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Identification of the Mouse Karyotype by Quinacrine Fluorescence, and Tentative Assignment of Seven Linkage Groups

(*Mus musculus*/autosomes/X and Y chromosomes)

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ABSTRACT **A karyotype of the mitotic chromosomes of the house mouse has been prepared based upon quinacrine fluorescence patterns. All 19 pairs of autosomes and the X and Y chromosomes have been identified. Examination of the chromosomes of the following translocation stocks, T(11;?)1Ald, T(3;?)6Ca, T(2;9)138Ca, T(2;12)163H, and T(9;13)190Ca, have led to the tentative assignments of autosomal linkage groups (LG) to chromosomes as follows: LGII to chromosome number 10 (or 13), LGIII to 12 or 15, LGIX to 16, LGXI to 6, LGXII to 19 and LGXIII to 1. By definition, LGXX is on the X chromosome.**

Meiotic studies of the house mouse, *Mus musculus*, have been very informative (1), but mitotic studies have been restricted by the limited variation in morphology of the chromosomes, all of which are acrocentric with small gradations in length. The only translocations that could be detected in stained preparations of mitotic chromosomes involved a marked change in length, such as the presence of a minute chromosome in the T6 (2) and one very long and one very short chromosome in the T190 (3), or the formation of a metacentric chromosome, as in the T163 (4). Bennett (3), who gives a brief review of prior cytologic studies, used the secondary constrictions in several chromosomes to help divide the complement into pairs. She was unable to detect any abnormality in T138.

It was recently reported that quinacrine and quinacrine mustard are bound to the DNA of human chromosomes in such a highly specific manner that each chromosome can be identified by its pattern of quinacrine fluorescence (5). Furthermore, the fluorescence patterns of chromosomal segments do not appear to be altered by translocation (6).

The accuracy of the quinacrine fluorescence technique of chromosome identification, and the availability of numerous potentially overlapping translocations in the house mouse, suggested to us the possibility of assigning the known murine linkage groups (LG) involved in these translocations to their specific chromosomes.

MATERIALS AND METHODS

Standard inbred and hybrid mice were purchased from The Jackson Laboratory, as were translocation stocks T(3;?)6Ca and T(2;9)138Ca. Translocation stocks T(2;12)163H, T(11;?)-1Ald, and T(9;13)190Ca were obtained from the M.R.C. Radiobiology Research Unit, Harwell, through the courtesy of Dr. Mary Lyon. These will be called T1, T6, T138, T163, and T190. All except T190 were studied in the homozygous state. All the translocations were radiation-induced and the origins (7), genetics (8), and configuration at the pachytene stage of meiosis (9) of most of these translocations have been described. The breeding of animals, establishment of primary tissue cultures from embryos, and the preparation of metaphase cells for karyotyping were done at The Roche Institute. Examination of the cells for patterns of quinacrine fluorescence and analysis of karyotypes were done at Columbia University.

Primary tissue cultures were established from mice by trypsinization of 11- to 13-day embryos in saline–citrate to obtain suspensions of single cells (10). Cells were grown in HEPES-buffered Nutrient Mixture F-10 (Gibco No. 239) containing 10% calf serum and 5% fetal calf serum. Falcon tissue-culture flasks (75 cm^2) were used and each flask was seeded with 10–12 ml of a suspension containing 5×10^5 viable cells/ml. After 2 or 3 days at 37°C, cells (including a large proportion in metaphase) were collected by washing the cell surface twice with calcium- and magnesium-free phosphate-buffered saline, followed by a 5-min treatment of the surface with 3 ml per flask of 0.25% Viokase. Cells from 5–7 flasks were pooled, and Colcemid was added to a final concentration of 0.5 μg/ml. Cells were immediately pelleted by centrifugation at 500 *g* for 10 min, resuspended in 10 ml of 0.032 M KCl, and incubated at 37°C for 5 min. 0.5 ml of freshly prepared fixative (methyl alcohol–glacial acetic acid, 3:1) was added, and the suspension was centrifuged at 200 $\times$ *g* for 5 min. The pelleted cells were resuspended in 5 ml of fresh fixative for up to 3 hr at room temperature, and the fixative was later changed three times. One to two drops of the cell suspension were dropped on cold, wet slides tilted at an angle of 30° and air-dried. This procedure produces metaphase spreads in which the two chromatids of each chromosome lie close together and are relatively long. Slides were stored at 4°C. The prepared slides were stained by immersion in a solution of quinacrine mustard (approx. 50 μg/ml) or quinacrine dihydrochloride ("Atabrine," Winthrop, 4.5 mg/ml) for 8 and 5 min, respectively, at room temperature, washed for 3 min in running water, and rinsed in Tris–maleate buffer, pH 5.6 (11). The slides were wet-mounted in the Tris–maleate buffer under a coverslip and the edges were sealed with clear nail polish. Metaphase chromosomes were observed with a Zeiss microscope, using epi-illumination with an HBO 200-W

Abbreviation: LG, linkage group.

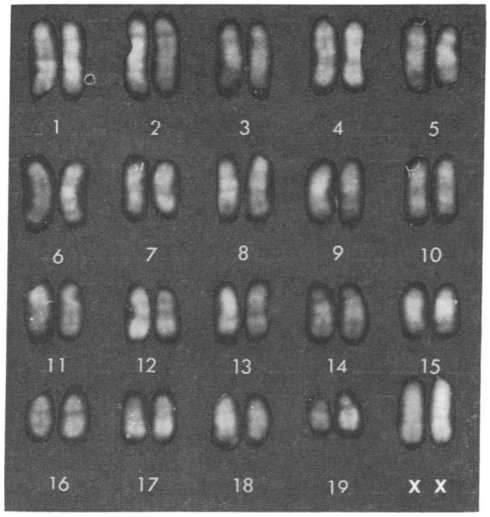

FIG. 1. Karyotype of a female mouse cell.

mercury lamp, a 530 barrier filter, a BG12 exciter filter, and a 100× Planapochromatic objective containing an iris diaphragm. Well-spread metaphases were photographed on Kodak Panatomic X film, using exposure times of 60 ± 10 sec, and printed on Ilford R4-1P paper of contrast grades 3 or 4. 10–40 karyotypes were prepared for the normal mouse and for each translocation.

RESULTS

Mouse chromosomes, like those of other species, show distinctive banding by the quinacrine fluorescence method. We have been able to pair homologous chromosomes on the basis of these banding patterns and to construct a consistent mouse karyotype. A typical fluorescent karyotype of a female mouse cell is shown in Fig. 1. The chromosome pairs have been arranged in order of approximate length and have been numbered 1–19, with the sex chromosomes occupying position 20. An ideogram, based on the analysis of numerous karyotypes, is shown in Fig. 2. A detailed description of the normal mouse karyotype will be published elsewhere.

Most of the homologous pairs can be unequivocally identified. The most difficult distinction has been between chromosomes number 10 and 13. The longer pair is designated number 10. Identification of the sex chromosomes is based upon finding only paired chromosomes in some cells (XX) and a single unmatched pair in others (XY). Measurement of the sex chromosomes in 10 cells showed that the X is about the fourth longest and the Y is usually the shortest chromosome. The two X chromosomes are of equal length. The mouse Y chromosome does not have a very bright fluorescent tip as does the human Y (5), though both X and Y are brightly fluorescent over their entire length.

Five mouse translocations were examined (Figs. 3–7). Two of these involve Robertsonian centric fusions. The translocation in T1 involves chromosomes 6 and 15, while that in T163 involves chromosomes 10 (or 13) and 19. The remaining three translocations were tandem. The translocation in T6 involves chromosomes 12 and 15, that in T190 involves chromosomes 1 and 16, while that in T138 involves numbers 10 (or 13) and 16.

Since it has been possible to specify the chromosomes involved in each translocation, we can now correlate the cytologic findings with the known linkage groups involved in these translocations (Table 1).

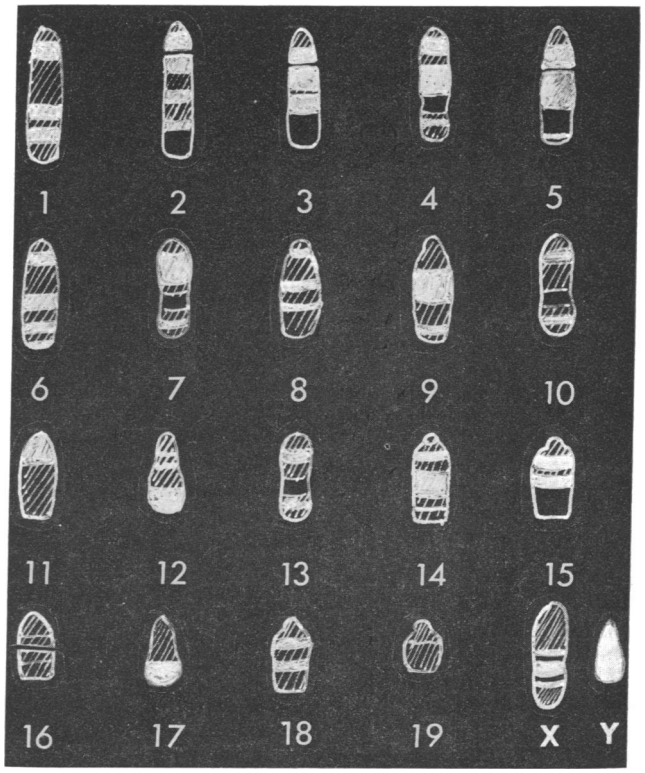

FIG. 2. Ideogram of mouse mitotic chromosome complement.

T6 involves LGIII and chromosomes 12 and 15. LGIII must, therefore, be located on either chromosome 12 or 15. T1 involves LGXI and chromosomes 6 and 15. Since the T6 translocation does not contain LGXI (8), this linkage group cannot be on chromosome 15, which T1 and T6 have in common. We therefore conclude that LGXI is located on chromosome 6 (Table 2).

T163 and T138 each contain LGII and chromosome 10 (or 13?). The fluorescence patterns do not enable us to deter-

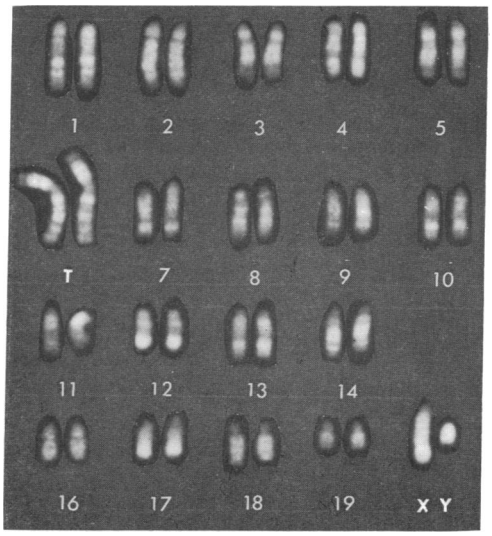

FIG. 3. Karyotype of a cell from a T(11;?)1Ald mouse. The translocation chromosomes (T) were the result of a centric fusion type of translocation of chromosomes 6 and 15.

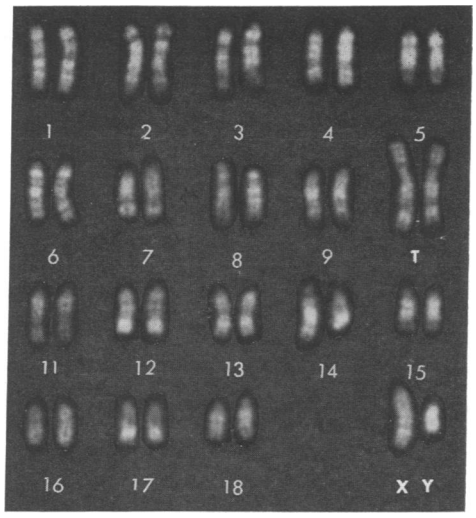

FIG. 4. Karyotype of a cell from a T(2;12)163H mouse. The translocation chromosomes were the result of a centric fusion of chromosomes 10 and 19.

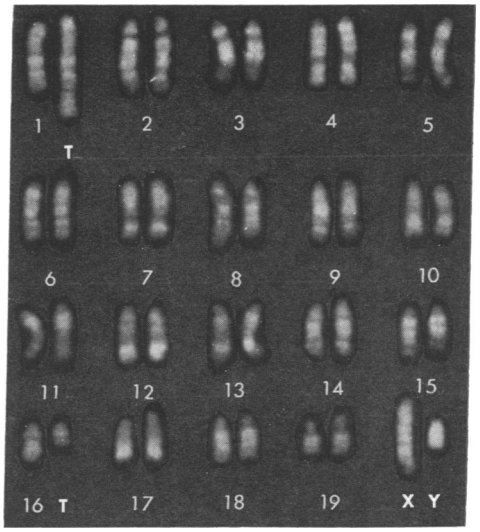

FIG. 6. Karyotype of a cell from a T(9;13)190Ca mouse. A segment of chromosome 16 is located on the distal end of chromosome 1.

mine whether chromosome 10 or 13 is involved. However, in their original report, Evans *et al.* (4) suggested that the length of the long arm of T163 was equivalent to the tenth longest acrocentric chromosome in regularly stained mitotic preparations. We have tentatively assigned LGII to chromosome 10. The other chromosome involved in T163 is number 19, which therefore carries LGXII. Similarly in T138, LGIX can be assigned to chromosome 16.

T190, like T138, also contains LGIX and chromosome 16, which confirms the correctness of this assignment. It follows that LGXIII, the remaining linkage group involved in T190, must be on chromosome 1.

DISCUSSION

The cytological identification of the 20 pairs of mouse mitotic chromosomes represents a major advance in mammalian genetics. There are nineteen known linkage groups in the mouse, 18 on the autosomes and one (linkage group XX) on the X. This is the first time that it has been possible to identify cytologically the mitotic chromosome that contains each linkage group. We have made tentative assignments of six of the autosomal linkage groups as well as identifying the X. As other translocations are studied, it will be possible to identify the mitotic chromosomes associated with the remaining linkage groups, thus resolving the question of whether each linkage group is on a separate chromosome. Since none of the linkage groups is as long as the mouse chromosomes, which range from 75 to 125 centimorgans, it has remained theoretically possible that two linkage groups are on a single chromosome (7).

A few investigators have prepared maps of mouse chromosomes at the pachytene stage of meiosis (1, 9) and made tentative assignments of a number of linkage groups. The accuracy of this technique is in doubt, especially since two of the chromosomes were reported to be metacentric, whereas

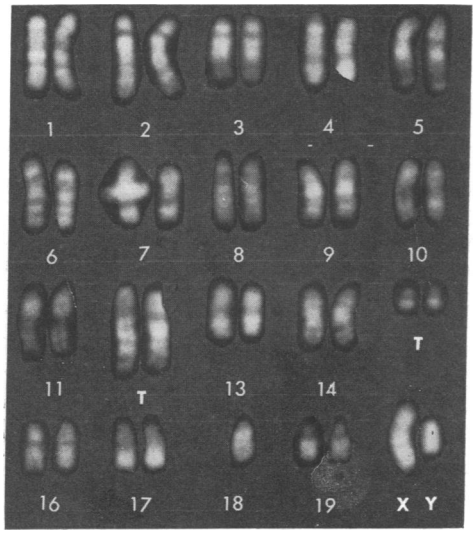

FIG. 5. Karyotype of a cell from a T(3;?)6Ca mouse. Most of chromosome 15 is visible on the distal end of chromosome 12. One chromosome 18 overlays a chromosome 7.

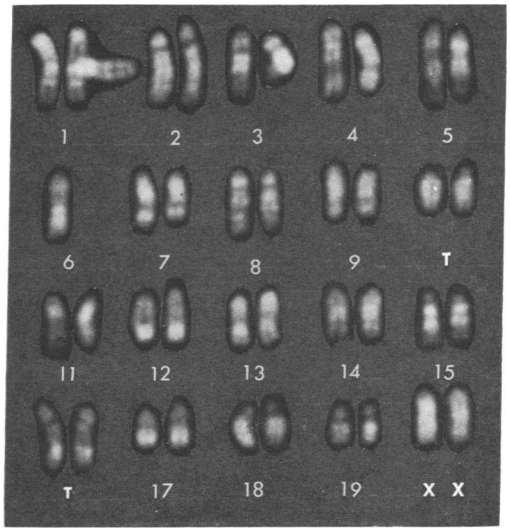

FIG. 7. Karyotype of a cell from a T(2;9)138H mouse. A distal segment of chromosome 10 is visible on the distal end of chromosome 16. One chromosome 6 overlays a chromosome 1.

TABLE 1. *Chromosomes and linkage groups involved in mouse translocations*

Translocation	Chromosomes	Linkage groups
T(3;?)6Ca	12,15	III, ?
T(11;?)1Ald	6,15	XI, ?
T(2;12)163H	10,19	II,XII
T(9;13)190Ca	1,16	IX,XIII
T(2;9)138Ca	10,16	II,IX

TABLE 2. *Chromosome assignment of linkage groups*

Linkage group	Chromosome
II	10
III	12 or 15
IX	16
XI	6
XII	19
XIII	1

other evidence overwhelmingly supports the acrocentric or telocentric nature of all the chromosomes in the normal mouse karyotype. Furthermore, pachytene maps are difficult to prepare and to analyze, and they have not proved to be generally useful. More importantly, they cannot be used to study the mitotic chromosomes of somatic cells.

The study of somatic cells, frequently maintained in culture, is becoming increasingly useful in analyzing many problems in the genetics of higher organisms, including the mouse. Genetic variants are known for at least 20 markers which can be studied in somatic cells, and this number could be greatly increased by the development of interspecific somatic cell hybrids from which mouse chromosomes are preferentially eliminated (12). Since the quinacrine fluorescence patterns of chromosomes appear to be unchanged in interspecific hybrids (13), specific mouse chromosomes could be identified and their presence correlated with that of proteins of mouse origin. In this way, many additional genetic functions could be identified and each gene assigned to its linkage group, thus adding to the known genetic loci of morphological, antigenic, and biochemical markers which have already been assigned to linkage groups of the mouse (14).

The availability of a method for identifying each mouse chromosome should make it possible to resolve other problems in mouse cytogenetics. For example, only two translocations of the Robertsonian type have been detected using semisterility as an indication of a potential translocation heterozygote. Since in the reported case of T163 fertility was not markedly reduced (4), it is possible that this type of translocation is more common but has remained undetected. Analysis of mitotic chromosomes can complement meiotic studies of structural rearrangements. The introduction of an accurate method for identifying mitotic chromosomes will now make it possible to analyze heteroploid mouse cells, whether tumor cells or established cultured cell lines.

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1. Griffen, A. B., in *Biology of the Laboratory Mouse*, ed. E. L. Green (McGraw-Hill, New York 1966), pp. 51–85.
2. Ford, C. E., J. L. Hamerton, D. W. H. Barnes, and J. F. Loutit, *Nature*, **177**, 452 (1956).
3. Bennett, D., *Proc. Nat. Acad. Sci. USA*, **53**, 730 (1965).
4. Evans, E. P., M. F. Lyon, and M. Daglish, *Cytogenetics*, **6**, 105 (1967).
5. Caspersson, T., L. Zech, and C. Johansson, *Exp. Cell Res.*, **62**, 490 (1970).
6. Miller, D. A., P. W. Allderdice, O. J. Miller, and W. R. Breg, *Nature*, in press.
7. Carter, T. C., M. F. Lyon, and R. J. S. Phillips, *J. Genet.*, **53**, 154 (1955).
8. Carter, T. C., M. F. Lyon, and R. J. S. Phillips, *J. Genet.*, **54**, 462 (1956).
9. Slizynski, B. M., *J. Genet.*, **55**, 122 (1957).
10. Kouri, R. E., and J. H. Coggin, *Proc. Soc. Exp. Biol. Med.*, **129**, 609 (1968).
11. *Handbook of Biochemistry; Selected Data for Molecular Biology*, ed. H. A. Sober (The Chemical Rubber Co., Cleveland, 1968), p. J-196.
12. Pontecorvo, G., *Nature*, **230**, 367 (1971).
13. Miller, O. J., P. W. Allderdice, D. A. Miller, W. R. Breg, and B. R. Migeon, *Science*, in press.
14. Green, M. C., in *Biology of the Laboratory Mouse*, ed. E. L. Green (McGraw-Hill, New York, 1966), pp. 87–150.