

Evidence that Linkage Group IV as well as Linkage Group X of the mouse are in chromosome 10*

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SUMMARY

Four translocations in which one breakpoint was known to be at or closely linked to the LGIV marker Steel, *Sl*, were studied in an attempt to identify cytologically the as yet unassigned Linkage Group IV of the mouse. The cytological results indicate that chromosome 10, which had previously been matched with LGX, is involved in each of the four translocations, the break being in 10D. Recombination between *Sl* and the LGX marker grizzled, *gr*, was determined to be 6-14%. The combined cytological and genetic results indicate that LGIV and LGX are both in chromosome 10, and that the order may be centromere-*gr-Sl*.

1. INTRODUCTION

The application of new chromosome banding techniques to the cytological study of genetically identified chromosome rearrangements of the mouse has led to rapid progress in the matching of linkage groups with chromosomes (see *Mouse News Letter* 1974, for up-to-date results). Two chromosomes, however, including the third-longest in the complement, have not so far been identified with linkage groups. Since one of the as yet unassigned linkage groups, LGIV, contains the marker *Sl* (Sarvella & Russell, 1956), and since we have found at our laboratory several rearrangements closely linked to this marker, we initiated experiments that we hoped would match LGIV to one of the two 'empty' chromosomes. Instead, to our surprise we found that *Sl* appears to be in chromosome 10, which had already been matched with LGX; and evidence indicates that linkage groups IV and X may have to be partly or wholly superimposed on each other. Preliminary information was presented earlier (Cacheiro & Russell, 1974; Russell & Cacheiro, 1975).

2. MATERIALS AND METHODS

There have been more than 30 independent occurrences of 'Steeloid, *Sld*' mutations at our laboratory (Russell, 1972). These are dominants that, in heterozygous condition, resemble *Sl/+* (slight dilution of coat colour, small headspot). In cases where *Sld/Sl* is fertile, crosses to *+/+* have yielded no wild-type progeny in several hundred young, indicating that at least some of the *Sld*'s (including 1XY, see below) are allelic or closely linked to *Sl*. In a number of the independent lines, *Sld/+* mice are partially sterile; and for some of these, evidence for reciprocal translocation had been clearly established some years ago (Wickham & Weaver, 1963). Four *Sld* translocations were used in the present experiment: two of these, 1XY and 35R60L, were discovered in offspring of

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neutron-irradiated males (spermatogonial stage); one, 39LL, in offspring derived from X-irradiated spermatozoa; and one, 74G, occurred in nonirradiated controls.

For cytological studies, short-term tissue cultures were prepared from kidneys of adult *Sld*/+ males and females in each of the four translocation stocks. Karyotypes were prepared from mitotic metaphases stained with quinacrine mustard by the technique described by Miller *et al.* (1971a).

Recombination frequencies were scored for *Sld* and breakpoint in each of the translocations; for *Sld* and *W^v* in 74G; and for *Sl* and *gr*, using stocks with intact chromosomes, namely the inbreds SL (F68) and GR (F32).

3. RESULTS AND DISCUSSION

The mitotic karyotypes of the four stocks (Plate 1, Figs. 1 and 2) indicate that chromosome 10 is involved in each of the four translocations, and that the approximate location of the break is near the distal end of the chromosome, in the 10D region (nomenclature of Nesbitt & Francke, 1973). The other reciprocally translocated chromosome is No. 5 in 35R60L, No. 12 in 39LL, No. 17 in 74G, and No. 18 in 1XY. The four translocations will therefore be designated T(5;10)9Rl, T(10;12)10Rl, T(10;17)11Rl, and T(10;18)12Rl, respectively. Break locations are in 5E, 12A, 17C, and 18D, respectively. More precise localization of breakpoints is now being attempted by the use of trypsin and Giemsa banding.

In an attempt to estimate recombination frequencies between *Sld* (probably equivalent to *Sl*) and the breakpoint in the four translocations, stock maintenance data were analysed for average litter size produced by *Sld*/+ animals in *Sld*/+ × +/+ crosses. No recombination between the *Sld*/+ phenotype and partial sterility has to date been observed. The number of *Sld*/+ animals tested for fertility was as follows: for T(5;10)9Rl, 20 ♂♂, 17 ♀♀; for T(10;12)10Rl, 70 ♂♂, 74 ♀♀; for T(10;17)11Rl, 82 ♂♂, 97 ♀♀; and for T(10;18)12Rl, 198 ♂♂, 132 ♀♀. It may thus be assumed that the *Sld*'s are at or very near the translocation breakpoint.

In the case of one of the translocations, T(5;10)9Rl, the cytological diagnosis of the second chromosome involved has been genetically verified by obtaining recombination data of *Sld* and *W^v*, a LGXVII (chromosome 5) marker. From crosses of T *Sld*/+ × *W^v*/+, we recovered double heterozygotes, recognizable by their very light mottled coat. Crosses of such double heterozygotes to wild type (repulsion cross) yielded 93 *Sld*/+, 78 *W^v*/+, 4 wild-type (not checked for translocation), and 1 double heterozygote that carried the translocation and thus represented a *W^v*-breakpoint recombinant. A line has been set up from this animal, and subsequent coupling crosses have so far yielded only one possible recombinant (which died young) in 60 young. The combined repulsion and coupling data thus indicate 5 or 6 *Sld*-*W^v* recombinants in a total of 236 offspring, or a frequency of 2.1 to 2.5%. If the *Sld*-breakpoint recombination in T(5;10)9Rl turns out to be as low as it is in the other translocations, most or all of this frequency would be due to *W^v*-breakpoint recombination. This will be checked in future work.

The finding that all four of the translocations involved chromosome 10 was unexpected in view of the fact that chromosome 10 had already been matched with LGX by Miller *et al.* (1971b), using T(10;13)199H and T(10;18)18H. Since *Sl* was supposedly in LGIV (Green, 1966), we initiated crosses to determine whether or not *Sl* might be linked to the LGX marker *gr*, grizzled. In crosses of *Sl*/+; +/*gr* ♀♀ × +/+; *gr*/*gr* ♂♂, only 8 of 106 offspring were wild type. The remaining 96 offspring could not always be definitively classified as to either *Sl*/+ or *gr*/*gr*, but a tentative classification gave 61 and 37, respectively; none were clearly double mutant. The 8 wild-type offspring observed should be compared with the expectations for random segregation, namely 26.5 (i.e. 1/4); or, 35.3

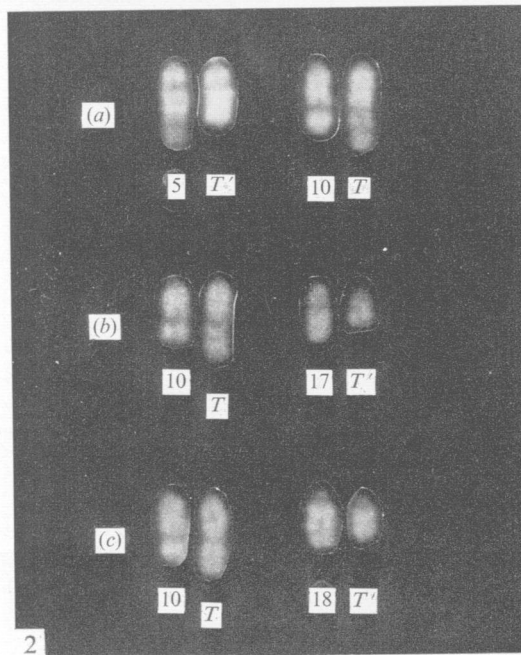
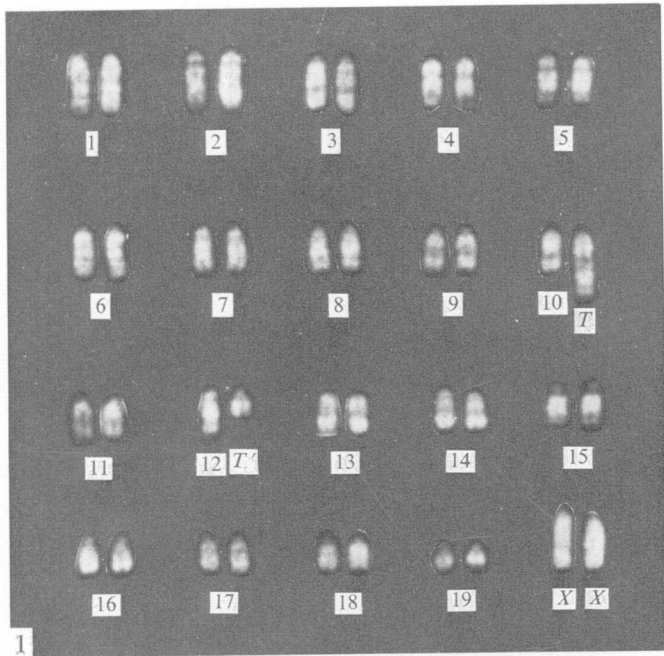


Fig. 1. Complete karyotype from a female mouse heterozygous for a translocation closely linked to *Sl*, Steel. The chromosomes have been arranged according to the classification of the Committee on Standardized Genetic Nomenclature for Mice (1972). *T* and *T'* are the products of the reciprocal translocation T(10;12)10R1.

Fig. 2. Partial karyotypes from three males. Each is heterozygous for a translocation closely linked to *Sl*. (a) T(5;10)9R1; (b) T(10;17)11R1; and (c) T(10;18)12R1. The translocation chromosomes are at the right of each pair, marked *T* and *T'*, respectively.

(i.e. 1/3) if $Sl/+;gr/gr$ is lethal. The data show Sl and gr to be clearly linked, with recombination calculated to be about 7% if $Sl\ gr/+gr$ is lethal, or about 15% if $Sl\ gr/+gr$ was not recognized among the non-wild-type.

Since there is considerable direct evidence for the linkage of Sl to other 'LGIV' markers, specifically av , si , pg , and dy (Green, 1966), it is very likely that most if not all of LGIV is located in chromosome 10, but the orientation of this entire linkage group with respect to the chromosome-10 centromere remains to be determined. The gr locus has been located in 10C by Dev *et al.* (1974). Since we have located Sl in 10D, the order is probably centromere- gr - Sl .

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