CHROMOSOME MARKERS IN *MUS MUSCULUS*: STRAIN DIFFERENCES IN C-BANDING*

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ABSTRACT

The mitotic chromosomes of several inbred strains of mice and a series of F_1 hybrids have been analyzed by quinacrine staining and further characterized by the centromeric heterochromatin banding (C-banding). Inbred strains had the same amount of C-banding material on homologous chromosomes but showed variation in the amount on different chromosomes. F_1 hybrids showed characteristics of each parent and it appears that the amount of C-banding on each chromosome is a simple inherited polymorphism. In this study 12 different chromosomes could be distinguished by their C-banding, and these can be used as normal chromosome markers.

IN the laboratory mouse, *Mus musculus*, 8–10% of the DNA has a lower buoyant density in a CsCl gradient than the main band DNA (1.690 and 1.701 g/ml, respectively) and is visible as a low density satellite peak (KIT 1961). *In situ* molecular hybridization has been used (JONES 1970; PARDUE and GALL 1970) to show that this satellite DNA is located in the centromeric region of mouse chromosomes other than the Y. PARDUE and GALL noted that after the series of treatments required for the *in situ* hybridization, Giemsa staining of the chromosomes in several unspecified mouse cell lines was limited to the regions occupied by the satellite DNA. This was confirmed in Swiss mice by HSU *et al.* (1971), who commented on the even distribution of this staining, now called C-banding, on all the chromosomes except the Y. Quite comparable C-banding regions have been observed in a sympatric species of mice, *M. cervicolor* (DEV *et al.* 1973).

The C-banding technique has been used to distinguish chromosomes of human and mouse origin in man-mouse hybrid cells (CHEN and RUDDLE 1971; ALLDERDICE *et al.* 1973) because the C-banding region at the centromere of human chromosomes is generally much smaller than that on mouse chromosomes. Prominent C-banding regions are seen only at the secondary constriction near the centromere of human chromosomes 1, 9 and 16, regions which appear to be polymorphic (CRAIG-HOLMES, MOORE and SHAW, 1973). Similar polymorphisms have been

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described in the wild mouse, *Peromyscus maniculatus* (DUFFY 1972), and it would not be surprising if such differences were found to exist in *Mus musculus*.

The purpose of this report is to describe the presence of differences in the C-banding regions of many chromosomes in various strains of the laboratory mouse and to show that they appear to be inherited as simple polymorphic traits which can be used as chromosome markers.

MATERIALS AND METHODS

Mice of the following inbred lines were obtained from the Jackson Laboratory: C57BL/6J, C57L/J, C3H/HeJ, DBA/2J, A/J, and SM/J (for a history of the lines, see STAATS 1972). These will be referred to as C57BL, C57L, C3H, DBA, A and SM, respectively. Females of the AKR and 129 lines, mated to AKR males, were obtained from DR. LLOYD OLD of the Sloane-Kettering Institue. The F_1 embryos of C57L, C3H, DBA and SM females mated to C57BL males are the same as described in our earlier report on strain differences in secondary constrictions (DEV *et al.* 1971). In most cases primary cultures were set up from pooled 14–17 day embryos from a single pregnant female, and metaphase chromosome spreads prepared, stained with quinacrine mustard to produce Q-banding and photographed by fluorescence microscopy as described earlier (MILLER *et al.* 1971). After removing the coverslip the slides were soaked in methanol for 1–3 hours, heated in 95° formamide in SSC at 65° for one hour and stained with a 2% solution of Giemsa in citrate-phosphate buffer at pH 6.8 to produce C-banding, as described by DEV *et al.* (1972). The cells which were previously photographed with Q-banding were rephotographed. In this way, Q-banding and C-banding photographs of a series of metaphase spreads were obtained and 3–10 double karyotypes prepared for each inbred strain or F_1 hybrid, identifying each chromosome solely by its fluorescent Q-banding pattern.

RESULTS

In most of the inbred strains every chromosome except the Y was found to have C-banding material. The size of the C-banding region was the same for both homologous chromosomes in each inbred strain, but the size of this region on some chromosomes varied from one strain to another. In C57BL, the amount was approximately the same on each chromosome (Figure 1) and we have used this strain as a standard for comparison. The table summarizes the differences we observed among the various strains. For example, in C57L chromosomes 3, 10 and 13 had smaller amounts of C-banding material than C57BL and chromosomes 5 and 12 had larger amounts.

This between-strain difference in the size of the C-banding regions was maintained in F_1 hybrids and was most easily demonstrated in such hybrids. Figure 2 illustrates the differences observed between C57L and C57BL. The five chromosomes of known maternal (C57L) origin are indicated by arrows. In other strains different chromosomes had differing amounts of C-banded material, as illustrated in Figure 3 for a cross between 129 and AKR mice, in which four chromosomes of maternal origin have been indicated. In all, 12 of the 20 mouse chromosomes could be distinguished in one or more of the eight inbred strains studied.

The amount of C-banding material present in a chromosome remains constant during prolonged *in vitro* culture of mouse cell lines. The C3H strain differs from C57BL in having very little C-banding material on chromosome 14, and relatively little on chromosome 18 (Figure 4a). We examined the chromosomes of

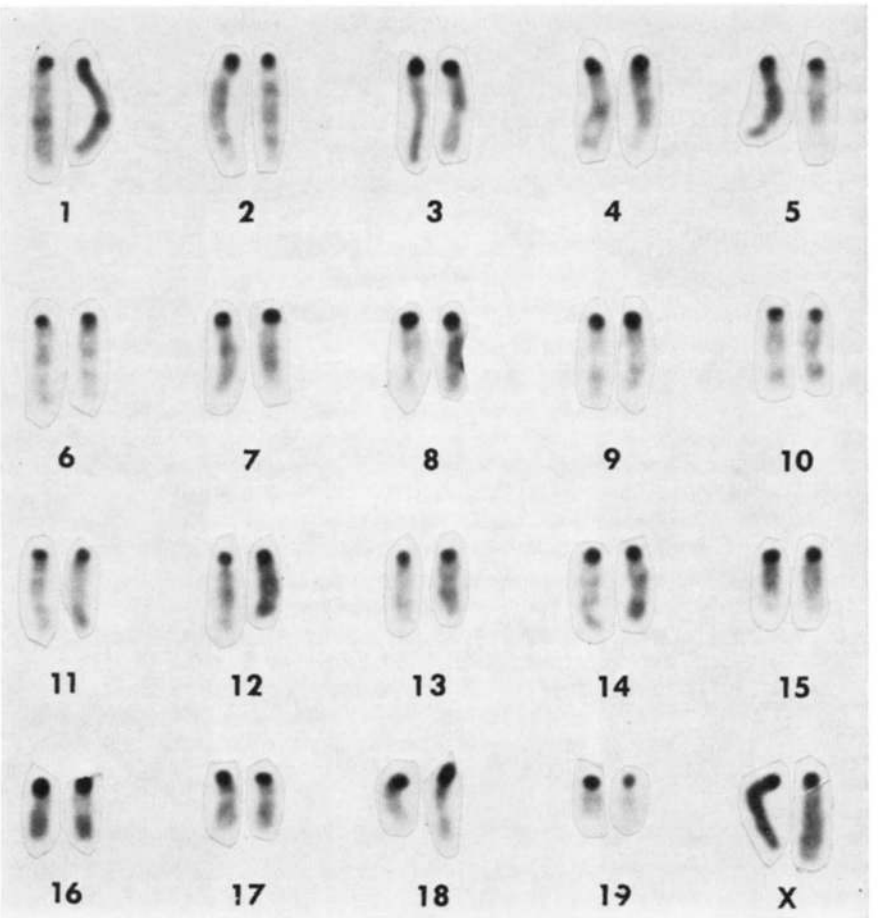

FIGURE 1.—C-banded karyotype of a C57BL/6J cell. Each chromosome was identified by Q-banding.

the A9 cell line, an HGPRT-deficient cell line derived from L cells, which are of C3H origin. As shown in Figure 4b, chromosome 14 in the A9 cells, like that in C3H, has little C-banding material. (The A9 cells do not have a normal 18 for comparison.) Number 14 is the only A9 chromosome which lacks C-banding material, with the exception of two markers. One of these is very small and its origin indeterminate. The other, M5, has the same fluorescent pattern as chromosome 14 in its proximal two-thirds, but has additional material at the distal end (Figure 4b. The two staining methods therefore provide strong evidence that the M5 chromosome originated from a chromosome 14. Figure 4b also shows a marker M1, which has C-banding material in non-centromeric locations.

DISCUSSION

Our results indicate that the amount of C-banding material on each chromo-

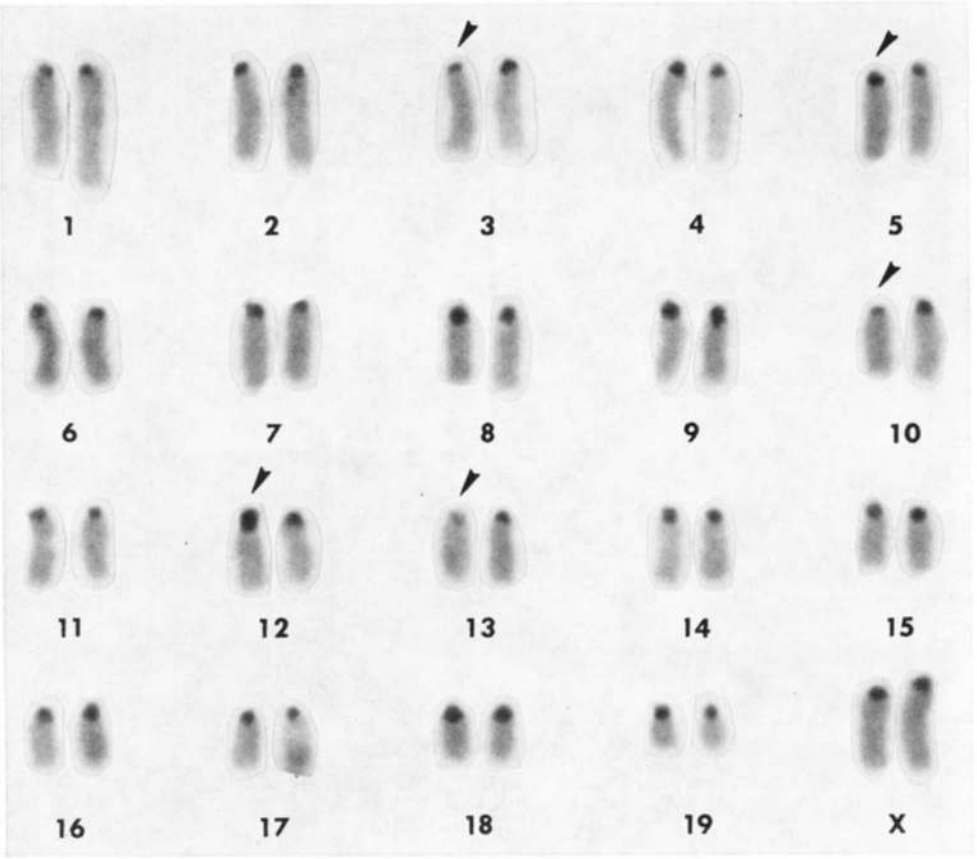

FIGURE 2.—C-banded karyotype of a C57L/J × C57BL/6J F_1 hybrid cell. Each chromosome was identified by Q-banding. Arrows indicate chromosomes of C57L/J origin.

some is characteristic for each inbred strain of mice. The existence of obvious differences in the distribution of C-banding material among the chromosomes in different inbred strains, and the persistence of the differences in F_1 hybrids, suggest that the amount of C-banding material on a chromosome is a polymorphic trait that is inherited in a simple fashion. This characteristic can therefore be used as a marker to distinguish between two normal homologous chromosomes. Several of the chromosomes can also be distinguished by the presence or absence of large non-staining secondary constrictions located adjacent to the C-banding region (Table, DEV *et al.* 1971). The presence of a secondary constriction region on chromosome 19 was used in mapping LG XII (EICHER 1971).

It is noteworthy that, with only a few strains examined (Table 1), 12 of the 20 chromosomes have shown markers of one or both types mentioned. Some of these, such as the number 14 with little C-banding material in both C3H and DBA mice, can be attributed to a common ancestor. However, inbred strains derived from a single set of parents can exhibit a surprising degree of diversity, as shown

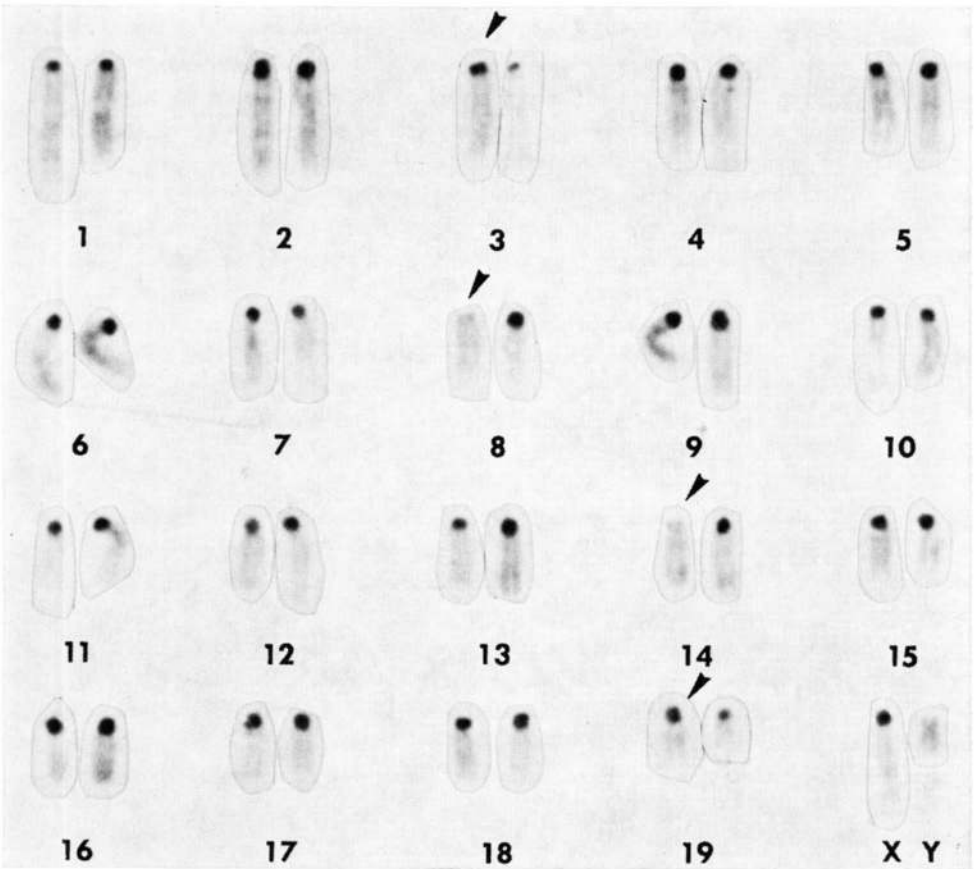

FIGURE 3.—C-banded karyotype of a 129 × AKR F_1 hybrid cell. Each chromosome was identified by Q-banding. Arrows indicate chromosomes of 129 origin.

with C57BL and C57L. As more inbred strains are examined, additional markers will undoubtedly be identified, perhaps involving every chromosome. We have found that almost every chromosome of *M. m. mollossinus* differs from that of *M. musculus* in the size of C-banding region (Table 1). Since *M. m. mollossinus* is interfertile with *M. musculus*, markers for every mouse chromosome except the Y are potentially available.

C-banding variants should be useful in genetic linkage studies, particularly those involving genes near the centromere. These normal variants could also be used in place of abnormal chromosomes, such as the T6 translocation marker (FORD 1966), in studies which require distinguishing between parental cell types (WIENER *et al.* 1972).

Information is still too fragmentary to permit any conclusions about the range in the amount of C-banding material which can be tolerated on specific chromosomes or in the entire mouse genome. It is not clear, for example, whether *M. m. mollossinus* has less C-banding material than *M. musculus*, or whether it is

TABLE 1

Chromosome markers revealed by C-banding (centromeric heterochromatin) and Q-banding (secondary constrictions)

Strain	Stocks examined Homozygote	F_1	Chromosomes with distinguishing markers C-banding*	Q-banding†
C57BL/6J	+	..		
C57L/J	+	+‡	3—,5+,10—,12+,13—	12,19
C3H/HeJ	+	+‡	14—,18—	..
DBA/2J	—	+‡	1+,14—	..
SM/J	+	+‡	16—	16,18
A/J	+	—	4—	18
AKR	+	+§	3—,19—	18
129	—	+§	8—,14—	16,19
M. m. mollossinus¶	?		1+,2+,3—,4+,5—,6+ 7—,9—,10—,11+,12+, 13—,14—,15—,16—,17—, 18+,X—	..

* C-bands larger (+) or smaller (—) than in C57BL/6J.
† Dev *et al.* 1971.
‡ Crossed with C57BL/6J ♂.
§ 129 ♀ × AKR ♂.
¶ Seth *et al.* 1973.

merely redistributed. Selective breeding of the two subspecies could be used to obtain laboratory mice with smaller or larger amounts of C-banding material, and, presumably, of satellite DNA, in the total genome.

Changes in the amount of C-banded material carried by a particular chromo-

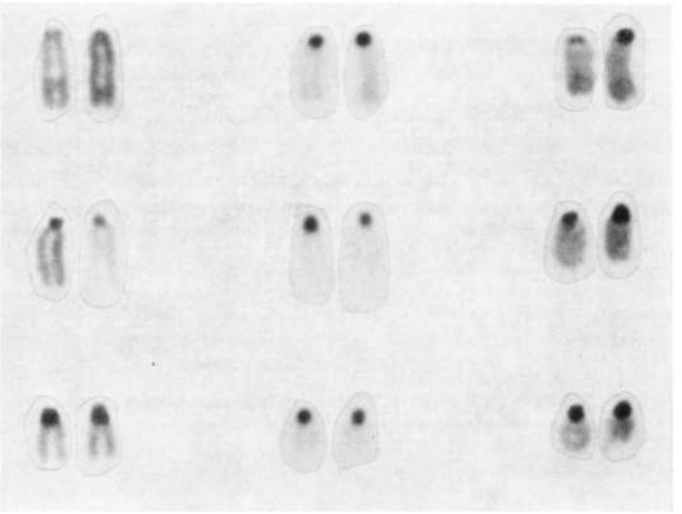

Figure 4a.—Partial karyotype comparing C-banding of chromosomes from a C3H/J cell (left), a C57BL/6J cell (middle) and an F_1 hybrid cell (right). Each chromosome was identified by Q-banding. The C3H chromosome 14 (first row), which has very little C-banding, and the number 18 (second row), which has a moderate amount, can be distinguished from the homologous C57BL chromosomes in the F_1 hybrid. Chromosome 19 (third row), which cannot be distinguished by the amount of C-banding, has been included for comparison.

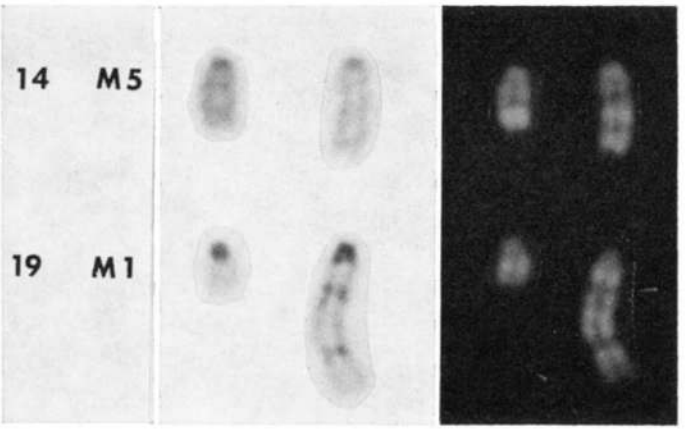

FIGURE 4b.—Chromosomes 14, 19, M1 and M5 from an A9 cell stained to show C-banding (left) and Q-banding (right). Note the absence of C-banding material and the similarity of Q-banding of chromosome 14 and its probable derivative M5. Marker M1 has C-banding material in two non-centromeric locations. Chromosome 19 is included for comparison.

some might be produced by one of the common types of chromosomal change such as deletion or translocation. Evidence that a translocation can be involved comes from cell lines in which markers, such as the A9 marker M1, have been shown to have C-banded material in regions not adjacent to the centromere (CHEN and RUDDLE 1971; ALLDERDICE *et al.* 1973). We have studied one reciprocal translocation, *T(10;13)199H*, in which the breakpoint is so close to the centromere of chromosome 13 that virtually all the C-banded material has been transferred to the other chromosome (unpublished data).

Changes in the amount of C-banding material may also be the result of unequal crossing over, which would be expected to occur in chromosomal regions which contain highly repetitive nucleotide sequences. The C-banding regions of mouse chromosomes are composed mainly of highly repetitious DNA, called satellite DNA (PARDUE and GALL 1970), consisting of millions of copies of a very short nucleotide sequence, or minor variants of it (SOUTHERN 1970).

The nucleotide sequences of the satellite DNA's of related mouse and rodent species have been shown to be quite different (HENNIG and WALKER 1970; SUTTON and MCCALLUM 1972.) No differences have been found in satellite DNA from several inbred strains of *Mus musculus* (C57, C3H, DBA, Swiss, and New Zealand black) (FLAMM and WALKER, unpublished data referred to by FLAMM 1972). Our data suggest, however, that the C-banding region of the chromosomes of inbred lines is variable and that comparison of other strains might provide evidence for within-species differences in the amount or type of satellite DNA.

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