THE CHROMOSOME COMPLEMENT OF THE BONNET MONKEY (*MACACA RADIATA*) DETERMINED IN LEUCOCYTES

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I. INTRODUCTION

ANATOMICAL, biochemical and to a large extent the behavioral studies contribute to the phylogeny and classification of primates, while cytological data provide additional criteria in elucidating the intraspecies relation as well as evolution of these animals.

The morphology and number of chromosomes are characteristic of the species. Assigning the taxonomic position or tracing of phylogeny of a particular group depends on the study of rearrangements and breakages of chromosomes. The karyotype—the number, length and relative arm size—is an important and specific morphological character and is associated with the intimate genetic make-up of the species.

Since most mammals possess several small chromosomes, counting and observing individual chromosomes face several hazards. The recent development of advanced techniques for the culture of somatic cells and the improved cytological methodology have made the assessment of chromosome number and morphology quite tangible.

Utilizing these techniques, investigations have been made on the karyotype of somatic chromosomes of many higher primates, including man, by several investigators like Chu *et al.* (1962), Bender *et al.* (1958, 1963), Klinger (1963), Chiarelli (1963 *a*) and others. Based on these observations, the Catarrhines have been divided into two superfamilies, Cercopithecoidea and Hominoidea (Chiarelli, 1966). The superfamily Cercopithecoidea embraces genus *Macaca*.

The cytology of rhesus macaque (*Macaca mulatta*) has been extensively studied (Rothfels and Siminovitch, 1958) but little about this aspect has been investigated for bonnet monkey (*Macaca radiata*). This paper presents the cytological observations on the karyotype of bonnet macaque with interest centered upon the female karyotype as the male has earlier been reported (Chiarelli, 1962 *d*).

II. MATERIALS AND METHOD

Several cytological advances have yielded remarkable results in mammals and especially man. The karyotypes, in primates especially, have been studied by culturing small pieces of skin, kidney, etc., obtained by biopsy or necropsy. Bone marrow obtained by laceration of ribs provides good results. The leucocyte culture is an ideal material to study the mitotic nuclear complement.

Though our efforts with bone marrow squashing yielded promising results, the best results were obtained by culturing the white blood corpuscles.

Blood from 2 males and 3 females was collected. The sterilised syringe coated with Heparin (anticoagulant) was used and 2.5 to 3 ml. of blood was collected by sending the needle directly into the heart of 4 animals and from the radial vein of one female. The blood cells were incubated for 72 hours in chromosome medium IA dissolved in Phytohaemagglutinin (GIBCO) at 37° C.

Four to five hours before the termination of incubation period, 0.2 ml. of blood culture colchicine (5 mg. colchicine in 500 ml. distilled water) was added to the medium. After the incubation the culture was transferred into a siliconed centrifuging tube and centrifuged at 5 settings for 4 minutes. The leucocytes were freed of the supernatant fluid and to the cells was added 6 ml. of 0.75 M, KCl (1.4 gm. KCl in 250 ml. of distilled water and 2 ml. of heparin, with pH adjusted to 7.0 by adding a few grains of sodium bicarbonate) kept at 37° C. The cells were thoroughly agitated to prevent clumping and were kept at 37° C. for 20 minutes. KCl was removed by centrifugation at the same rate and time as before, and the cells were fixed in freshly prepared fixative (3 : 1 methanol and glacial acetic acid) and agitated. The fixative was removed by recentrifuging and fresh fixative was added to the cells and kept in freezing chamber or with ice cubes around for about 20 minutes. After 20 minutes, the cells were separated from the fixative by centrifugation and

about 0.4 to 0.5 ml. (depending upon the desired number of slides) of fixative was added and the cells were thoroughly suspended in the fixative by sucking in and squirting out of a fine siliconed pipette.

Slides, pre-cleaned with 95% alcohol and chilled distilled water, were used and a few drops of cell suspension were taken on the slides and dried at 35° C. on a hot plate.

The cells were stained with 2% natural Orcein for about 2 hours and taken through 2 grades of 95% alcohol and one grade of absolute alcohol (for about 2 minutes in each grade) and mounted using diaphan.

III. RESULTS

(1) *Chromosome Number*

In suitable preparations, a very clear picture of the chromosomes was seen. In fairly well-spread plates the nucleus presented a normal diploid number. It was not uncommon to see a nuclear deficiency of 1 or 2 chromosomes from the normal but never a polyploid condition was encountered. Such normal, non-polyploid cells showed a count of 42 chromosomes and are in conformity with the other members of Cercopithicinae showing the same number of chromosomes. The chromosomes of all species of Cercopithicinae have a number of common morphological features such as sex chromosome dimorphism, *i.e.*, XX in female and XY in male. Thus there are 40 autosomes and 2 X chromosomes in a female and 40 autosomes and X & Y chromosomes in male.

(2) *Chromosome Measurement*

Well-spread chromosomes with well-defined centromeres in a nucleus were chosen to obtain measurement for quantitative analysis (Fig. 1). They were microphotographed using ZHR 74 filter and printed. The enlarged chromosomes were measured in mm. with the aid of a map measurer calibrated in fifths of a mm. For each chromosome, mean of the two short and two long chromatid arms was recorded in mm. From the mean length of the long and short arms, the arm ratio was calculated by dividing the length of the short arm into that of the long arm. The homologous pairing was done based on the relative length and arm ratio. The mm. units were then converted into micron units with a micrometer. The chromosomes were then numbered by Roman numerals in decreasing order of length. The X chromosome thus ranked VIII in the series and the sat-chromosome bearing the secondary constriction was IX.

The chromosomes could be conveniently divided into four types:

(1) Large submetacentric chromosomes, (2) Submetacentric with secondary constriction, (3) Metacentric chromosomes, (4) the sex chromosomes.

Of the 42 chromosomes, 12 pairs are large and submetacentric and they are I, II, III, IV, V, VII, X, XI, XII, XIII, XIV and XV; one pair bearing the secondary constriction, *i.e.*, IX; seven pairs are metacentric, *i.e.*, VI, XVI, XVII, XVIII, XIX, XX and XXI; and one pair of sex chromosomes, *i.e.*, VIII.

Measurement of individual pair of chromosomes is given in Table I. Based on these measurements an idiogram is constructed (Fig. 2) that diagrammatically represents the relative length of the chromosomes.

TABLE I

Identification of homologous chromosomes by length and arm ratio

Chromosome No.	Long arm in units	Short arm in units	Total length in units	Relative length	Index
I. 1	4.0	2.3	6.3	3.8	.58
2	3.6	2.2	5.8	3.5	.61
II. 1	3.8	2.0	5.8	3.5	.52
2	3.4	2.0	5.4	2.7	.58
III. 1	3.0	1.6	4.6	2.8	.53
2	3.6	1.4	5.0	3.1	.39
IV. 1	3.0	1.6	4.6	2.8	.53
2	3.6	1.4	5.0	3.1	.39
V. 1	3.0	1.6	4.6	2.8	.53
2	3.0	1.8	4.8	2.9	.60
VI. 1	2.8	2.2	5.0	3.1	.79
2	2.6	1.8	4.4	2.7	.69
VII. 1	2.4	2.0	4.4	2.7	.83
2	2.8	1.8	4.4	2.7	.59
VIII. 1	3.6	1.2	4.8	2.9	.33
2	2.6	1.2	3.8	2.3	.46

TABLE I (Contd.)

Chromosome No.	Long arm in units	Short arm in units	Total length in units	Relative length	Index
IX. 1	2.4	2.0	4.4	2.7	.83
2	2.0	1.6	3.6	2.2	.80
X. 1	2.2	1.8	4.0	2.4	.82
2	2.4	1.6	4.0	2.4	.69
XI. 1	2.4	1.6	4.0	2.4	.69
2	2.2	1.4	3.6	2.2	.63
XII. 1	2.8	1.2	4.0	2.4	.43
2	2.4	1.2	3.6	2.2	.50
XIII. 1	2.4	1.2	3.6	2.2	.50
2	2.4	1.2	3.6	2.2	.50
XIV. 1	2.4	1.2	3.6	2.2	.50
2	3.0	1.4	4.4	2.7	.47
XV. 1	2.0	0.8	2.8	1.7	.40
2	2.4	1.2	3.6	2.2	.50
XVI. 1	1.6	1.6	3.2	1.9	1.0
2	1.6	1.6	3.2	1.9	1.0
XVII. 1	1.6	1.2	2.8	1.7	.75
2	1.6	1.2	2.8	1.7	.75
XVIII. 1	1.4	1.2	2.6	1.6	.86
2	1.4	1.2	2.6	1.6	.86
XIX. 1	1.6	1.2	2.8	1.7	.75
2	1.2	1.0	2.2	1.4	.83
XX. 1	1.2	1.2	2.4	1.5	1.0
2	1.4	0.8	2.2	1.4	.57
XXI. 1	1.0	0.8	1.8	1.1	.80
2	1.0	0.8	1.8	1.1	.80

IV. DISCUSSION

The chromosome complement of male and female bonnet monkeys has been assessed by leucocyte culture. The chromosome number is 42.

The X chromosome is placed in the 8th place of decreasing order. The Y of male is the smallest. The 40 autosomes form a decreasing series, the longest measuring 6.3 microns and the shortest 1.8 microns. The chromosome IX carries a non-stainable secondary constriction close to the centromere and it is believed to be the nucleolar chromosome.

The karyotype of bonnet monkey is similar to that of rhesus (Rothfels and Siminovitch, 1958) but there is a considerable difference in the size of chromosomes as the rhesus chromosomes are longer than those of the bonnet and measure from 3 to 15 microns. In possessing a satellite chromosome, the bonnet monkey resembles *Papio*, *Theropithecus*, *Cercocebus*, *Cercopithecus*, *Presbytis* and *Hylobates* (Chiarelli, 1962 *d* and 1963 *a*). The number 42, that is quite common for all the members of Cercopithecoidea, can hence be taken as the 'type number' (White, 1948) for Cercopithecoidea.

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FIG. 1. Photomicrograph of metaphase nucleus from Bonnet Monkey.

FIG. 2. Idiogram of haploid chromosome complement of Bonnet Monkey.