IDENTIFICATION BY QUINACRINE FLUORESCENCE OF THE CHROMOSOME CARRYING MOUSE LINKAGE GROUP I IN THE CATTANACH TRANSLOCATION

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CATTANACH's flecked (fd) translocation, $T(1;X)Ct$ is thought to be a 3-point nonreciprocal translocation in which an inverted segment (SLIZYNSKI 1967) of the mid-portion of linkage group I (LG I, EICHER 1967) has been inserted into the mid-portion of the X (CATTANACH 1966). One of the break points in LG I is between the $sh-1$ and Hbb loci (EICHER 1967; WOLFE 1967). The second break point is between the qv and $ru-2$ loci. The length of the segment of LG I which has been inserted into the X is thus 21–32 centimorgans (cM) long, i.e. 30–46% of the 70 cM distance separating the outside markers of LG I, fr and nv (MNL 1970).

It is now possible to identify each chromosome in the mitotic metaphase complement of the mouse by its quinacrine fluorescence pattern (MILLER, *et al.* 1971). Using this technique, we have identified the X and Y chromosomes and numbered each pair of autosomes in order of their approximate length. Using a series of translocations, we have been able to assign LG II to chromosome 10, LG III to 12 or 15, LG IX to 16, LG XI to 6, LG XII to 19, and LG XIII to 1. We report here the assignment of LG I to chromosome 8 and confirmation of our earlier identification of the X (by definition, LG XX) by quinacrine fluorescence study of the Cattanach translocation.

MATERIALS AND METHODS

Primary cultures of pooled, trypsinized 11–13 day embryos were prepared. The mating from which embryos were obtained was of the type: $(Df/c^{ch} + X^{fd}/X) \text{♀} \times (c^h/c^{ch} + X/Y) \text{♂}$, where c^h = Himalayan, c^{ch} = Chinchilla, Df = the chromosome carrying LG I with the deficiency, and X^{fd} = the X chromosome carrying the transposed segment from LG I. The breeding of animals, establishing of primary cultures, and preparing of metaphase spreads were done at the Roche Institute, as described in our earlier report (MILLER *et al.* 1971). The quin-

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FIGURE 1.—Karyotype of a male cell. Arrows indicate the 8^{Df} and X^{fd} chromosomes.

acrine fluorescence microscopy, photography and karyotyping were done at Columbia University, as described in the same report.

RESULTS AND DISCUSSION

One hundred twenty fluorescent metaphase spreads were photographed and approximately 50 karyotypes prepared. A representative karyotype of a male cell with both the X^{fd} and Df chromosomes is shown in Figure 1. The Df chromosome, derived from LG I, is chromosome number 8. The normal number 8 has a bright band near the centromere and two small bright bands in the central region. The 8^{Df} chromosome is two-thirds as long as the normal number 8 based on measurements in 28 cells. It has a bright band near the centromere and a very narrow band in an otherwise dull distal region. The pattern of fluorescence of both its proximal and distal portions matches the corresponding segments of its

FIGURE 2.—Partial karyotypes from three female (top row) and three male (bottom row) cells. The translocation chromosome, 8^{Df} or X^{fd} , is the left member of each pair. The last case in the bottom row has two normal chromosomes 8 and no 8^{Df} chromosome.

normal homologue. The normal X chromosome is about the fourth longest chromosome and brightly fluorescent along its entire length, with two very bright bands visible at the distal end of the middle third of the chromosome (MILLER *et al.* 1971, and Figure 2). The X^{fd} chromosome is usually the longest chromosome in the complement. It is one-third longer than the normal X, based on measurements in 17 cells. The X^{fd} chromosome has a relatively dull segment inserted into the distal third of the chromosome, adjacent to the bright bands. The fluorescent pattern of the inserted segment appears to correspond to the segment deleted from chromosome number 8. Partial karyotypes of the normal and abnormal chromosomes 8 and the sex chromosomes are shown in Figure 2. This method is not sufficiently accurate to distinguish the break points or to confirm that the inserted segment in X^{fd} is inverted.

Four of the eight genetic combinations possible from the mating of heterozygous females to normal males which produced the embryos used in this study were found in the sample of 42 unequivocal karyotypes examined. Most of the karyotypes (23 female and 11 male cells) contained both X^{fd} and 8^{Df} chromosomes, while six (all male) had two normal 8's and the X^{fd} chromosome and two (both female) had only normal chromosomes. Since our cultures were of pooled embryos from the relevant mating, the relative number of cells of the different types probably has no significance.

The results obtained in this study confirm the identification of the X chromosome we reported earlier (MILLER *et al.* 1971), and extend from seven to eight the number of linkage groups which we have been able to assign to cytologically

identifiable mitotic chromosomes. These eight linkage groups have all been assigned to different chromosomes, but the possibility remains that others may be assigned to the same chromosome. It is interesting in this regard that the cytological evidence provided by the flecked translocation fits so well with the linkage data. The translocation involves one-third of the chromosome and 30–46% of the known linkage group, suggesting that the outside markers are perhaps near the end of chromosome 8.

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