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Meiotic disjunction in mouse translocations and the determination of centromere position

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SUMMARY

If heterozygotes for a reciprocal translocation are intercrossed, some of their viable balanced progeny result from the fusion of unbalanced gametes with complementary duplications and deficiencies of the translocated segments. Therefore, if one parent in such an intercross is homozygous for a genetic marker on one of the segments concerned, some homozygous offspring will be produced even if the other parent does not have the marker. The expected frequency of such exceptional offspring among live-born is one-sixth if the marker is on the distal (non-centromeric) side of the point of exchange and single chiasmata normally occur in each interstitial segment. Much lower frequencies are expected if the marker is on the centromeric side, since duplications and deficiencies of proximal segments occur only as a consequence of adjacent-2 disjunction, in which homologous centromeres proceed to the same pole. This is rarer than normal disjunction. Thus, by comparing the frequencies of offspring homozygous for markers on one or other side of the point of exchange, it is possible (i) to determine which marker is in the centromeric segment, (ii) to estimate the frequency of adjacent-2 disjunction, given information on the nature of meiotic configurations in the translocation concerned.

By this method, it is shown that the frequency of adjacent-2 disjunction is similar in heterozygotes for mouse translocations (*T5;18)26H*, *T(13;?) 70H* and *T(14;17)264Ca*, averaging 13 %. Centromeres were located at the *Sd* end of linkage group V (confirming previous findings), the *fz* end of XIII and the *bg* end of XIV.

1. INTRODUCTION

It is now well established that the 19 pairs of autosomes and the *X* and *Y* chromosomes of the house mouse (*Mus musculus* L.) have terminal or closely subterminal centromeres (see Levan, Hsu & Stitch, 1962). Moreover, the *X*-chromosomal and 18 autosomal linkage groups have now been described (see Green, 1966). Yet the task of correlating the genetical and cytological maps has not proceeded very far. A first essential is the determination of centromere position in relation to the linkage groups. Several methods have been used with varying success and will be discussed later. The present paper describes another method (briefly outlined by Searle, 1968) which is based on the intercrossing of translocation heterozygotes carrying genetic

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tags, to allow identification of zygotes derived from complementary unbalanced gametes. By this method it is possible to identify a group of progeny resulting from that type of 2-and-2 segregation of the translocation quadrivalent which has been called 'adjacent-2 disjunction' by McClintock (1945). Because homologous centromeres go to the same pole of the spindle at first meiotic division adjacent-2 disjunction leads to the production of unbalanced gametes only. Its frequency can be estimated by the methods to be described and the position of the centromere on the genetically marked chromosome can be deduced. This has been done for linkage groups V, XIII and XIV. The amount of adjacent-2 disjunction seems roughly the same in the three translocations studied despite their different cytological properties.

In this paper 'translocation' means reciprocal translocation except where otherwise stated. 'Segment' denotes one of the two parts into which a chromosome is divided at the point of exchange but an 'interstitial segment' lies between the centromere and point of exchange. 'Arm' denotes two homologous segments subsequent to pairing.

2. METHOD

Translocation heterozygotes give rise to both balanced gametes and unbalanced ones carrying duplications and deficiencies. The more common types of disjunction known as alternate and adjacent-1 (McClintock, 1945) become effectively equivalent in mouse translocations, because of chiasma formation in the interstitial segments. They produce duplications and deficiencies that affect only the distal (non-centromeric) segment of the chromosomes involved in the translocation, whereas with adjacent-2 disjunction the centromeric segments (proximal to the point of exchange) are duplicated or deficient. As Snell (1946) showed, when translocation heterozygotes are intercrossed, the fusion of unbalanced gametes complementary to each other (i.e. with a duplication and deficiency in one corresponding to a deficiency and duplication in the other) gives rise to a viable zygote. Thus if the proximal and distal segments of translocated chromosomes are appropriately tagged with genetic markers, then it is possible to distinguish two groups of exceptional progeny arising from this complementation process. The more common one will involve duplication of a distal segment and the rarer, duplication of a proximal segment. This is the principle by which one can deduce in which tagged segment the centromere lies.

(i) *Expected frequencies of zygotic classes*

Text-fig. 1 shows diagrammatically the standard quadrivalent configuration found in stages from diplotene to metaphase in a mouse translocation heterozygote involving two non-homologous chromosomes AB and CD, where A and C represent the proximal and B and D the distal segments. Chiasmata are represented in all four arms, which is the most usual condition and leads cytologically to formation of a ring of four elements (R IV) at first meiotic division. Failure of association in one arm to give a chain quadrivalent (C IV), or in two opposite arms to give two bivalents (2 II), is also common. Failure of association in two adjacent arms to give trivalent plus univalent (III + I) occurs less frequently, except in particular translocations. Other derivative forms (II + 2 I, 4 I) are rare.

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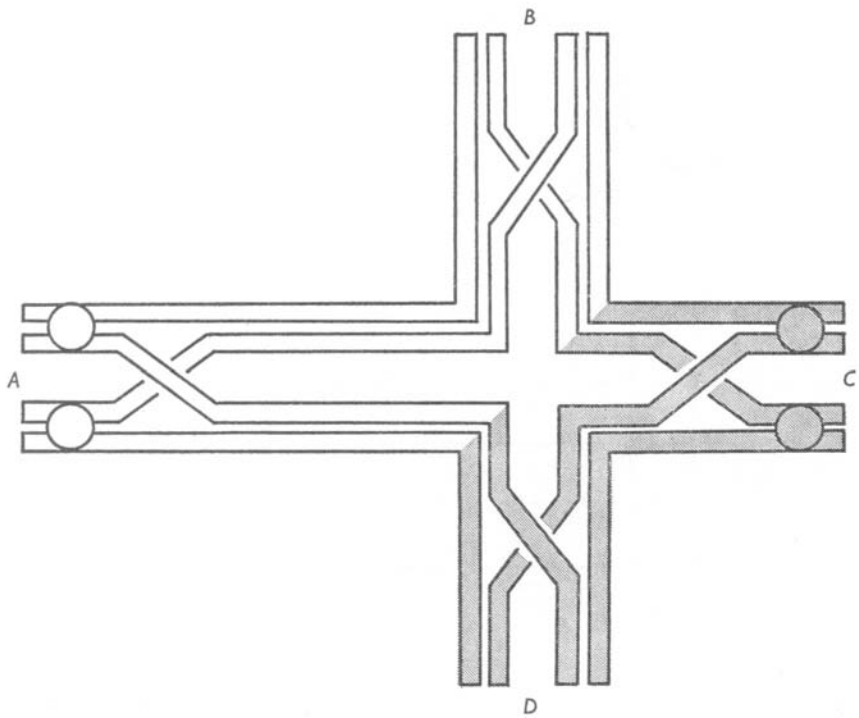
Normal disjunction of homologous centromeres is expected to yield equal numbers of *AB.CD*, *AD.CB*, *AB.CB*, and *AD.CD* gametes,* provided there is chiasma formation in at least one of the interstitial segments (see Lewis & John, 1963; Ford, 1969; Ford *et al.* 1969). The fact that double chiasmata of the compensating 2- or 4-strand type have the same segmental consequences as the absence of chiasmata may provide very rare exceptions to this general rule. The last two types of gamete listed above are unbalanced, but if an *AB.CB* gamete from one parent fuses with a complementary *AD.CD* gamete from the other then a balanced zygote will result which is heterozygous for the reciprocal translocation. It will also be homozygous for any genetic marker carried on both *B* segments of the *AB.CB* gamete regardless of the genotype of the parent contributing the other gamete.



Text-fig. 1. Diagram of standard cross-configuration at diplotene for a translocation heterozygote involving chromosomes *AB* and *CD* (the latter stippled). The centromeres are shown at *A* and *C* and there are single chiasmata in all four arms.

Adjacent-2 disjunction has quite different consequences (Ford & Clegg, 1969). Let us consider these with reference to the *A* centromeres of Text-fig. 1. If both of these go to the same pole and there was a chiasma in the corresponding interstitial segment, then two *AB.AD* dyads will be included in the interphase nucleus. With normal disjunction at the 2nd division the resultant gametes and ratios would be 1 *AB.AB*:2 *AB.AD*:1 *AD.AD*. However, if no chiasma is formed in *A* (but one or more are formed in *C*), then *AB.AB* and *AD.AD* dyads will go the same pole at anaphase, so

* I.e. gametes carrying chromosomes *AB* and *CD*, etc.

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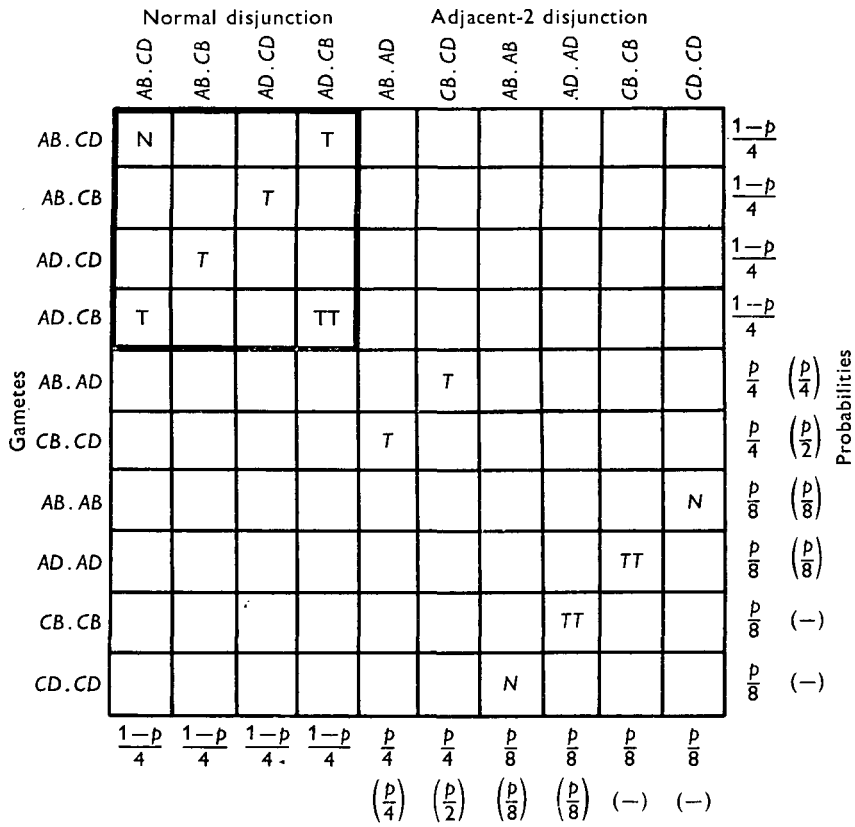
that the resultant gametes from these dyads will all be $AB.AD$. Where two chiasmata are formed the consequences will depend on whether 2, 3 or 4 strands are involved. With 2 or 4 strands the resultant gametes will have the same segmental constitution as when there is no chiasma, but with three they will follow the 1-chiasma pattern. On the assumption that chromatid interference does not occur, 3-strand doubles will be as frequent as 2-strand and 4-strand doubles combined. Thus in calculations the double chiasma class can be divided equally between the 0 and 1 chiasma classes.



Text-fig. 2. Checkerboard showing the types of balanced zygote to be expected from intercrossing translocation heterozygotes of type $AB.CD/AD.CB$ as shown in Fig. 1. Products of numerical non-disjunction are omitted. Blank squares represent unbalanced zygotes, normally inviable. Gametic probabilities after normal disjunction and after adjacent-2 disjunction are expressed in terms of p, the overall frequency of adjacent-2 disjunction. The probabilities in parentheses apply when there is adjacent-2 disjunction and no chiasma in the interstitial segment of arm C but 1 chiasma in arm A. N, T and TT signify zygotes which are normal, heterozygous and homozygous for the translocation, respectively. These are italicized when the zygote results from complementation of two unbalanced gametes.

A special situation arises when both points of exchange are near the centromere, so that interstitial segments are short and frequently have no chiasmata. Two bivalents with heterologous centromeres would normally result. If they segregate independently as expected, homologous centromeres would go to the same pole as

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frequently as heterologous ones, so that adjacent-2 disjunction (if the term can still be used) and alternate disjunction would be equally frequent, while adjacent-1 disjunction should be very rare. Disregarding the latter category, resultant gametes would be $AB.AD$, $CB.CD$, $AB.CD$ and $AD.CB$ in equal numbers. The first two are unbalanced, so that on outcrossing one would expect about 50% extra embryonic mortality. Further consideration of this category of translocations will be deferred to a later paper.

Table 1. *Expected frequencies of various events among offspring of translocation heterozygotes in which* (*i*) *both interstitial segments have a single chiasma, or* (*ii*) *one particular interstitial segment has a chiasma while the other has not*

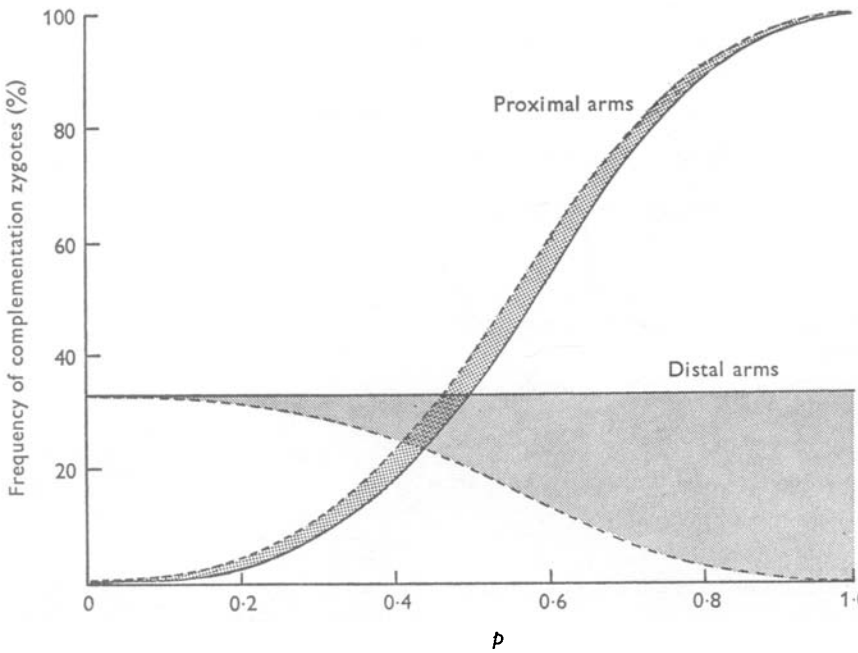
(Frequency of adjacent-2 disjunction = p. The values in III–VI must be halved to give the expected proportions of classified offspring homozygous for a particular marker on a distal or proximal arm, when one parent is homozygous for it and the other homozygous normal.)

	Both with chiasma	One with chiasma
I. Viability of zygotes after outcrossing, relative to normal	$\frac{1-p}{2}$	$\frac{1-p}{2}$
II. Viability of zygotes after intercrossing, relative to normal	$\frac{3}{8}-\frac{12p-9p^2}{16}$	$\frac{3}{8}-\frac{6p-5p^2}{8}$
III. Proportion of intercross survivors resulting from complementation involving distal arms	1/3	$\frac{1-2p+p^2}{3-6p+5p^2}$
IV. As III: involving proximal arms	$\frac{p^2}{2-4p+3p^2}$	$\frac{2p^2}{3-6p+5p^2}$
V. As III, if translocation homozygote lethal	$\frac{4-8p+5p^2}{10-30p+15p^2}$	$\frac{2-4p+2p^2}{5-10p+9p^2}$
VI. As IV, if translocation homozygote lethal	$\frac{p^2}{2-4p+3p^2}$	$\frac{4p^2}{5-10p+9p^2}$

The expected consequences of intercrossing translocation heterozygotes are best shown by means of a checker-board (Text-fig. 2) in which the blank squares represent unbalanced zygotes, normally dying *in utero*. Numerically unequal (3:1 and 4:0) segregations of the four elements of the translocation complex are considered to be much less likely and have been ignored. The frequency of adjacent-2 disjunction is taken as p, so that the frequency of gametes with duplication of a particular proximal segment and associated genetic marker is $p/2$. The probabilities in brackets relate to gametes arising from adjacent-2 disjunction in which there was one chiasma in the interstitial segment of A but none in that of C. Those zygotes which arise as the result of complementation of unbalanced gametes are italicized. As already stated, with disjunction of homologous centromeres complementation only involves the distal arms of chromosomes, but with adjacent-2 disjunction it may involve either the proximal arms or both distal and proximal.

The expected frequencies of various events on intercrossing and on outcrossing translocation heterozygotes can be calculated from the checker-board and are shown in Table 1 for two alternative possibilities with respect to chiasma formation in

interstitial segments. Normally, of course, one will have a mixture of these. In this table it is assumed that no unbalanced zygotes survive to birth and that there is no gametic selection (see Ford *et al.* 1969). It is interesting to note that where interstitial segments have single chiasmata (which is the usual situation) the expected proportion of survivors which result from complementation with respect to distal arms remains at 1/3 whatever the frequency of p. Thus, if a translocation heterozygote fulfilling



Text-fig. 3. Expected frequencies of balanced complementation zygotes homozygous for proximal arms and associated markers or for distal arms and markers, with increasing frequencies of adjacent-2 disjunction (p). The solid lines show expectations with single chiasmata always present in both interstitial segments and the interrupted lines apply in the absence of such chiasmata from one particular interstitial segment. The stippled areas represent the range of expectations between these extremes.

this condition is homozygous for a recessive marker situated in a distal arm of one of the two chromosomes involved, and is intercrossed to a translocation heterozygote not carrying that marker, then one would expect 1/6 of the progeny to be homozygous for the marker.

Text-fig. 3 shows how the expected proportions of balanced zygotes homozygous for proximal markers or for distal markers as a result of complementation vary with increasing frequency of adjacent-2 disjunction, and with presence of chiasmata in both or in one interstitial segment. It can be seen that even if there is regularly no chiasma in the interstitial segment of arm A of a translocation heterozygote $AB.CD/AD.CB$, though the opposite segment has one, the expected proportion of offspring homozygous for a distal marker (on BB) as the result of complementation will be higher than that of those homozygous for a proximal marker (on AA), provided p

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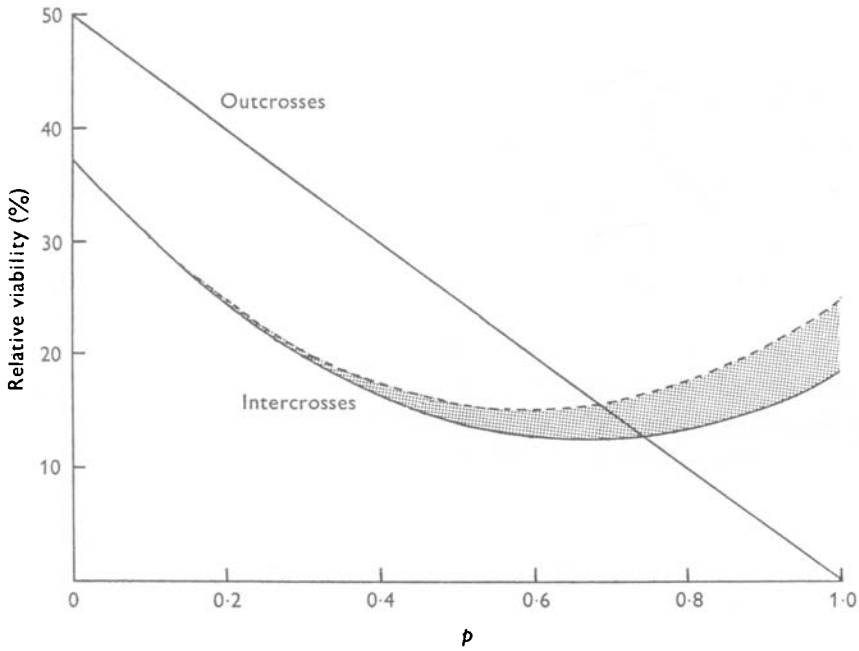
does not exceed 0·4. The area of discrimination extends as far as a p value of nearly 0·5 if there is regularly one chiasma in both interstitial segments concerned. Therefore this method of distinguishing between proximal and distal arms should be applicable to most known mouse translocations, since they seem to have a fairly low rate of adjacent-2 disjunction, as judged by relative survival of outcross progeny (Carter, Lyon & Phillips (1955) and the present paper) and by cytological evidence (Ford, Carter & Hamerton, unpublished). It is likely to work best with translocations in which the point of interchange is nearly central on the chromosome concerned, since this will make it more probable that a chiasma is formed in both interstitial segments.



Text-fig. 4. Expected viabilities relative to normal in outcross and intercross progeny of translocation heterozygotes, with increasing frequencies of adjacent-2 disjunction. For intercross progeny, the solid line represents the situation in the presence of single chiasmata in both interstitial segments, while the interrupted line refers to absence of chiasmata from one of the two segments. The stippled area represents the range of expectations between these extremes.

(ii) *Interpretation of survival data*

Text-fig. 4 shows how rates of survival of outcross and intercross progeny relative to normal depend on the frequency of adjacent-2 disjunction. For intercross progeny they also depend on the presence or absence of chiasmata in interstitial segments, with minimum survival at $p = 0{\cdot}66$ if a chiasma is always present and at $p = 0{\cdot}60$ if a chiasma is present in one arm but not the other. Thus the relative survival of intercross progeny can also lead to an estimate of the extent of adjacent-2 disjunction, especially if the cytological picture has been studied. It has been assumed in these calculations that the frequency of numerical non-disjunction is negligible in

translocation heterozygotes, as it is in normals. This may not hold for certain translocations, especially those giving III + I configurations.

(iii) *Experimental procedure*

It was decided to use the method described for determining centromere positions in mouse linkage groups V, XIII and XIV, and for finding the frequency of adjacent-2 disjunction in the intercrossed translocation heterozygotes. The experimental procedure was as follows.

(1) The spectrum of cytological configurations at meiosis was studied in males heterozygous for the translocations. The approximate position of the point of exchange on one or both of the linkage groups involved had been determined previously by genetic methods. The relative viabilities of progeny from translocation heterozygotes and normals were estimated from the ratios of live embryos to number of uteri, as in Carter *et al.* (1955).

(2) Translocation heterozygotes were mated to homozygotes for markers on each side of the point of exchange. Recombinants were obtained between translocation and marker, so that marker homozygotes carrying the translocation could be produced.

(3) Translocation heterozygotes were intercrossed, one of the pair being homozygous for one or more of the markers, while the other was homozygous normal with respect to those markers.

(4) The frequency of homozygosity for the markers in a large number of offspring was determined and the results compared with expectation if each marker was (*a*) distal, (*b*) proximal (Text-fig. 3). Table 1 and Text-figs. 3 and 4 were used to make different estimates of the frequency of adjacent-2 disjunction.

3. RESULTS

(i) *Linkage group V*

Translocation *T(5;18)26H*, derived from a specific locus experiment involving chronic neutron exposures (Batchelor, Phillips & Searle, 1966), was used to determine centromere position in linkage group V. This translocation is of particular interest because it has a phenotypic effect on agouti coloration, the homozygote being dark agouti with dark pinna-hairs. The heterozygote is umbrous agouti with light pinna-hairs if *a* or a^t is on the other chromosome. No recombination has been found between the translocation and agouti alleles, so the point of exchange seems to be at the agouti locus (see Text-fig. 5).

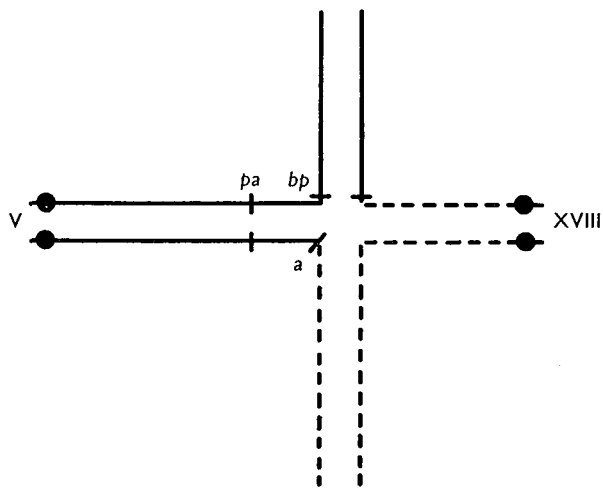
The recessive genes pallid *pa* and brachypodism *bp*, which are on opposite sides of the agouti locus (see Green, 1966) were chosen as suitable markers. Both can be easily classified at birth and are fully penetrant. Backcrosses of *pa* + *bp* + *T26H* + mice to *pa* + *bp*/*pa* + *bp* gave the following phenotypes (results from both sexes combined):

+ ? +	*pa* ? *bp*	*pa T26H* +	+ + *bp*	*pa* + +	+ *T26H bp*
96	91	6 + 1?	11	1	2

Only the recombinant types with respect to the markers were tested for presence of T26H. The one queried mouse gave an inconclusive result in her semi-sterility test.

These results agree with expectation if the translocation point of exchange does lie at the *a* locus.

T26H pa/+ *pa* and *T26H bp*/+ *bp* mice were then crossed to *T26H*/+ wild-type. Both types of complementation homozygote were obtained (Table 2) but the frequency of offspring with brachypodism was significantly higher than the frequency of pallids. The classification for brachypodism in late foetal life were carried out when it was discovered that no *bp*/*bp* offspring survived longer than 5 days after birth and some were dead when first found. This was surprising, since they normally show little or no reduction in viability. The higher foetal frequency suggests that some are stillborn and eaten by the mother before the litter can be recorded.



Text-fig. 5. Diagram of pachytene cross in *T(5;18)26H* heterozygote, to show approximate positions of loci used in linkage tests and intercrosses. Linkage groups are shown in Roman numerals and centromere locations (as deduced from the present experiments) are indicated by solid circles. The actual distances of centromeres from nearest markers are not known.

Table 2. *Frequencies of offspring homozygous for recessive markers in heterozygous* T26H *intercrosses where one parent is homozygous for the marker and the other homozygous normal*

Marker	Time of classification	No. homozygous	Total classified	Frequency (%)
bp	Late pregnancy	17	110	15·5
	Birth	37	377	9·8
pa	Birth	3*	189	1·6

For classifications at birth, $\chi_1^2 = 11{\cdot}8$, $P = 0{\cdot}0006$.
* Two semi-sterile, 1 with test inconclusive.

Cytological studies of primary spermatocytes of *T26H* heterozygotes gave the following spectrum of configurations: 1 cell with 20 bivalents, 41 with chains of IV, 158 with rings of IV. The translocation was between a long chromosome and a

medium one; failure of association normally involved the proximal arm of the medium chromosome. Plate 1 shows typical configurations. Clearly the long chromosome is the one involving linkage group V, which is one of the longest in the mouse genome as judged both by cytological (Griffen, 1960; Slizynski, 1949, 1952) and genetical (see Green, 1966) data. Eleven per cent of the rings and 12% of the chains had two chiasmata in one arm, which was normally the proximal arm contributed by the linkage group V chromosome. It can be deduced that most zygotes will be derived from gametes produced by spermatocytes which had one chiasma in each interstitial segment at meiosis.

Further evidence for the occurrence of two chiasmata in the linkage group V proximal arm came from the results of the *bp* outcrosses. The mice used were in fact *bp+pa/bpT26H+*, mated to wild-type *T26H/+*. Besides the 37 brachypod offspring (Table 2) there were also two pallids (0·5%), one of which was proved to be heterozygous for *T26H*. The only way in which such an offspring could arise seems to be by the formation of two chiasmata in the linkage group V interstitial segment, one on each side of *pa*, followed by adjacent-2 disjunction. In this way *AB.AB* and *AD.AD* dyads, both *+pa*, would be formed (see Text-fig. 1) and one of the four resultant gametes would be *AB.AD papa*.

The relative total (pre- and post-implantation) viability did not differ significantly in embryos from outcrossed *T26H/+* males and females (Table 4), the mean being 0·44. As expected, the total viability in the offspring of intercrossed *T26H+* was much lower, being 26% of the mean of the two control viabilities. From Text-fig. 4, the values of *p* corresponding to these estimates are 0·12 (outcross) and 0·19 (intercross). With these values the expected frequency of homozygotes for a distal marker is around 16%. This is a good fit to the frequency of 15·5% *bp/bp* with late foetal examination; thus *bp* must be a distal marker and *pa* proximal. The 1·6% frequency of pallid offspring would correspond to a *p* value of 0·20 if single chiasmata are always present or 0·18 if one interstitial segment had a chiasma and the other not. Since the frequencies of 0 and 2 chiasmata were low the former estimate was used. Combining the three estimates leads to an average value for *p* of 0·17 (Table 7). Since *pa* is clearly on the proximal side of the point of interchange, the centromere must lie at the *Sd* end of linkage group V.

Because *T26H* homozygotes had a distinct phenotype (dark agouti with dark pinna-hairs) it was possible to determine their frequency in the two sets of intercross progeny. 27/111 (24·3 ± 4·1%) were classified as having the homozygous phenotype in the *T26H* intercrosses involving *pa*, in which a frequency of about 1/6 would be expected (Text-fig. 2), while 52/159 (32·7 ± 3·7%) were found in the intercross involving *bp*, in which a frequency of about 1/5 would be expected because of the early death of *bpbp* mice. Both observed frequencies are higher than expectation, the latter significantly so; the reason for this is unknown.

The unexpected neonatal death of *bp/bp* offspring in the *T26H/+* intercrosses is still being investigated. It seems to be connected with presence of *bp* rather than just with formation of a particular type of complementation zygote. For if the latter was true one would expect extra neonatal mortality in the intercrosses involving *pa*

as well as those involving *bp*. This was not so, the figures being 13% mortality in the *pa* outcross compared with 25% in the *bp* outcross.

Translocation *T(2;5)11H*, which has the point of exchange about 2 units from *pa*, has been used to see if the same mortality occurs when the *bp* locus is further from the point of exchange. Crosses of *T11H bp/+ bp* and *T11H +/+ +* gave 19/123 newborn offspring with brachypodism (15·4%). So far, six offspring have survived beyond the neonatal period. Fertility tests have been made on five, all of which proved semi-sterile, as expected. The improved survival and higher proportion of recorded *bp/bp* offspring at birth in the T11H crosses suggests that the distance of *bp* from the breakpoint may well be an important factor. The results also confirm the location of the centromere at the *Sd* end of linkage group V.

Table 3. *Frequencies of offspring homozygous for recessive markers in heterozygous* T70H *intercrosses*

Marker	No. homozygous	Total classified	Frequency (%)
fz	1*	91	1·1
ln	10†	56	17·9

For exact 2×2 test, $P = 0{\cdot}0003$.
* Semi-sterile. † Eight semi-sterile, 2 fertile.

(ii) *Linkage group XIII*

Translocation *T(13;?)70H*, derived from an experiment in which hybrid male mice were exposed to two fractions of 600 R acute X-irradiation 8 weeks apart (Lyon, Phillips & Searle, 1964), was used to determine centromere position on linkage group XIII. Backcrosses of *+ T70H +/fz + ln* mice to homozygous fuzzy leadens (*fz ln/fz ln*) gave offspring of the following phenotypes:

+ T70H +	*fz + ln*	*fz T70H +*	*+ + ln*	*+ T70H ln*	*fz + +*	*fz T70H ln*	*+ + +*
47	41	4	2	10	7	2	1

The estimated recombination frequency between the point of exchange and *fz* is 7·9%, the figure for *ln* being 17·5%. Since the map distance between *fz* and *ln* is about 41 units (see Green, 1966) the point of exchange appears to lie between these two loci (Text-fig. 6). Figures for presumptive double crossovers agree with this but there are signs of some crossover suppression.

In *T70H/+* intercrosses combined with outcrosses of either *fz* or *ln* homozygotes, a much higher proportion of leaden than fuzzy offspring were recorded (Table 3). Two leaden offspring were fully fertile; these were not tested for *T70H* homozygosity, but were presumed to be homozygous normal. Evidence from *T70H fz/+ +* intercrosses strongly suggested that the translocation homozygote was lethal; however, an undoubted homozygote has now been obtained from different intercrosses. It seems likely that a lethal gene close to the point of exchange was responsible for the earlier findings, but this is being investigated further.

Cytological examination of spermatocytes of *T70H/+* males revealed the following

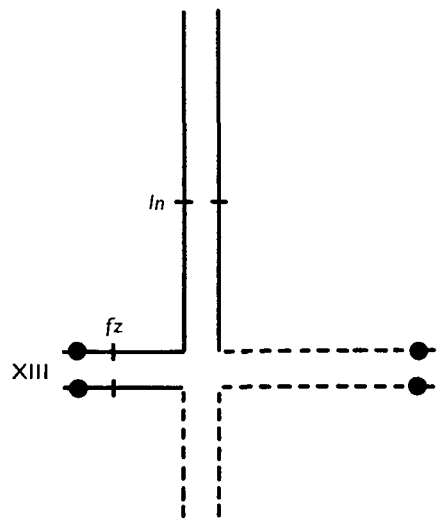
Table 4. *Relative post-implantation (A) and total (B) viability of embryos in outcrosses and intercrosses of* T26H *heterozygotes, as compared with outcrosses of normal members of the same stock*

Mating	Sex	No. of uteri	Live embryos	Total implants	Live embryo / Total implant	Ratio (A)	Live embryo / No. uteri	Ratio (B)
T26H outcross	♂	40	139	333	0·411	0·468 ± 0·032	3·48	0·463 ± 0·048
Control outcross	♂	40	300	336	0·898		7·50	
T26H outcross	♀	20	71	173	0·405	0·423 ± 0·039	3·55	0·420 ± 0·059
Control outcross	♀	20	169	174	0·971		8·45	
T26H intercross		55	110	424	0·259	0·282 ± 0·023	2·00	0·256 ± 0·027

Table 5. *Relative post-implantation (A) and total (B) viability of embryos in outcrosses of presumptive* T70H *heterozygotes (semi-sterile), as compared with fully fertile members of the same stock*

Outcross	Sex	No. of uteri	Live embryos	Total implants	Live embryo / Total implant	Ratio (A)	Live embryo / No. uteri	Ratio (B)
T70H outcross	♂	68	201	515	0·390	0·437 ± 0·025	2·96	0·445 ± 0·038
Control outcross	♂	61	405	453	0·894		6·64	
T70H outcross	♀	50	167	479	0·349	0·358 ± 0·023	3·34	0·325 ± 0·031
Control outcross	♀	30	308	316	0·975		10·27	

spectrum of configurations in 317 cells: 22 rings of IV; 256 chains of IV; 39 chains of III+I. As Plate 1 shows, the translocation was unequal, between long and medium chromosomes, giving rise to one very short element. A detailed examination of spermatocytes in first and second metaphase showed that failure of association was mainly in a proximal arm, probably in the one carrying part of linkage group XIII (Text-fig. 6). The existence of some crossing over in this arm is borne out by the two fully fertile leadens already mentioned. These presumably resulted from fusion of an *AB.AB* with a *CD.CD* gamete, both of which would result from adjacent-2 disjunction associated with a single chiasma in the interstitial segment.



Text-fig. 6. Diagram of pachytene cross in *T(13;?)70H* heterozygote. See Fig. 5 for further explanation.

Table 5 shows that in *T70H*, as in *T26H*, survival in heterozygotes was less than 50% normal. The absence of any good genetic tag means that decisions on presence of the translocation are based only on fertility tests, using the criteria of Carter *et al.* (1955). It is highly likely that a number of females giving an inconclusive result, and therefore omitted from the data of Table 5, will really have carried the translocation, thereby introducing a bias. The bias will be small or absent in males, each of which was mated to at least two females in order to give an accurate diagnosis. Thus the relative total viability in outcross progeny of semi-sterile males (0·445) has been used to give one estimate of the extent of adjacent-2 disjunction, namely 0·11. At this level, the expected frequency of homozygotes for a distal marker will be 19–20% if the translocation homozygote does not survive. The observed figure of 17·9% homozygous progeny in the leaden cross (Table 3) is in good agreement with this and shows that *ln* is in the distal arm of linkage group XIII. The low frequency of 1·1% homozygotes in the *fz* crosses is also a good agreement with expectation if *fz* is in the proximal arm. From Table 1 this frequency leads to another estimate of adjacent-2 disjunction frequency, namely 0·14.

The genotype of the fuzzy mice used in the *T70H+* intercrosses (Table 3) was *fzT70H+/fz+ln* while their mates were homozygous normal at both the *fz* and *ln* loci. Nevertheless, the offspring included two leaden mice, both heterozygous for *T70H*, as well as the single fuzzy. These leadens must have resulted from crossing over between *ln* and the point of exchange in the distal segment of the chromosome carrying linkage group XIII, followed by complementation of an *AB.BC fzlnln* gamete by an *AD.DC+* gamete. About 20% of the 91 offspring would be expected to result from complementation involving the distal end of this linkage group (see previous paragraph), while one-quarter of such complementation zygotes would show the leaden phenotype if there was always one chiasma between *ln* and the point of exchange. Thus a maximum of 4 or 5 leaden offspring would be expected. The fact that two were found suggests that single chiasmata were formed in this region in about half the spermatocytes. The corresponding recombination frequency should be roughly 25%, in agreement with linkage test results (13/76 or 17%).

(iii) *Linkage group XIV*

The translocation used was *T(14;17)264Ca*, first described by Carter *et al.* (1955). The point of exchange in linkage group XVII is closely tagged by W^v, while in group XIV it has been reported (Phillips, 1961) to lie about 8 units from flexed (*f*). Linkage tests between *T264* and *pe*, in which W^v was used as genetic tag for the former, gave $11/50 = 22 \pm 6\%$ recombinations in male heterozygotes. Similar tests between *T264* and *sa* and *bg*, also on linkage group XIV, gave the following phenotypes in backcrosses of heterozygous females:

W^v++	$+sabg$	$+++$	W^vsabg	W^v+bg	$+sa+$	Total
12	12	5	5	0	8	42

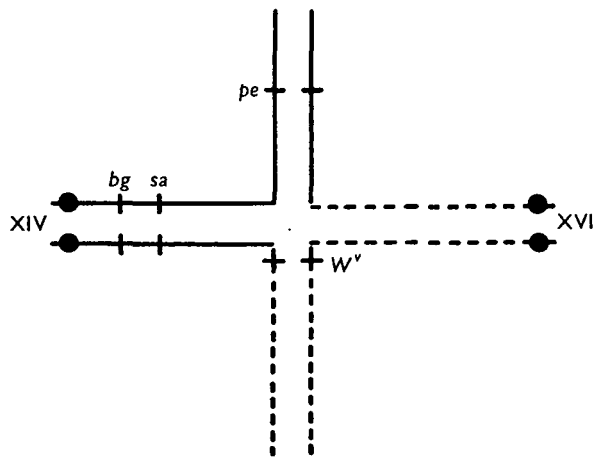
The recombination frequency between the W^v tag and *sa* is $24 \pm 7\%$ and between the W^v tag and *bg* is $43 \pm 8\%$. Lyon & Meredith (1969) have shown that the order of loci in this linkage group with respect to the genes under consideration is (map distances in brackets, sexes separate):

bg... (♀ 8·5, ♂ 3·5) ...*sa*... (♀ 15, ♂ 16) ...*f*... (♀ 25, ♂ 16) ...*pe*

Phillips (1961) found that the order was *T264–f–cr*. Since crinkled (*cr*) is close to beige (Lane, 1965), the data are all consistent with a location of the *T264* point of exchange between *f* and *pe*, probably closer to *f*. Thus it is between *sa* and *pe*, so these two recessives were chosen as genetic tags (see Text-fig. 7).

Table 6 shows that there was again a significant difference between the frequencies of the two types of homozygote in progeny from the test-matings. Carter *et al.* (1955) found that the total viability of embryos in outcrosses of *T264/+* males relative to normal was 0·475 (no data are available for females). From this figure the value of p can be estimated as 0·05 (Table 1). The cytological spectrum of multivalent configurations in 100 cells was 82 rings of IV, 8 chains of IV, 3 chains of III+I, 7 with 20 bivalents. In a previous survey, 392 rings of IV and 9 chains of IV were observed in 401 cells (Ford, 1969). The rings normally had one chiasma per interstitial segment (Plate 1). It can be seen from Fig. 3 that when $p = 0{\cdot}05$ the frequencies of homo-

zygotes for proximal and distal markers are close to 0% and 16·7% respectively whether chiasmata are formed or not. Thus the intercross results (Table 6) show that *sa* is at the proximal and *pe* at the distal end of the linkage group XIV. From Tables 6 and 1, assuming single chiasmata in each interstitial segment for the purposes of calculation, we can obtain another estimate of p, namely 0·13.



Text-fig. 7. Diagram of pachytene cross in *T(14;17)264Ca* heterozygote. The probable distal position of the W^v locus with respect to the point of exchange on linkage group XVII is based on the work of Roderick (1971).

Table 6. *Frequencies of offspring homozygous for recessive markers in* T264 W^v/+ + *intercrosses*

(As W^v/W^v mice could not be classified for *pe*, they are omitted from the *pe* results.)

Marker	No. homozygous	Total classified	Frequency (%)
sa	1*	145	0·7
pe	18†	109	16·5

For exact 2×2 test, $P = 5{\cdot}9 \times 10^{-6}$.
* Semi-sterile. † All W^v+, 8 tested were semi-sterile.

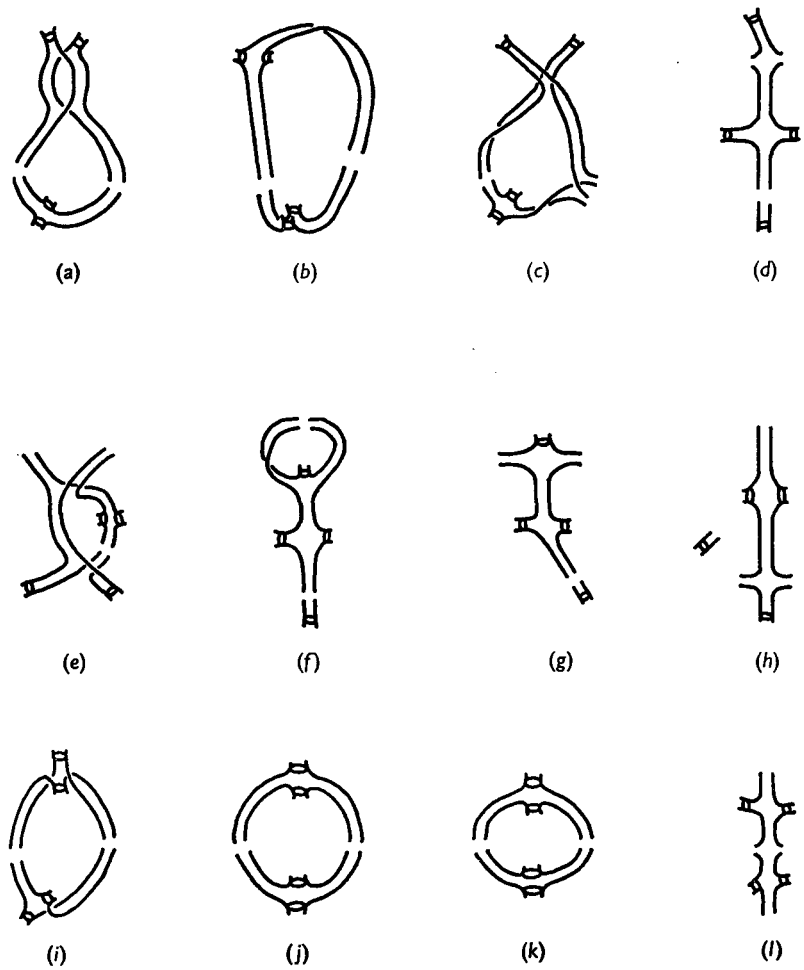
Since the pearl phenotype could not be classified on W^v homozygotes (black-eyed white) the expected frequency of *pe/pe* offspring is the same as if the translocation homozygote was lethal (Table 1), i.e. around 20%. Thus the actual frequency found is lower than expected, though not significantly so.

4. DISCUSSION

(i) *Centromere position*

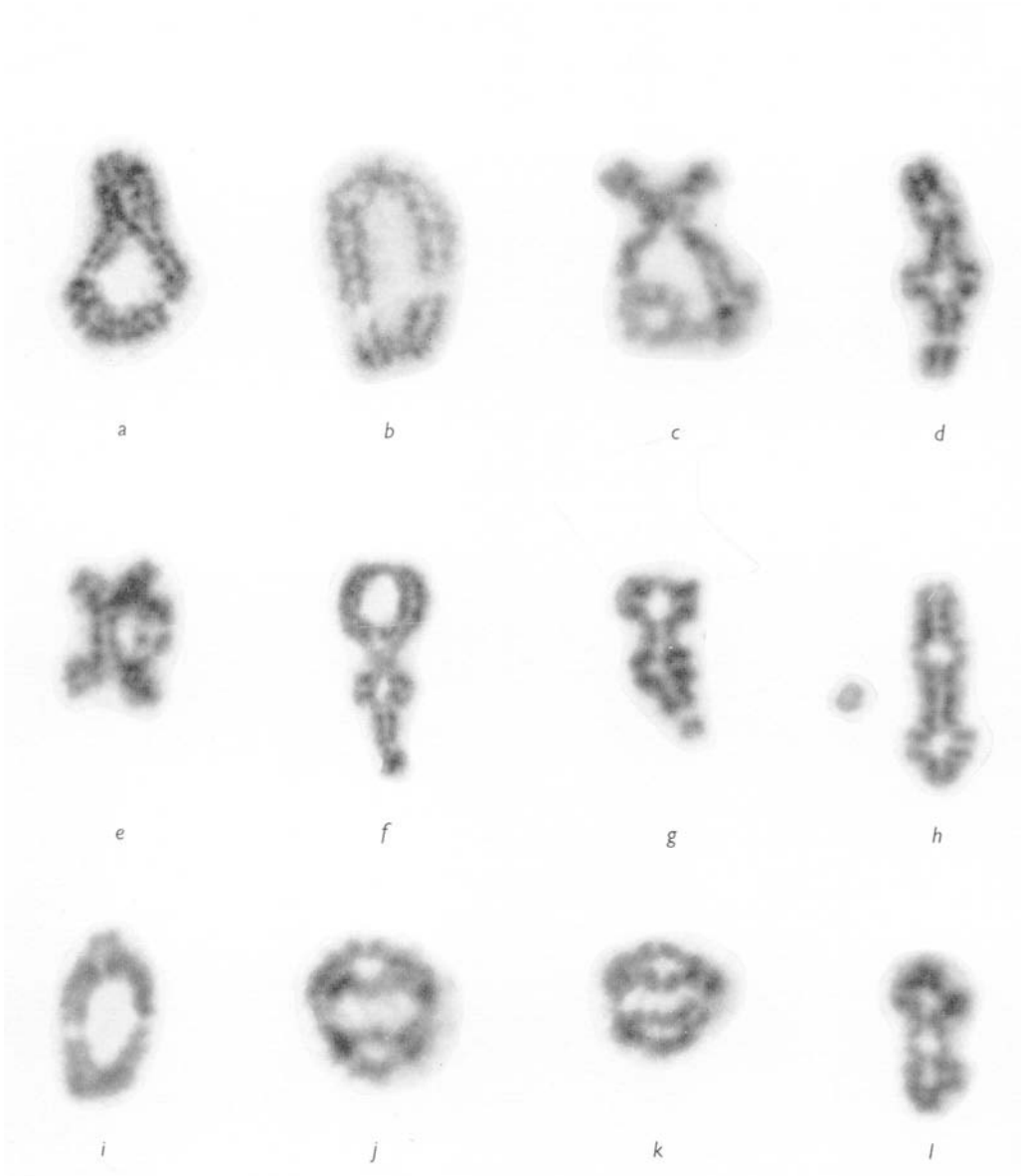
Previous attempts to fix centromere positions on mouse chromosomes have used rather different methods. Early work on the subject was based on the phenomenon of affinity (Michie & Wallace, 1953) in which the quasi-linkage of genes on

non-homologous chromosomes was supposed to result from the existence of more than one kind of centromere, those of the same kind tending to go to the same pole at meiosis. Markers close to such centromeres would segregate as if linked even if they were on non-homologous chromosomes. By this means, Wallace (1961) placed the centromere of linkage group V between *Sd* and *fi*, about 16 units from *Sd*. By the



Text-fig. 8. Chromatid diagrams of configurations shown in the Plate, with presumptive centromere positions.

same method, the centromere was placed very close to *se* in linkage group II, 8 units from *W* in linkage group III (now moved to group XVIII) and 17 units from *Ca* in linkage group VI (see Wallace, 1961). The postulated central centromeric positions for groups II and XVII conflict with the fact that centromeres on all mouse chromosomes are normally terminal or subterminal, as described earlier. Even in linkage group V, Wallace's findings are difficult to reconcile with the cytological picture of at most a very short arm beyond the centromere and the lack of chiasmata in this



Translocation configurations at diakinesis – metaphase I. *a–d*, T26H; *e–h*, T70H; *i–l*, T264Ca.

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region (Ford & Evans, 1964). However, our own findings do place the centromere at the *Sd* rather than the *Ra* end of linkage group V.

Evidence of centromere positions in linkage group V and VIII was obtained by Ford, Carter & Hamerton (1956) by cytological examination of Snell's translocation, *T(5;8)Sn*. They found that heterozygotes for this translocation form ring quadrivalents at meiosis in less than 30% of cells, other cells containing bivalents with failure of association in non-centromeric arms. Therefore the maximum amount of recombination to be expected between markers in adjacent non-centromeric arms was 15%. Since the *a* locus marked the point of exchange and since Snell (1946) had reported more than 30% recombination between *a* and *b* in *T(5;8)Sn* heterozygotes, therefore the *b* locus must lie in the centromeric arm of linkage group VIII, proximal to the translocation's point of exchange. Moreover, Snell had obtained two pallid brown offspring from crosses of *paTSn+/pa++* × *+TSnb/++b* and had deduced that the loci for *pa* and *b* were on opposite rather than adjacent arms. If so *pa*, like *b*, would be in a centromeric arm and thus the centromere would be at the *Sd* end of linkage group *V*. It should be noted from Text-fig. 2 that pallid browns could also be obtained even if *pa* and *b* were on adjacent rather than opposite arms, provided single chiasmata were formed. However, higher frequencies of whichever marker was distal would then be expected, whereas in fact no pallid non-browns or brown non-pallids were observed. Moreover, the low frequencies of pallid offspring (2 among 504 classified for pink eyes = 0·4%) and of brown (2/322 = 0·6%) found by Snell also suggest that these are proximal to the point of exchange. This would mean that centromeres are at the *Sd* end of linkage group V and the *wd* end of linkage group VIII, and these positions can be regarded as firmly established.

Translocations giving rise to metacentric chromosomes are likely to prove very useful for determining centromere positions in the mouse because of the necessarily close proximity between the point of exchange and the centromere. Thus linkage tests to determine the position of the former will also locate the latter. In this way Lyon, Butler & Kemp (1968) have used translocation *T(2;12)163H*, which results in a submetacentric chromosome, to locate the position of the centromere at the *cw* end of linkage group II. With this information it was possible also to deduce centromere position in linkage group IX, by studying segregation in homozygotes for translocation *T(2;9)138Ca*. The genes *d* and *T* are linked in these homozygotes, which shows that they must be on the same rearranged chromosome, but on opposite sides of the point of exchange. Since *d* is known to be distal to the point of exchange for *T138Ca* in linkage group II then *T* must be on the proximal side for linkage group IX.

Thus several different methods involving translocations have proved useful for the location of centromeres. Only the metacentric method has so far given information on the map distance from the centromere to the nearest genetic marker, though it should be theoretically possible to obtain such information from translocation intercrosses in which one of the parents is heterozygous for a proximal marker and the other homozygous normal. Only when there is crossing over between the centromere and these markers will there be a chance for offspring homozygous for the marker to be obtained. This phenomenon occurred with *pa* in the *T26H* experiments (*bp/bp*

outcrosses) but clearly a very large and laborious experiment would be needed in order to obtain accurate estimates of map distance by this method. Where a translocation has a good genetic tag, close to the point of exchange, and has cytologically distinguishable arms, then chiasma counts in good preparations of spermatocytes at diakinesis should allow one to calculate the map distance from the marker to the centromere. Thus, by cytological examination of *T26H*/+ spermatocytes, Ford (unpublished) has estimated the distance from *a* (the point of exchange) to the proximal end of the linkage group V chromosome to be 56 cM. It is interesting to compare this with Wallace's (1961) estimate of the a^t-*Sd* map distance in males, made by applying Kosambi's (1944) method to her own balanced recombination data, as 55·8 cM. This suggests that the linkage group V centromere is not far from *Sd*.

(ii) *Adjacent-2 disjunction*

Adjacent-2 disjunction can only give a balanced zygote when an unbalanced gamete from one parent complements an unbalanced one from the other. Therefore, the possibility of sex differences in its frequency should be considered. Evidence in favour of such differences comes from the data of Carter *et al.* (1955) on the relative viabilities of embryos in matings of male and female heterozygotes from seven different translocation stocks. In all seven, both the total survival and the post-implantation survival were lower for female heterozygotes, although the summed differences did not quite reach a significant level. If lowered relative survival is mainly the result of increased adjacent-2 disjunction, as seems probable, then its frequency may well tend to be higher in females.

The gametic spectrum produced by translocation heterozygotes should also depend on chiasma frequencies, as we have seen. There is some uncertainty on the extent of sex differences in this character, though recombination frequencies are generally higher in females than in males. Dunn & Bennett (1967) reviewed all the recombination data in the mouse from this point of view and found that only in linkage group VI did recombination frequencies tend to be significantly higher in males. For 18 out of 19 significant differences in other linkage groups the recombination frequency was higher in females.

Only a large-scale experiment, involving translocation heterozygotes in which all four arms are marked and crosses are made in both directions, will provide sufficient data for disentangling all the variables and seeing if there is agreement between breeding results, observations on chiasma frequency and relative survival, and theoretical expectations. However, by the use of Table 1 and the cytological and genetical data available, rough estimates can be made of the frequency of adjacent-2 disjunction associated with each of the three translocations used in the present experiment. Table 7 shows that the values of p obtained by different methods agree reasonably well on the whole, as do the mean values for different translocations, even though *T70H* forms mainly chain quadrivalents while the other two form mainly rings. The overall frequency of adjacent-2 disjunction is about 13%.

Further information is available from studies on Snell's translocation. Ford and Hamerton (unpublished data) have made direct cytological observations at late

metaphase to early anaphase of adjacent-2 disjunction in male *TSn* heterozygotes. They found that its frequency was about 10%, in very good agreement with their calculations from Snell's (1946) genetic data.

Table 7. *Estimates of the frequencies of adjacent-2 disjunction in three different translocation heterozygotes by three different methods*

	Method			
Translocation	Frequency of homozygotes for proximal markers	Relative viability of outcross progeny	Relative viability of intercross progeny	Mean value
T26H	0·20	0·12	0·19	0·17
T70H	0·14	0·11*	—†	0·13
T264Ca	0·13	0·05*	—†	0·09

* Based on outcrossed males only.

† Omitted through lack of suitable data from fully fertile relations.

These frequencies are all quite high, but the data of Carter *et al.* (1955) on relative survival in various translocation stocks indicates that a much higher frequency may hold for certain translocations. In outcrosses of heterozygotes for the *T6* marker translocation, for instance, the mean relative viability is about 0·37, which formally corresponds to an overall frequency of adjacent-2 disjunction of 26%. However, lowered survival frequencies may depend on other factors besides adjacent-2 disjunction. Thus *T6* gives mainly III + I configurations, so numerical non-disjunction is likely to be common. The most accurate estimates of adjacent-2 disjunction are likely to come from the use of genetic tags combined with translocation intercrosses as in the present experiment, since it is difficult to envisage any other way in which homozygous progeny can have arisen from outcrosses of homozygotes with such a relatively high frequency.

It may be of interest to compare the frequency of adjacent-2 disjunction with that of non-disjunction in the mouse. Information on the latter is meagre, but simultaneous non-disjunction has been invoked as the most likely explanation for the occurrence of normal dilute short-ear offspring (*dse/dse*) from a cross of wild-type mice with members of a specific locus stock (Russell & Russell, 1960) homozygous for *d* and *se*, which are closely linked genes on linkage group II. Four have occurred in a control series, which totals over 531 500 mice (Russell, 1960). Since only one-quarter of zygotes resulting from simultaneous non-disjunction would be detectable, the frequency of non-disjunction for the linkage group II chromosome may be around 0·5–0·6%. However, Russell & Russell state that the spontaneous non-disjunction frequency for the other four autosomes marked in these specific locus experiments could not be as high as the frequency for chromosome II. Therefore the overall frequency of non-disjunction in all autosomes of the mouse is likely to be decidedly lower than the frequency of adjacent-2 disjunction associated with particular translocations.

Cytogenetic studies on plants by McClintock (1945) and Burnham (1950) seemed to show that adjacent-2 disjunction did not occur when there was crossing-over in the interstitial segment of a translocation heterozygote (see also Lewis & John, 1963). Our findings from the mouse do not agree with this conclusion, since nearly all the spermatocytes examined in heterozygotes of all three translocations showed the presence of one or more chiasmata in at least one of the two interstitial segments. Yet the mean frequency of adjacent-2 disjunction was 13%. In addition, the recovery of two fully fertile leaden progeny from the *T70H* intercrosses can only be simply explained by adjacent-2 disjunction associated with chiasmata in the corresponding interstitial segments.

The frequency of adjacent-2 disjunction must depend ultimately on the factors influencing orientation of multivalent configurations on the spindle. These are likely to include relative arm-lengths, the number and position of chiasmata and perhaps factors specifically influencing centromeric behaviour, such as the temporal relationship between the onset of centric repulsion and congression on the metaphase plate (see Darlington, 1937). These may well differ considerably between different species. Studies of the characteristics of mouse translocation heterozygotes which give widely different frequencies of adjacent-2 disjunction may help to unravel these factors.

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REFERENCES

Batchelor, A. L., Phillips, R. J. S. & Searle, A. G. (1966). A comparison of the mutagenic effectiveness of chronic neutron- and γ-irradiation of mouse spermatogonia. *Mutation Research* **3**, 218–229.

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

Carter, T. C., Lyon, M. F. & Phillips, R. J. S. (1955). Gene-tagged chromosome translocations in eleven stocks of mice. *Journal of Genetics* **53**, 154–166.

Dunn, L. C. & Bennett, D. (1967). Sex difference in recombination of linked genes in animals. *Genetical Research* **9**, 211–220.

Darlington, C. D. (1937). *Recent Advances in Cytology*, 2nd ed. London: Churchill.

Ford, C. E. (1969). Meiosis in mammals. In *Comparative Mammalian Cytogenetics* (ed. K. Benirschke), pp. 91–106. New York: Springer-Verlag.

Ford, C. E., Carter, T. C. & Hamerton, J. L. (1956). Cytogenetics of a mouse translocation. *Heredity* **10**, 284 (abstr.).

Ford, C. E. & Clegg, H. M. (1969). Reciprocal translocations. *British Medical Bulletin* **25**, 110–114.

Ford, C. E. & Evans, E. P. (1964). A reciprocal translocation in the mouse between the *X* chromosome and a short autosome. *Cytogenetics* **3**, 295–305.

Ford, C. E., Searle, A. G., Evans, E. P. & West, D. J. (1969). Differential transmission of translocations induced in spermatogonia of mice by irradiation. *Cytogenetics* **8**, 447–470.

Green, M. C. (1966). Mutant genes and linkages. In *Biology of the Laboratory Mouse*, 2nd ed. (ed. E. L. Green), pp. 87–150. New York: McGraw-Hill.

Griffen, A. B. (1960). Mammalian pachytene chromosome mapping and somatic chromosome identification. *Journal of Cellular and Comparative Physiology* **56** (suppl. 1), 113–122.

Kosambi, D. D. (1944). The estimation of map distances from recombination values. *Annals of Eugenics* **12**, 172–175.

Lane, P. W. (1965). Personal communication. *Mouse News Letter* **32**, 47.

LEVAN, A., HSU, T. C. & STICH, H. F. (1962). The idiogram of the mouse. *Hereditas* **48**, 677–687.

LEWIS, K. R. & JOHN, B. (1963). *Chromosome Marker*. London: Churchill.

LYON, M. F., BUTLER, J. M. & KEMP, R. (1968). The positions of the centromeres in linkage groups II and IX of the mouse. *Genetical Research* **11**, 193–199.

LYON, M. F. & MEREDITH, R. (1969). Muted, a new mutant affecting coat colour and otoliths of the mouse, and its position in linkage group XIV. *Genetical Research* **14**, 163–166.

LYON, M. F., PHILLIPS, R. J. S. & SEARLE, A. G. (1964). The overall rates of dominant and recessive lethal and visible mutation induced by spermatogonial X-irradiation of mice. *Genetical Research* **5**, 448–467.

MCCLINTOCK, B. (1945). Neurospora. I. Preliminary observations of the chromosome of *Neurospora crassa*. *American Journal of Botany* **32**, 671–678.

MICHIE, D. & WALLACE, M. E. (1953). Affinity: a new genetic phenomenon in the house mouse. *Nature* **171**, 26.

PHILLIPS, R. J. S. (1961). Personal communication. *Mouse News Letter* **24**, 34.

RODERICK, T. H. (1971). Producing and detecting paracentric chromosome inversions in mice. *Mutation Research* **11**, 59–69.

RUSSELL, L. B. & RUSSELL, W. L. (1960). Genetic analysis of induced deletions and spontaneous nondisjunction involving chromosome II of the mouse. *Journal of Cellular and Comparative Physiology* **56** (suppl. 1), 169–188.

RUSSELL, W. L. (1960). The effect of radiation dose rate and fractionation on mutation in mice. In *Repair from Genetic Radiation Damage* (ed. F. H. Sobels), pp. 205–217. Oxford: Pergamon.

SEARLE, A. G. (1968). The determination of centromere position in the mouse by translocation intercrosses. *Heredity* **23**, 629–630 (abstr.).

SLIZYNSKI, B. M. (1949). A preliminary pachytene map of the house mouse. *Journal of Genetics* **49**, 242–245.

SLIZYNSKI, B. M. (1952). Pachytene analysis of Snell's T(5:8)*a* translocation in the mouse. *Journal of Genetics* **50**, 507–510.

SNELL, G. D. (1946). An analysis of translocations in the mouse. *Genetics* **31**, 157–180.

WALLACE, M. E. (1961). Affinity: evidence from crossing inbred lines of mice. *Heredity* **16**, 1–23.