is the case with air-dried preparations, the orientation of a
multivalent cannot be inferred from metaphase I - anaphase I observations, two
approaches stand open to this problem.
a) The comparison of the segregational behavior of translocations with different
   spectra of multivalent configurations.
b) The comparison between members of different families, heterozygous for the
   same translocation.
One can then relate the variation found with respect to multivalent configuration
formation with the segregation pattern observed. For T26H/+ , RIV was the most fre-
quent configuration found (53.1%) although the summed frequency of CIV's almost
equals this (44.3%). From the low % of adjacent II segregations and the low pro-
bability of numerical non-disjunction (see table VIII) it can be inferred that
chains do not differ appreciably from rings with respect to segregation. The
majority (90.3%) of the chains is of type II (following the classification of
Lewis and John, 1963) 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. "Unstable"
because of the indirect relation between the centromeres of 8 and 8^2 with at
least three chiasmata between these two. Alternate/adjacent I segregation is
preferred by the T26H/+ chains, however.

T70H/+ males mostly show CIV of type II (60.6%) followed by CIII+I con-
figurations (33.8%). Considerable heterogeneity was found for the spectrum of
multivalent configurations among males (see the section on diakinesis - meta-
phase I). In contrast to this, no significant heterogeneity was found in the
males for the frequencies of adjacent II secondary spermatocytes, with percen-
tages of 22, 19, 24, 20 and 32% respectively. 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. A relation between the frequency of CIII+I configurations and numerical non-disjunction per male is absent as well. The same reasons as mentioned above plus the fact that numerical non-disjunction cannot be traced with 100% certainty in metaphase II's (see table VI) restrain us from a full appreciation of the relation between multivalent configuration and numerical non-disjunction.

The two translocations differ, both with respect to their multivalent configuration spectrum and the frequencies of adjacent II segregation. Notably the work of Burnham (1950) and Lewis and John (1963) have lead to the proposal of a few rules based on translocation work with mainly submeta- and metacentric chromosomes, which should be helpful to explain the differences in segregational behavior 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 and Lewis and John, 1963).

Both rules do not always seem to be obeyed by our two reciprocal mouse translocations between acrocentric chromosomes. The almost consistent occurrence of a chiasma in 13_i does not prevent adjacent II segregation. On the other hand, the majority of type II chains ($CIV8_i$, $CIV1_i$) must segregate alternate/adjacent I. In our view, the observation of the precocious slipping off of chiasmata in the segments 2_t and 8_t of T26H RIV's offers a key to explain the segregational behavior 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 coorientation. If, however, one or both translocated segments are long (as 1_t of T70H), (a) chiasma(ta) in this segment retain(s) the power of leading to coorientation. Then, adjacent II segregation might depend on the position of the chiasma in 13_i. The more proximal it is, the higher the chance that non-disjunction for the 13-centromeres will follow. Summarizing, the segregational behavior 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.

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.

c) Univalents (at least of T70H origin) and observed in air-dried preparations retain a remarkable capability of coorientation.

Unfortunately, the genetic background is an important factor for multivalent behavior. This point receives more attention in the section on the estimation of the adjacent II segregation frequency via different methods.

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 non-centric heterochromatin containing segments. Although in T26H, 8_i and 2_t are almost equally long (de Boer and van Gijsen, 1974), the frequency of a chiasma in 2_t was appr. 1.5 times as high as the frequency of a chiasma in 8_i. Similarly, despite 1_i being twice as long as 13_t, a chiasma is almost exclusively situated in 13_t. This agrees with the tendency found in a meiotic study of tertiary trisomics $(Ts(1^{13})70H$, de Boer and Groen, 1974). In this study, 1^{13} was bound to 13 in 92.7% of the cells where it was bound at all (22%). For the T70H/+ males this was 93.7% (of the 64.7% where it was bound). Cross-over suppression between marker genes close to a translocation breakpoint has been observed in T(14;15)6Ca (Eicher and Green, 1972). The comparison between T(1;13)70H and $Ts(1^{13})70H$ meiotic behavior together with the fact that the minute segment 13_t (0.55% of an "average" haploid set of 20 chromosomes) has such a high frequency of being bound, especially in the less condensed chromosomes, lead us to suppose that the disturbance of synapsis in the centre of the translocation cross at least of T70H/+ heterozygotes is only modest. One can think of the possibility that the centric heterochromatin exerts an influence on chiasma formation over a longer segment than the heterochromatic block as such. Segment 13_i was not bound in 1.5% of the cells. It occupies 4.07% of the total length of the "average" haploid complement (de Boer and van Gijsen, 1974). This distance should be long enough to accommodate at least one chiasma, knowing that the smallest autosome (19) occupies 2.65% of a haploid female complement (Nesbitt and Francke, 1973). Disturbed synapsis in the centre of the cross should not play an important role with segments of this length. The frequency of univalence for all autosomes, not involved in T70H was 1.3%. In this translocation, the relative absence of chiasmata in 13_i is of significance and it might indicate the power of centric heterochromatic segments to reduce chiasma frequencies in their neighborhood.

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These results do not entirely agree with those of Henderson (1963) and Fox (1973) with regard to the acrocentric *Schistocerca gregaria* chromosomes. Here, the region adjacent to the centric heterochromatin was frequently involved in chiasma formation. Klášterská et al. (1974), in a study of heterochromatin distribution and chiasma localization in the grasshopper *Bryoderma tuberculata*, observed the single chiasma to be situated next to the centric heterochromatin when the blocks were small but at the telomeres when the blocks were large. The latter situation points to a parallel with the one encountered here. In this study, the influence of the centric heterochromatin on a certain segment is expressed as the chance that no chiasma is formed at all.

In agreement with the tendency towards distally localized chiasmata found here are the findings with human gene mapping, particularly with chromosome 1 (W. Burgerhout, pers. communication; Cook et al., 1974).

Chiasma frequencies and Giemsa-banding

A question which has received attention only recently is that of a possible relation between Giemsa-positive regions and a lowered chiasma frequency relative to the Giemsa-negative regions. According to Pathak et al. (1973) in the fruit bat *Carollia perspicillata*, the order of replication in the S-phase of the cell-cycle is Giemsa-negative, Giemsa-positive, constitutive heterochromatin, facultative heterochromatin. Comings (1974) suggests that the Giemsa-positive regions constitute a special class of heterochromatin. In *Schistocerca gregaria* and especially in the small chromosomes, a Giemsa-positive band coincides with a drop of the chiasma frequency (Fox et al., 1974). Furthermore, H.J. Evans (sited in the same paper), studying human material, observed a tendency of the chiasma frequency per bivalent to fall when the share of Giemsa-positive material per bivalent increased. The present material is not decisive in this respect. The telomeric ends of chromosome 2 and chromosome 13 show a small and weak Giemsa-positive band as is the case with most of the mouse chromosomes (Nesbitt and Francke, 1973). Reciprocal mouse translocations can help to elucidate the significance of Giemsa-positive material with respect to chiasma formation. T(14;15)6Ca parallels T(1;13)70H with respect to a morphologically recognizable small marker chromosome (15^{14}), a high frequency of univalents at diakinesis – metaphase I (47.9%, see table IX) and the fact that both produce tertiary trisomic offspring. The frequency of univalents is remarkably high if one knows that 15^{14} is largely of 14-distal origin. This phenomenon might be explained on the basis of the very strong Giemsa-positive character of the distal end of chromosome 14.

Probably, the weak Giemsa-positive bands 2H2, 2H4 (of 2_t) and 13 D_2 (of 13_t) do not interfere with chiasma formation.

Two examples of a-chiasmate centric association have been observed in this investigation, although both with a low frequency (around 2%). The first type is between the homologous centric heterochromatic blocks of $CIV8_i$ chains. 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 and Groen, 1974), turned up again in the T70H/+ males. The general nature of this phenomenon has been discussed before (de Boer and Groen, 1974) and a demonstration of it in grasshopper chromosomes is given by Klásterská et al. (1974).

The fate of univalents at anaphase I

We observed earlier (de Boer and Groen, 1974) that univalents 1^{13} display a strong tendency to move to either pole at anaphase I. This phenomenon is confirmed by the present data on T70H/+ males (table VI). When we leave out the 14 doubtful metaphase II cells and cells with a single 1^{13} chromatid, 209 cells possess chromosome 1^{13} and 225 do not. If univalents 1^{13} get lost at anaphase I, it must be with a low frequency.

Similar observations which hitherto remained unpublished were made by Dr. E.P. Evans using males heterozygous for the T(14;15)6Ca translocation. Table IX gives his results.

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

configuration	metaphase I n=1600			
	RIV	CIV	CIII+I	II+II
	1.7%	50.1%	47.9%	0.3%
chromosome number	metaphase II n=2069			
	20	$20(19+15^{14})$	19	$21(20+15^{14})$
	49.0%	45.0%	3.0%	3.0%

Despite a very high percentage of CIII+I configurations, numerical non-disjunction only takes place in a small minority of the primary spermatocytes at anaphase I.

The small marker chromosome 15^{14} does not seem to get lost in those cells, reaching the secondary spermatocyte stage. The difference between all meta-phase II cells including 15^{14} and those without the T6Ca small marker chromosome is not entirely significant (χ^2_1 = 3.17, 0.05 < P < 0.10). So, we can conclude that our findings in T70H/+ males agree with the picture presented by T6Ca/+ males. One has to remember, however, that T6Ca/+ males have an impaired spermatogenesis with death of germ cells occurring from the pachytene stage on (Baranov and Dyban, 1968). We have evidence that epididymal sperm counts are somewhat lower in T70H/+ males versus controls as well. This effect hampers a true appraisal of the congruence between the meiotic behavior of the two types of translocation heterozygous males.

The estimation of the adjacent II segregation frequency via different methods

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 des-cribed by Searle et al. (1971).
a) The viability of T/+ outcross progeny relative to normal (see table VIII).
b) The viability of T/+ x T/+ intercross progeny relative to normal.
c) The frequency of homozygotes for interstitially located gene markers.
All three methods suffer from a bias caused by the possibility of a selection against small litters during gestation. It is not entirely clear at which level (litter size) this selection starts to act but we believe it to be present in the mouse (de Boer and Groen, 1974) and in fact it has been clearly shown to exist in other mammals, for instance in the pig (Polge et al., 1966).

Table X summarizes the adjacent II frequency estimates so far reported in the mouse. The estimates based on the frequency of complementation for inter-stitially located marker genes assumes that complementation does not follow numerical non-disjunction, although theoretically, this is a possibility. An estimate on the basis of the relative viability of outcross progeny includes em-bryonic and fetal death caused by numerical non-disjunction. There is a tendency for the second method to yield lower estimates than the first one (table X), especially if one knows that for T6Ca, estimate b is biased by a proportion of aneuploid offspring (Baranov and Dyban, 1970). One expects such a difference if there is a selection against small litters during gestation. A reason for the production of reasonably large litters of T/+ intercrosses is a relative large share of complementation-produced zygotes among all viable zygotes. The higher the adjacent II percentage of a translocation, the more likely the outcross

Table X. Adjacent II estimates for reciprocal mouse translocations so far obtained. For further explanation, see the text.

| | method | | |
| | a | b | c |
translocation	frequency of homozygotes for interstitial marker genes	relative viability of outcross progeny	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 and Green (1972)

[***] calculated from Lyon et al. (1972)

[+] figures for T/+ males only

litters carried to term form a selected sample from the litters at the zygote stage. The first method thus yields an overestimate, the second one an underestimate.

If one compares the results for T26H and T70H in table X with those given in table VIII, the conclusion seems to be warranted that the change of genetic background (to the Swiss stock) has altered the behavior of T/+ male multivalent configurations. The T26H/+ males behave in a more regular (alternate/adjacent I) way now while the frequency of adjacent II segregations has gone up for T70H. This change remains unexplained as such here, although the phenomenon is common. 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 II, the chiasma frequency must have dropped quite strongly during the process of outcrossing T/+ animals to the Swiss random-bred stock. Within the Swiss T/+ stock, variation of the chiasma frequency between males did not result in a parallel variation with regard to segregation. Other factors must be responsible for this change in multivalent configuration behavior, but these have not been considered here.

Reciprocal translocations between acrocentric chromosomes have been studied earlier although not in much detail. Sarkar (1955) studied a translocation hetero-

zygote in the grasshopper *Gesonula punctifrons*. Numerical non-disjunction was a relative frequent event (15-20%) while adjacent II segregation was around 5%. Kayano and Nakamura (1960) could find neither of these two special classes of segregation in a reciprocal translocation in *Acrida lata*. The comparison between translocations in different species is even more difficult than intraspecies comparisons. One has to remember that each translocation is unique and may show an individual behavior.

Acknowledgements

References

Bakker, H.: Effect of selection for relative growth rate and bodyweight of mice on rate, composition and efficiency of growth. Thesis, Wageningen (1974).

Baranov, V.S. and Dyban, A.P.: Analysis of spermatogenic and embryogenic abnormalities in mice, heterozygous for the chromosome translocation T6. Genetika (U.S.S.R.) 4: 70-83 (1968).

Baranov, V.S. and Dyban, A.P.: Correlation between abnormal embryonic development and the type of chromosome abnormalities arising during meiosis in mice that are heterozygous with regard to T6 translocation. Soviet J. Develop. Biol. 1: 196-205 (1970).

Boer, P. de: Fertile tertiary trisomy in the mouse (*Mus musculus*). Cytogenet. Cell Genet. 12: 435-442 (1973).

Boer, P. de and Groen, A.: Fertility and meiotic behavior of male T70H tertiary trisomics of the mouse (*Mus musculus*). A case of preferential telomeric meiotic pairing in a mammal. Cytogenet. Cell Genet. In press (1974).

Boer, P. de and Gijsen, M. van: The location of the position of the breakpoints involved in the T26H and T70H mouse translocations with the aid of Giemsa-banding. Can. J. Genet. Cytol. In press (1974).

Burnham, C.R.: Chromosome segregation in translocations involving chromosome 6 in maize. Genetics 35: 446-481 (1950).

Carter, T.C.; Lyon, M.F. and Phillips, R.J.S.: Gene-tagged chromosome translocations in eleven stocks of mice. J. Genet. 53: 154-166 (1955).

Chandley, A.C. and Fletcher, J.M.: Centromere staining at meiosis in man. Human-genetik 18: 247-252 (1973).

Comings, D.E.: Cytochemistry of chromosome structure. Leiden chromosome conference, Leiden, July (1974).

Cook, P.J.L.; Robson, E.B.; Buckton, K.E.; Jacobs, P.A. and Polani, P.E.: Segregation of genetic markers in families with chromosome polymorphisms and structural rearrangements involving chromosome 1. Ann. Hum. Genet. 37: 261-274 (1974).

Eicher, E.M. and Green, M.C.: The T6 translocation in the mouse: its use in trisomy mapping, centromere localization, and cytological identification of linkage group III. Genetics 71: 621-632 (1972).

Evans, E.P.; Breckon, C. and Ford, C.E.: An air-drying method for meiotic preparations from mammalian testes. Cytogenetics, 3: 289-294 (1964).

Falconer, D.S.: Genetic aspects of breeding methods. In the U.F.A.W. Handbook on the care and management of laboratory animals. (Livingstone, Edinburgh and London, 1967).

Ford, C.E.; Carter, T.C. and Hamerton, J.L.: The cytogenetics of reciprocal translocations in the mouse: a survey of twelve stocks. Unpublished.

Forejt, J.: Pseudochiasmata in male meiosis? Mouse News Letter 49: 46 (1973).

Fox, D.P.: The control of chiasma distribution in the locust, *Schistocerca gregaria* (Forskål). Chromosoma 43: 289-328 (1973).

Fox, D.P.; Carter, K.C. and Hewitt, G.M.: Giemsa-banding and chiasma distribution in the desert locust. Can. J. Genet. Cytol. 16: 272-276 (1974).

Hamerton, J.L.: Human cytogenetics Vol. I (Academic Press, New York and London, 1971).

Henderson, S.A.: Chiasma distribution at diplotene in a locust. Heredity 18: 173-190 (1963).

Jacobs, P.A.: Human population cytogenetics. In J. de Grouchy, F.J.G. Ebling and I.W. Henderson eds.: Human genetics (Excerpta Medica, Amsterdam, 1972).

Kayano, H. and Nakamura, K.: Chiasma studies in structural hybrids V. Heterozygotes for a centric fusion and for a translocation in *Acrida lata*. Cytologia (Tokyo) 25: 476-480 (1960).

Klåsterská, I.; Natarajan, A.T. and Ramel, C.: Heterochromatin distribution and chiasma localization in the grasshopper *Bryodema tuberculata* (Fabr.) (*Acrididae*). Chromosoma 44: 393-404 (1974).

Lewis, K.R. and John, B.: Spontaneous interchange in *Chorthippus brunneus*. Chromosoma 14: 618-637 (1963).

Lyon, M.F.; Glenister, P.H. and Hawker, S.G.: Do the H-2 and T-loci of the mouse
have a function in the haploid phase of sperm? Nature 240: 152-153 (1972).

McClintock, B.: Neurospora. I. Preliminary observations of the chromosomes of
Neurospora crassa. Am. J. Bot. 32: 671-678 (1945).

Nesbitt, M.N. and Francke, U.: A system of nomenclature for band patterns of
mouse chromosomes. Chromosoma 41: 145-158 (1973).

Pathak, S.; Hsu, T.C. and Utakoji, T.: Relationships between patterns of chromo-
some banding and DNA synthetic sequences: a study on the chromosomes of the
Seba's fruit bat, *Carollia perspicillata*. Cytogenet. Cell Genet. 12: 157-164
(1973).

Polge, C.; Rowson, L.E.A. and Chang, M.C.: The effects of reducing the number of
embryos during early stages of gestation on the maintenance of pregnancy in
the pig. J. Reprod. Fert. 12: 395-397 (1966).

Sannomiya, M.: Chiasma studies in structural hybrids. X. Further studies in
Acrida lata. Japan. J. Genet. 43: 103-108 (1968).

Sarkar, I.: A translocation heterozygote in the grasshopper *Gesonula punctifrons*.
J. Hered. 46: 157-160 (1955).

Schreuder, I.: Een vergelijking van pré- en post-implantoire verliezen in op
vruchtbaarheid geselecteerde muizen en een controle populatie. Scriptie
Veeteelt, Wageningen (1972).

Searle, A.G.; Ford, C.E. and Beechey, C.V.: Meiotic disjunction in mouse trans-
locations and the determination of centromere position. Genet. Res., Camb.
18: 215-235 (1971).

Snell, G.D.: An analysis of translocations in the mouse. Genetics 31: 157-180
(1946).

Sumner, A.T.: A simple technique for demonstrating centromeric heterochromatin.
Exptl. Cell Res. 75: 304-306 (1972).

Summary and conclusions

In this section, the order of the articles has not been closely followed. Each point ends with the number(s) of the article(s) (as given in the contents), where the conclusion is based on.

1) Cytological meiotic studies of T(2;8)26H and T(1;13)70H heterozygotes and Ts(1^{13})70H tertiary trisomics indicate, that chiasmata are more often located in the distal (translocated) segments than in the proximal (interstitial) segments containing centric heterochromatin (3 and 5).

2) This study opens the possibility that the presence of centric heterochromatin decreases the probability of chiasma formation in its vicinity with a positive gradient distally (5).

3) The genetic lengths of the interstitial and translocated chromosome segments coincide rather well with the physical length of these segments as estimated with the aid of Giemsa-banding. This finding does not fit the tendency expressed in the conclusions 1 and 2. The apparent exception of this rule is segment 13_t which is overestimated when looking at genetic recombination. For cytological studies, the physical length of a segment is of a greater value (4).

4) Univalence for chromosome 1^{13} at metaphase I - anaphase I does not lead to an appreciable loss of this chromosome in the male, neither in the Ts(1^{13})70H tertiary trisomic karyotype nor in the T(1;13)70H heterozygote (3 and 5).

5) In the T70H/+ karyotype, there is strong evidence for coorientation of the 1^{13} univalent so that the four reciprocal translocation involved chromosomes segregate two by two. Occasionally, equational separation of the two 1^{13} chromatids may occur at anaphase I (5).

6) The segregational behavior of heterozygous translocation multivalent configurations can, within the genetic background concerned, be best explained by time differences of chiasma terminalization during metaphase I - anaphase I (5).

7) The genetic background most likely exerts an influence on the behavior of mouse reciprocal translocations (5).

8) The reliability of the formula which relates the summed frequencies of adjacent II disjunction and numerical non-disjunction and the relative viability of heterozygous translocation outcross progeny depends on the existence of selection against small litters during gestation. This is the more likely when the theoretically expected litter size decreases (5).

9) A-chiasmate non-homologous chromosome association of the centric heterochromatin of chromosome 1^{13} and the X-chromosome does occur (3 and 5).

10) The majority of male Ts(1^{13})70H tertiary trisomics are capable of producing offspring. Thus, tertiary trisomy does not invariably lead to sterility in the male mouse (2 and 3).

11) Tertiary trisomics for chromosome 1^{13} in the mouse display a variety of phenotypes. The condition can lead to death in utero, to death before weaning, to morphologically affected but viable animals and to animals with an unaltered appearance (2 and 3).

12) The ratio between morphologically affected and unaffected tertiary trisomics for chromosome 1^{13} at birth (live or dead) amounts to between 2 and 3. This ratio might depend on the genetic background concerned (2 and 3).

13) The most obvious abnormality of the morphologically affected tertiary trisomics of the Ts(1^{13})70H karyotype is a malformation of the bones of the skull which often leads to an abnormal growth of the upper and lower incisors (2).

14) The impaired fertility of Ts(1^{13})70H males is most probably due to a lowered production of functional spermatozoa and the consequences this has for the continuation of pregnancy. Thus, the elimination of "unbalanced" progeny is not the first cause (3).

Samenvatting en conclusies

In dit deel wordt de volgorde der artikelen niet aangehouden. Wel wordt na elk punt vermeld uit wel artikel of welke artikelen (aangegeven door de nummers in de "contents") de betreffende conclusie afkomstig is.

1) Zowel bij T(2;8)26H als bij T(1;13)70H heterozygoten en Ts(1^{13})70H tertiaire trisomen is gevonden, dat de distale segmenten van chromosoom 2 en 13 sterk verhoogde chiasma frequenties hebben in vergelijking tot de proximale segmenten van chromosoom 1 en 8, die beide centrisch heterochromatine bevatten (3 en 5).

2) Er bestaan aanwijzingen voor het feit dat de invloed, die centrisch heterochromatine heeft op de chiasma frequentie, namelijk het omlaag brengen ervan, zich uitstrekt tot meer distaal gelegen euchromatische chromosoomgedeelten (5).

3) Niettegenstaande de vorige conclusie is er een goede overeenkomst tussen de genetische lengten van de interstitiële en getransloceerde chromosoom segmenten en de fysische lengten gemeten in mitotische prometafase chromosomen. Dit gaat vooral op voor de chromosomen 1, 2 en 8. Voor chromosoom 13 blijkt het fysisch gemeten breukpunt meer distaal te liggen t.o.v. het genetisch bepaalde breukpunt. In het kader van de cytologische analyse van het meiotische gedrag van structurele chromosoomafwijkingen bieden de fysisch bepaalde lengten het meeste houvast (4).

4) Het feit dat chromosoom 1^{13} tijdens de 1e meiotische deling vaak als een univalent voorkomt leidt niet tot significante verliezen van dit chromosoom. Dit geldt zowel voor Ts(1^{13})70H tertiair trisome mannetjes als voor T(1;13)70H translocatie heterozygote mannetjes (3 en 5).

5) Tijdens de 1e meiotische deling in T(1;13)70H heterozygote mannetjes coörienteert het univalent 1^{13} in de meerderheid van de gevallen met het trivalent en wel zo dat de uiteindelijke segregatie 2 bij 2 is. Equatoriale splitsing van dit chromosoom tijdens de anafase I behoort tot de mogelijkheden maar is geen regel (5).

6) Het segregatiepatroon van multivalent configuraties tengevolge van translocatie heterozygotie kan het beste worden verklaard door aan te nemen dat er tijdens de metafase I - anafase I tijdsverschillen in chiasma terminalisatie optreden (5).

7) De genetische achtergrond waarin de reciproke translocatie zich bevindt heeft bij de muis naar alle waarschijnlijkheid een duidelijke invloed op de segregatie van de multivalenten.(5).

8) De betrouwbaarheid van een relatie tussen de som van de frequenties van adjacent II segregaties en numerieke non-disjunctie en de worpgrootte van translocatie heterozygote ouderdieren (gepaard met normale en vergeleken met normale dieren) hangt af van het eventuele bestaan van een prenatale selectie tegen kleine worpen. Het optreden van zo'n selectie is des te waarschijnlijker wanneer de theoretisch verwachte worpgrootte kleiner is (3 en 5).

9) Tijdens de 1e meiotische deling bestaat er een vorm van achiasmatische attractie tussen de non-homologe delen centrisch heterochromatine van het X-chromosoom en chromosoom 1^{13} (3 en 5).

10) De mannelijke muizen van het tertiair trisome $Ts(1^{13})70H$ karyotype zijn in grote meerderheid in staat nakomelingen te verwekken. Dit duidt erop dat mannelijke muizen met tertiaire trisomie of anderszins extra chromosomaal materiaal niet per definitie steriel zijn (2 en 3).

11) Tertiaire trisomie voor chromosoom 1^{13} bij de muis uit zich in een scala van fenotypen. Dit karyotype kan leiden tot de dood in utero, tot de dood tijdens de zoogperiode en daarna, maar ook kan de conditie levensvatbaar zijn en aanleiding zijn tot fenotypische effecten. Tenslotte komen er tertiair trisome dieren voor, die tijdens hun gehele leven onopgemerkt blijven (2 en 3).

12) De verhouding uiterlijk waarneembare tertiaire trisomen $Ts(1^{13})70H$ en niet opvallende trisomen ligt tussen de 2 en 3. Deze verhouding geldt op basis van het aantal levend en dood geboren jongen en is gebonden aan het restgenotype (2 en 3).

13) De meest in het oog lopende morphologische verandering die tertiaire trisomen $Ts(1^{13})70H$ kunnen ondergaan is een afwijking van de schedel, vaak resulterend in een abnormale stand en groei van de snijtanden (2).

14) De meest waarschijnlijke verklaring voor de lage en variabele worpgrootte van $Ts(1^{13})70H$ mannetjes is een verlaagde produktie van normaal functionerende spermatozoa en de gevolgen die dit heeft voor het verloop van de dracht. De rol, die gespeeld wordt door het in utero afsterven van genetisch ongebalanceerde embryo's is dus kleiner (3).

Curriculum vitae

Schrijver dezes werd in 1947 geboren te Maarssen. Hij bezocht de gemeente-
lijke H.B.S. te Utrecht en stond van 1964 tot 1971 ingeschreven aan de Landbouw-
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