

# The chromosomes of the Cynomolgus macaque (*Macaca fascicularis*)

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The Cynomolgus or crab-eating macaque, *Macaca fascicularis* (*M. irus*) has 42 chromosomes. The X chromosome is submetacentric and about 5 % in length of the complement. One of the X chromosomes is very late replicating in the female somatic cells. The other X is also relatively late replicating. The Y chromosome is a minute acrocentric. A short metacentric chromosome was also found to be late replicating. Chromosome no. 20 has an obvious secondary constriction which often associates in a characteristic way. The sex bivalent is identified at pachytene as a characteristic "sex vesicle". At diakinesis it shows an end-to-end association. The mean number of chiasmata per cell was 40 at diakinesis-first metaphase.

The Cynomolgus or crab-eating macaque was first named by CUVIER as *Macaca irus* (1818). NAPIER and NAPIER (1967) in their exhaustive revision of the biology and taxonomy of living primates have used, as the first appropriate name for this monkey, that of *Macaca fascicularis* RAFFLES (1821). Although this is a primate currently used in virology and cell line experiments only few studies on its chromosomes have been published (CHU and BENDER 1961; CHIARELLI 1962; FERGUSON and TOMKINS 1964). No studies on the DNA replication pattern and on the meiotic chromosomes of this species has been found in literature.

## Materials and methods

Five males and one female were used. Two or three blood cultures from each animal were prepared and chromosomes preparations were obtained from cell suspensions by the air-drying method.

For studies on the meiotic chromosomes the testes were processed by the method described by HULTÉN et al. (1966) and by squashing seminiferous tubules after staining with acetic orcein. The labelling of chromosomes in blood cultures

from one male and the female was done by adding 0.4 µc of tritiated thymidine (Amersham, specific activity 5 c/mM) per ml of culture medium during the last four and six hours of incubation in the female, and for four hours in the male. Colcemide® was added one hour before harvesting.

For autoradiography the slides were coated with Kodak AR 10 stripping film and exposed for seven days. The labelled mitoses were photographed before and after removal of the autoradiographic grains.

## Results

### 1. Somatic chromosomes

All animals had a diploid number of 42 chromosomes. The chromosomes were classified into two groups according to their size and position of the centromere. The first group was composed of 13 submetacentrics and the second of 6 metacentrics. The identification of the autosome pairs was difficult because of their almost continuously decreasing size. The only exception was pair no. 20, which was very easy to recognize because of its large secondary constriction on the short arm.

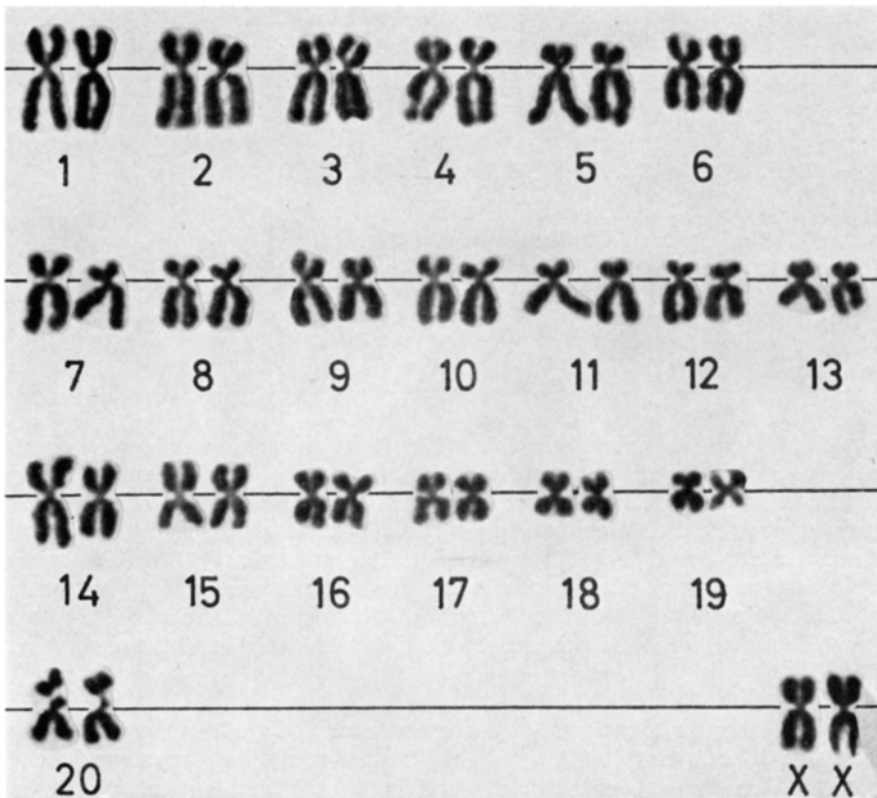


Fig. 1. Female karyotype. The chromosomes have been arranged into two groups (see text).

This pair was not included in any of the two groups because the variability in the length of the secondary constriction made it appearing either as a submetacentric or as a metacentric chromosome.

The Y chromosome was acrocentric and the smallest of the complement. Because of its minute size it was not always easy to recognize the position of the centromere (Fig. 2, 3 d, f). The X, as identified by autoradiography in the female cells, was a submetacentric chromosome very similar in size to no. 6 with a mean arm ratio of 1.6 (in 8 cells range 1.4—1.7) and representing 4.8 % of the absolute length of the karyotype (Fig. 1 and 2). A striking association of chromosomes 20 apparently at the secondary constriction was found in several metaphases from cultures from one of the males and the female (Fig. 3). This association was usually pronounced in the female cells (Fig. 3 a, b, c) while it more often appeared as a close

“contiguity” of the two chromosomes in the male (Fig. 3 d, e, f).

## 2. Male meiotic chromosomes

Chromosome counts made on first and second meiotic metaphases revealed 21 bivalents and 21 chromosomes, respectively. A peripherally located condensed and intensely stained body was seen in early zygotene and interpreted as the sex bivalent (Fig. 4 a). In the same stage minute heterochromatic bodies were seen on the autosomes. These heterochromatic bodies tended to disappear with progressive condensation of the autosomes in pachytene. In this stage, the sex chromosomes appeared as a “sex vesicle” (Fig. 4 b). In early and late diplotene it was usually difficult to distinguish the sex pair, although it was clearly visible in diakinesis and first metaphase showing an end to end association (Fig.

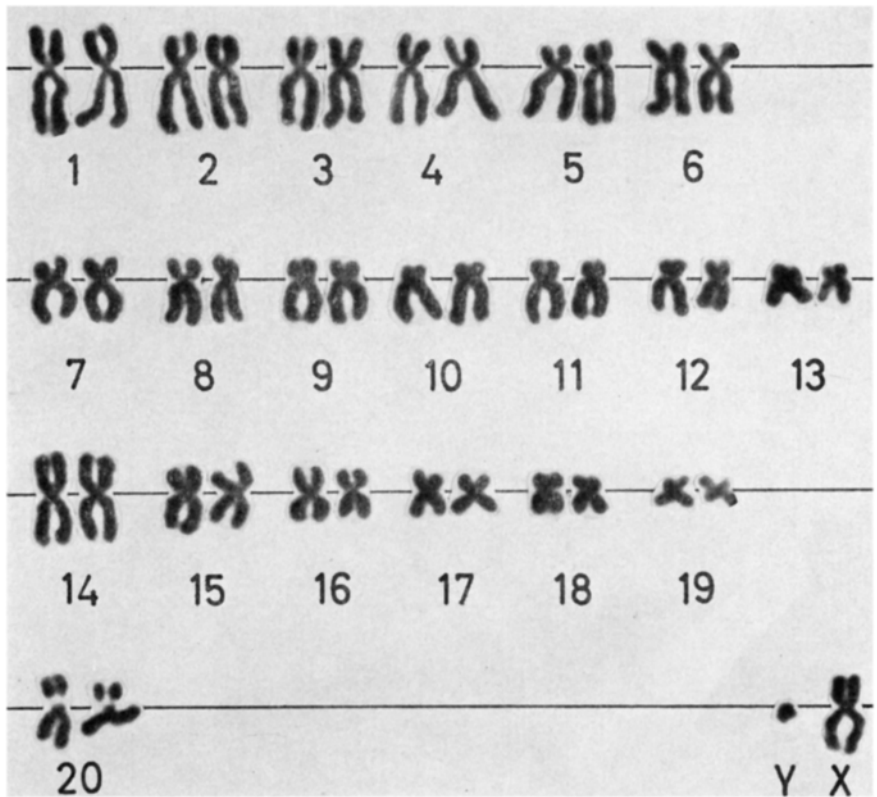


Fig. 2. Male karyotype.

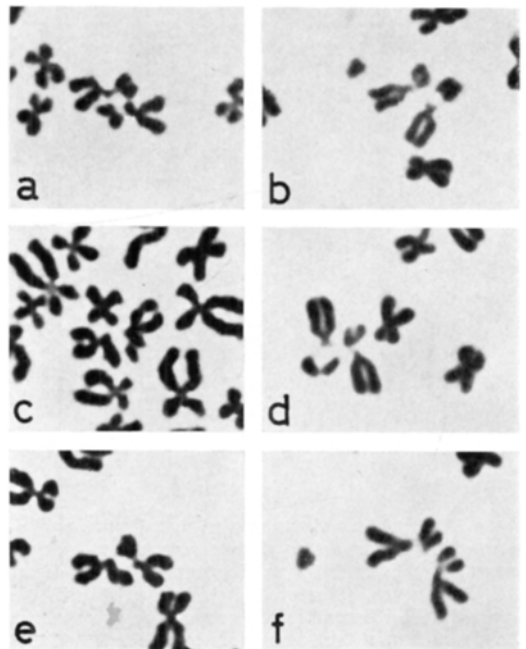


Fig. 3. Association of the chromosomes No 20: a—c: details from female metaphase plates with the chromosomes associated by the acromatic zone; d—f: details from male metaphases. The minute acrocentric in d and f is the Y chromosome.

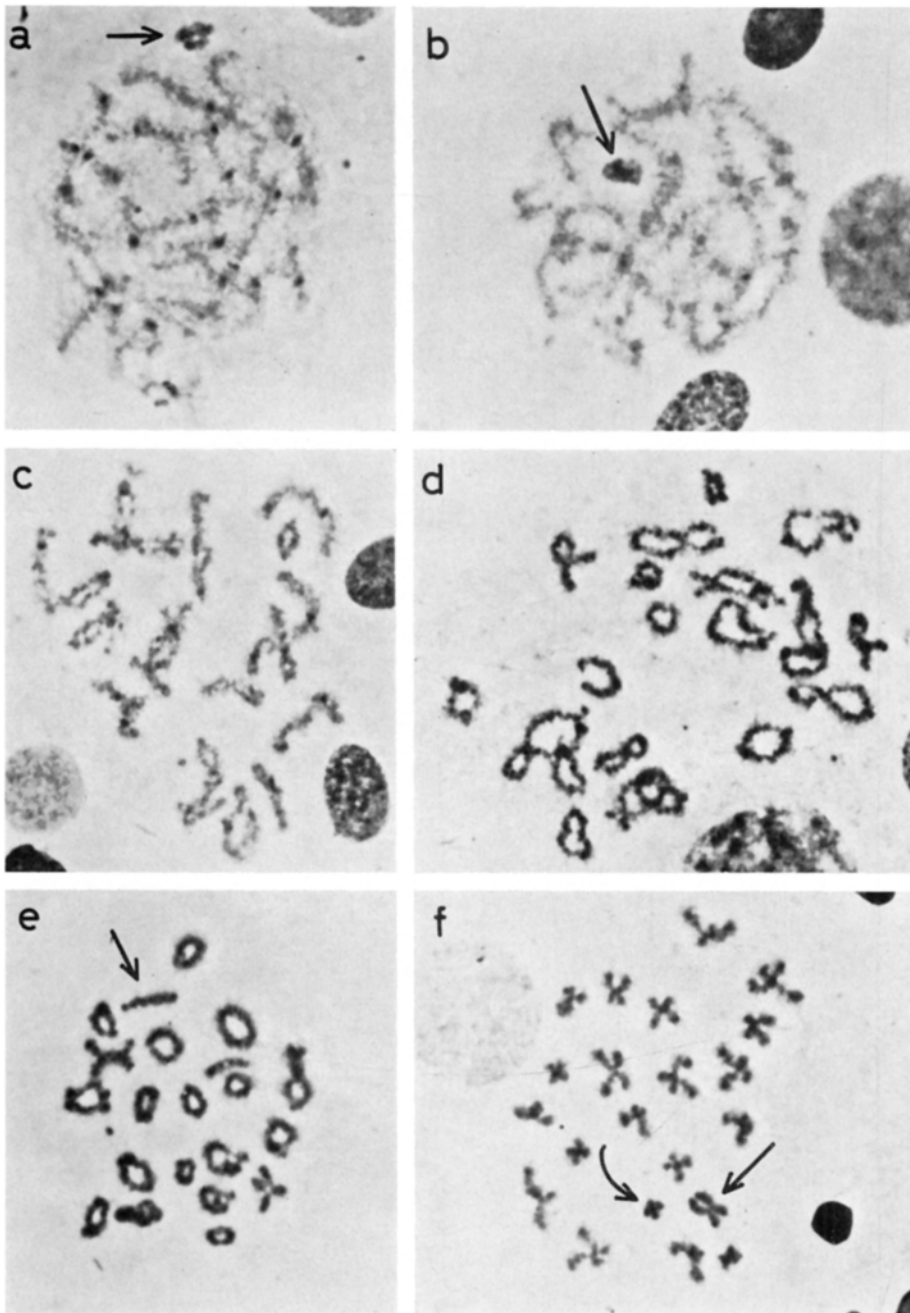


Fig. 4. Male meiotic chromosomes. a: Zygotene. The sex bivalent indicated by an arrow. Minute heterochromatic bodies on the autosomes. b: Pachytene. The sex bivalent forms the "sex vesicle" (arrow). c) Early diplotene. d: Late diplotene, 45 autosomal chiasmata. e: First metaphase with 21 bivalents, 40 autosomal chiasmata. The XY bivalent (arrow) shows an end to end association. f: Second metaphase with an X and 20 autosomes. The X (straight arrow) and a short metacentric (bent arrow) are more condensed than the other chromosomes.

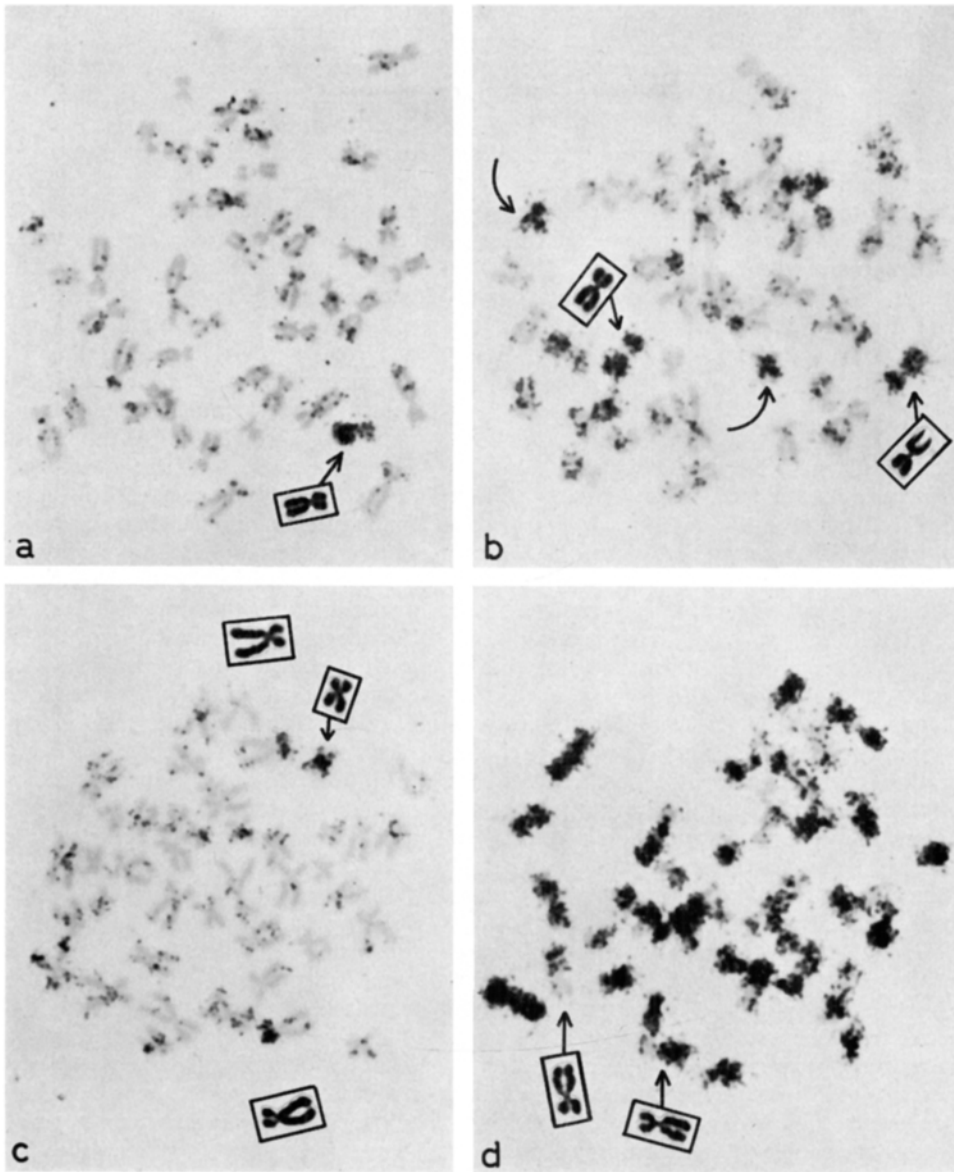


Fig. 5. Radioautographs of female metaphases from leucocytes cultures. a—c: From cultures with four hours of incubation with  $^3\text{H}$  thymidine. a: The late replicating X chromosome and the same chromosome without grains in the box. The long arm is more labelled than the short. b: Two late replicating X chromosomes in the boxes and the same chromosomes without grains. Note the differences in labelling between the long arm of the two labelled X chromosomes and the two late replicating short metacentric chromosomes (bent arrow). c: A small late replicating metacentric chromosome and chromosomes No 5 showing a heavily labelled short arms. The same chromosomes without grains in the boxes. d: Two X chromosomes in a heavily labelled metaphase after six hours of incubation with  $^3\text{H}$  thymidine. The short arms are unlabelled and the long arms differentially labelled. The two X chromosomes are less labelled than the rest of the complement.

4 e). The number of autosomal chiasmata per cell was estimated in 10 cells in diplotene, diakinesis and first metaphase. The result was 37, 38, 39, 40, 40, 41, 41, 42, 42, giving a mean number of 40 autosomal chiasmata per cell.

The chromosomes were generally difficult to identify in second metaphase except for the X and Y chromosomes which were relatively easy to recognize, X because it was more condensed than the autosomes and Y due to its small size (Fig. 4 i). A small metacentric chromosome as condensed as the X was observed in some of the second metaphases (Fig. 4 f).

### 3. Labelling pattern

Among 71 female metaphases which had been in contact with tritiated thymidine for the last four hours of culture, 41 were labelled. Three main types of labelling pattern were recognized. In the first one, a single late replicating submetacentric chromosome was found, which was interpreted as an X (Fig. 5 a). In a second group of cells another submetacentric chromosome, interpreted as the second X, was also heavily labelled although somewhat less. This chromosome displayed asynchrony in the labelling of the two arms the long arm being usually more labelled than the short (Fig. 5 a, d). A third group of metaphases showed one or two short metacentrics, pairs no. 17 or 18 in our classification, with a high grain count. These were sometimes the last to terminate replication (Fig. 5 c).

Among 20 cells from female cultures labelled for the last six hours, 14 were labelled. In some of the more heavily labelled cells from this sample one or two X chromosomes and one of the small metacentrics were among the less labelled chromosomes of the complement. In the male cultures incubated with tritiated thymidine for four hours, it was not possible to detect any significant pattern of replication because of the poor incorporation of tritiated thymidine and the small number of labelled metaphases recovered.

## Discussion

This study confirms that the diploid number of *M. fascicularis* is 42 (CHU and BENDER 1961), which is the same for all the species studied of the

genus *Macaca* (CHIARELLI 1962 b; BORGAONKAR 1966; EGOZCUE 1969). The late replicating X chromosome found in our experiments differed from the X chromosome identified by CHIARELLI (1962 b) on a morphological basis. The X chromosome identified by radioautography appeared more submetacentric (mean arm ratio 1.6 versus 1.3) and shorter (relative length 4.8 % versus 5.3 %) than that found by CHIARELLI (1962 a). For the rest of the karyotype there was in general good agreement between our results and those published earlier (CHIARELLI 1962 b; FERGUSON and TOMKINS 1964). The association between the chromosomes No. 20 is very similar to that between the acrocentric satellited chromosomes in man (OHNO et al. 1961; FERGUSON-SMITH and HANDMAKER 1961) and in some species of Hominoidea (HAMERTON et al. 1963). This association found in male and female metaphases might be interpreted as the persistence of nucleolar material in a chromosome zone which organizes a nucleolus during the interphase (OHNO et al. 1961). The morphological variations of this association in different samples of cells may be due to technical variations. It is also clear that the association is between the acromatic zone of the chromosomes and not between the satellites (Fig. 3 a, b, c). The unexpected pattern of late replication of a short metacentric in the female cells is in accordance with the relatively more condensed state observed in the same pair in the second meiotic metaphases of the male.

*M. fascicularis* represents the largest group of subspecies, 21, in the genus *Macaca*, and has a wide geographical distribution (NAPIER and NAPIER 1967). Recently FOODEN (1964) from studies on a sample of monkey, considered by this author as "intermediates" of the geographical range of the species *M. fascicularis* and *M. mulatta*, has proposed that these two groups of monkeys should be considered as a single species. The same author reports the occurrence in captivity of fertile hybrids of both sexes between *M. fascicularis* and *M. mulatta*. The well-known similarities of the karyotypes of these two species (CHU and BENDER 1961) tend to confirm this proposition. Studies on mitotic and meiotic chromosomes of the different subspecies of *M. fascicularis* and *M. mulatta* and *fascicularis/mulatta* hybrids, and comparative studies of labelling patterns, would be of definitive importance when testing the hypothesis of FOODEN and would also clarify

whether the natural populations and domestic colonies of this group of *Macaca* are polymorphic for minor chromosomal rearrangements.

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