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The positions of the centromeres in linkage groups II and IX of the mouse

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1. INTRODUCTION

Considerable information has been accumulated about mouse linkage groups (*Mus musculus* L.) but the position of the centromere is not yet known for any. In other organisms the positions of centromeres have been found by the use of chromosome structural changes. Recently a translocation has been found which permits the fairly accurate localization of the centromere in linkage group II of the mouse and also, in conjunction with information from another translocation, determines the order of loci relative to the centromere in linkage group IX.

The translocation concerned is T(2;?)163H (Evans, Lyon & Daglish, 1967), which results in a submetacentric chromosome formed by the joining of a small and a medium-sized acrocentric chromosome at their centromeric ends. One of the acrocentrics, presumed to be the medium one, was shown, partly by cytological studies of mice carrying two translocations, and partly by genetic studies with the linkage group II marker, short-ear, *se*, to carry linkage group II. The present paper describes further genetic studies with various linkage group II markers to determine which was most closely linked to the point of rearrangement and therefore to the centromere. It also describes results with linkage group II and linkage group IX markers in mice carrying translocation T(2;9)138Ca, from which the relative position of the linkage group IX centromere can be inferred, if that in group II is known. The linkage group II markers concerned were curly whiskers (*cw*), short-ear (*se*) and tail-kinks (*tk*), which Falconer & Isaacson (1966) showed to be arranged in that order with recombinations

$$cw\text{-}se,\ 38{\cdot}4 \pm 4{\cdot}8\%$$
$$se\text{-}tk,\ 5{\cdot}8 \pm 2{\cdot}3\%$$

2. METHODS

(*a*) *Breeding methods*

The genes used in this work were:

Linkage-group II		Linkage-group IX	
curly whiskers	*cw*	Brachyury	*T*
dilute	*d*	t-alleles	t^6
short ear	*se*	tufted	*tf*
tail kinks	*tk*		

Preliminary tests were made with short-ear, *se*, only, followed by four-point backcrosses using a stock carrying *cw*, *se* and *tk*. For the preliminary tests a T163T163 homozygote was crossed to *sese* and the T163+/+*se* offspring were backcrossed to *sese*. Progeny were classified for *se* and then, at or soon after weaning age, they were killed and examined cytologically for the presence of T163. Data from both male and female heterozygotes were collected. For the four-point tests a T163T163 mouse was crossed to *cwsetk/cwsetk* and male and female offspring were backcrossed to *cwsetk/cwsetk*. As before, progeny were classified for *cw*, *se* and *tk* and then examined cytologically.

In translocation T(2;9)138Ca, by linkage studies in heterozygotes, the order of loci relative to the chromosome breaks had been shown to be *tk-se-*(T138 break) in linkage group II (Green & Stimpfling, 1966) and *T-tf-*(T138 break) in linkage group IX (Lyon & Phillips, 1959). The present paper reports studies of linkage between *T* and *d* in homozygotes for T138, to find whether these loci were in the same translocated chromosome or not. The results were accumulated over several years, using several different types of parent, mated to homozygous *dd* T138T138. Some parents carried the *t*-allele t^6, which suppresses crossing-over in the *T-tf* region of linkage group IX (Lyon & Phillips, 1959).

(*b*) *Cytological methods*

To detect the presence of T163, corneal mitoses were studied by Fredga's (1964) method, using lacto-aceto-orcein as a stain in place of the aceto-orcein recommended by Fredga. About ten clear metaphase figures were scored for each animal.

3. RESULTS

The preliminary tests (Table 1) indicated 37–38% recombination between the *se* locus and T163 and from this the position of the centromere could not be

Table 1. *Recombination between short-ear* (se) *and T163 among the offspring of T163+/+se × +se/+se*

Sex of heterozygote	Offspring				Recombination
	T163+	T163*se*	++	+*se*	
♀	15	7	13	18	37·7 ± 6·7%
♂	19	16	5	16	37·5 ± 6·5%

concluded. The four-point tests, however, showed that *cw* was closely linked to the translocation (Table 2) and therefore was at the centromere end of the arm carrying linkage group II. The order could be either centromere-*cw-se*, or *cw*-centromere-*se*, in which case the *cw* locus would lie in a hypothetical short arm of linkage group II. It is not yet possible to distinguish between these two alternatives, since, as Fig. 1B and C shows, in either case a single chiasma would be sufficient to give crossovers of the two observed types. However, as the short arms of

mouse chromosomes are so small it seems more convenient to consider that all loci lie in the long arms until crucial evidence can be obtained. If one assumes this for linkage group II then the data of Table 2 suggest that the *cw* locus is at about 2 map units from the centromere. It is possible, however, that the presence of the translocation might have altered the recombination in this region and that the

Table 2. *Offspring of four-point backcrosses of the type T163+ + +/+*cwsetk

Phenotype of offspring	Heterozygous parent ♀	Heterozygous parent ♂	Phenotype of offspring	Heterozygous parent ♀	Heterozygous parent ♂
T163 + + +	20	28	T163 *cw se tk*	—	1
+ *cw se tk*	6	8	+ + + +	—	1
T163 + + *tk*	3	—	T163 *cw se* +	—	—
+ *cw se* +	3	2	+ + + *tk*	—	—
T163 + *se* +	—	—	T163 *cw* + *tk*	—	—
+ *cw* + *tk*	—	—	+ + *se* +	—	—
T163 + *se tk*	7	7	T163 *cw* + +	—	—
+ *cw* + +	13	15	+ + *se tk*	—	—

Recombinations		%	S.E.
T163-*cw*	2/114	1·75	1·18
T163-*se*	44/114	38·6	4·6
T163-*tk*	52/114	45·6	4·7
cw-se	42/114	36·8	4·5
se-tk	8/114	7·0	2·4

Table 3. *Recombination between* T *and* d *in T138T138 homozygotes*

Cross	Sex of heterozygote	Offspring *T*+	++	*Td*	+*d*	Total	R.F. (%)	S.E. (%)
T+/+*d* × +*d*/+*d*	♀	252	143	142	238	775	36·8	1·73
T+/*t*^{6}*d* × +*d*/+*d*	♀	52	33	26	59	170	34·7	3·65
T+/+*d* × +*d*/+*d*	♂	196	161	138	229	724	41·3	1·83
T+/*t*^{6}*d* × +*d*/+*d*	♂	62	109	25	189	385	34·8	2·43
T+/+*d* × +*d*/*t*^{6}*d*	♀	198	211	112	324	845	38·2	1·67
T+/+*d* × +*d*/*t*^{6}*d*	♂	371	292	234	400	1297	40·6	1·36
T+/*t*^{6}*d* × +*d*/*t*^{6}*d*	♀	1	0	1	3	5	—	—
T+/*t*^{6}*d* × +*d*/*t*^{6}*d*	♂	60	49	17	89	215	30·7	3·15

R.F. = recombination fraction; S.E. = standard error.

map distance might be different in normal animals. At present there is no means of testing this point, but data on recombination between *cw* and *se* in the presence of T163 may give some indication. The observed figures (Table 2) give a recombina- close to that found by Falconer & Isaacson (1966) in normal animals. They therefore suggest that if T163 is causing any reduction in crossing-over, it must be a slight one, at least in the *cw-se* region.

The offspring of the T138T138 homozygotes (Table 3) indicated significant linkage of *T* and *d* in animals of this chromosomal type and in this they confirm

the findings of Green (1967). In some of the crosses there were disturbed single factor ratios. In the case of male parents carrying t^6 this can be attributed to the well-known abnormal segregation of this allele in males, whereas in the other cases either incomplete penetrance or reduced viability of $T+$ may have been the explanation. The discrepancies are sufficient to make the estimates of recombination inaccurate, but not sufficient to invalidate the conclusion of linkage, especially for the first of the crosses, in which 775 offspring were obtained, with no disturbance in single-factor ratios. The data also suggest that the recombination in $T+/t^6d$ heterozygotes is less than that in $T+/+d$ heterozygotes of the same sex, which is in accord with the known effect of t^6 in suppressing crossing-over in the region from *T* to *tf* (Lyon & Phillips, 1959), a region which must lie between *T* and *d* in the translocated chromosome.

4. DISCUSSION

The results indicate that the *cw* locus is close to the centromere of T163H and that the order must be centromere-*cw*-*se*-*tk* or *cw*-centromere-*se*-*tk*. The question arises whether this also represents the order in normal chromosomes or whether linkage group II could have been inverted in the translocation (see Fig. 1). If the latter were the case then, as Fig. 1D shows, crossing-over in the inverted segment would lead to the formation of dicentrics and acentric fragments. This would be expected to lead to visible cytological abnormalities at meiosis, including abnormal second metaphases. In fact, Evans *et al.* (1967) observed no such abnormalities. Moreover, it would be expected to give apparent reduction in crossing-over between *cw* and *se*, and reduced fertility, since the crossover products would give inviable gametes or zygotes. The observed crossing-over was close to that in normal animals, and although there was some decrease in fertility in male heterozygotes for T163 the females were fully fertile (Evans *et al.* 1967). This is despite the fact that a trivalent was found in 100% of first meiotic metaphases, indicating that chiasmata were formed in 100% of cases. It is therefore concluded that there is no evidence for inversion in T163, and that the centromere is indeed at the *cw* end of linkage group II in normal mouse chromosomes. It is suggested that for the present it should be assumed that the whole of linkage group II lies in the same arm of the chromosome and that the centromere is terminal. Whether or not *cw* does lie in a hypothetical short arm of the chromosome might be determined by finding which linkage group occupies the other arm of the T163 metacentric chromosome and doing linkage tests with this group, *cw*, and T163. Investigations to find this other linkage group are still in progress.

This point does not affect the argument concerning the order of loci with respect to the centromere in linkage group IX. Green & Stimpfling (1966) found that in T138 the point of exchange in linkage group II lay on the side of *d* away from *tk*, i.e. on the proximal side of *d*. The finding of Green (1967) and of ourselves that *T* and *d* are linked in T138T138 homozygotes shows that the loci of these two genes must be on opposite sides of the translocation break. Therefore, if the break is proximal to *d* it must be distal to *T*. Lyon & Phillips (1959) showed that the T138

break was on the same side of *T* as *tf* and beyond *tf*. Therefore, the order must be centromere-*T*-*tf*-(T138 break). Since the *H-2* locus gives about 7% recombination with *tf* (Green & Stimpfling, 1966) and the T138 break gives 10–15% (Lyon & Phillips, 1959), the order is probably also centromere-*T*-*tf*-*H-2*-(T138 break), but the position of *H-2* cannot be considered certain without a direct three-point test

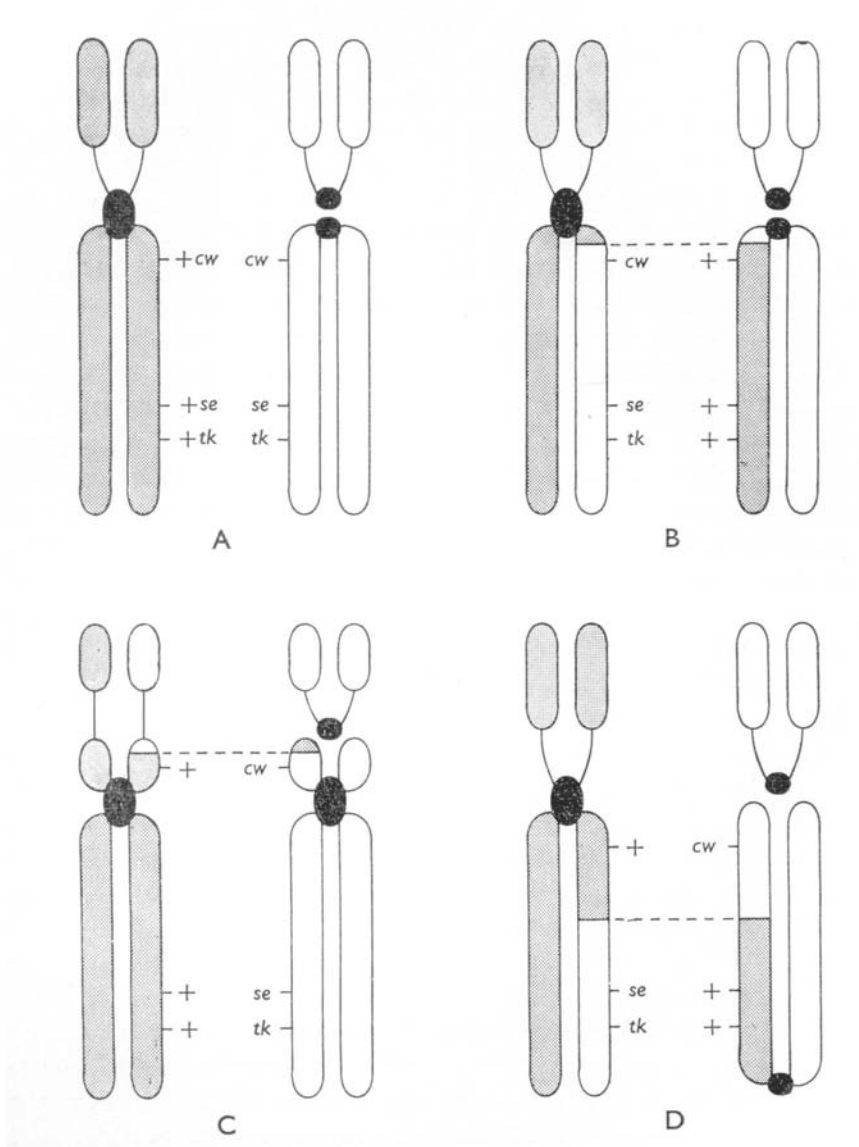

Fig. 1. Results of crossing-over in various positions in animals heterozygous for T(2;?)163H and linkage group II genes. A: the postulated gene arrangement without crossing-over. B and C: the positions of single chiasmata which would give rise to the observed types of T163-*cw* crossovers, if *cw* were in the long or the short arm of T163, respectively. D: the products of a chiasma between *cw* and *se* if T163 involved a major inversion.

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with *tf* and T138. A point about which the present data give no information is the distance of the *T* locus from the centromere.

These results concerning the order of loci in linkage group IX are interesting in relation to the work of G. Klein and his colleagues on variation at the *H-2* locus in tumour cells. Tumours originating in F_1 hybrids between two inbred strains were tested for their ability to grow in either of the parental strains, i.e. for loss of *H-2* specificities. Variants were obtained in which the alteration in specificity persisted through many cell generations, showing that some form of genetic change was involved. The *H-2* locus is a complex one with many alleles determining various specificities, and with crossing-over between the various alleles or specificities at meiosis. In particular, the specificities known as D and K, or 4 and 11 (Snell & Stimpfling, 1967), show about 0·2–0·5% meiotic crossing-over (Snell & Stimpfling, 1967). With tumours which were genetically D^+K^+/D^-K^- Klein & Klein (1958) found that loss of D was always accompanied by loss of K, but that K could be lost without D. The possibility that D was a precursor of K was disproved by the fact that loss of D without K did occur among variants from genetically D^+K^-/D^-K^+ tumours (Klein & Klein, 1964). E. Klein (1961) suggested that the variants arose by mitotic crossing-over, in which case the absence of D^-K^+ variants from D^+K^+/D^-K^- tumours would be explicable if D were proximal to K with respect to the centromere. Linkage tests by various workers, however, have indicated the order of loci to be *T-tf*-K-D (Allen, 1955; Green & Stimpfling, 1966; Shreffler, 1965). Therefore, if our present conclusion is correct, that the centromere is to the left of *T*, then D is distal to K and the explanation of mitotic crossing-over to account for the tumour variants will not hold. Klein & Klein (1964) have also suggested other possible explanations, including chromosomal deletion, and failure of transcription or translation of parts of a postulated *H-2* operon, but one cannot yet decide between these explanations.

It would be valuable if the conclusions of the present work could be confirmed with other translocations, since, although it seems unlikely, there is still a possibility that translocations T138 and T163 are more complicated than they appear and that inversions are involved. Work with other translocations proceeds and suitable ones will undoubtedly be found. The metacentric chromosome found by Léonard & Deknudt (1967), for instance, will no doubt prove as useful or more so than T163.

SUMMARY

In mice heterozygous for translocation T(2;?)163H, and also for linkage group II markers, the *cw* locus shows close linkage with the point of rearrangement (about 1–2% recombination). Since T163 was apparently formed by fusion of two chromosomes near their centromeres, this means that the centromere must lie at the *cw* end of linkage group II. In homozygotes for T(2;9)138Ca the genes *T* and *d* show significant linkage, indicating that their loci are on opposite sides of the translocation break. Since, from previous data, the break is known to be proximal to *d*, it must then be distal to *T* and, again from previous data, the order of loci in linkage group IX must be centromere-*T-tf*-T138 break.

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