Chromosome Banding of *Cercopithecus neglectus*

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Introduction

Chromosome analysis will aid in understanding the phylogenetic relationships of the Cercopithecoidae. Within the genus *Cercopithecus*, the chromosome number varies from 48 to 72 (2), indicative of numerous chromosomal rearrangements that have occurred during evolution. Consequently, karyotypic studies are needed to provide information on the chromosome number, morphology and banding pattern for these species. Previous efforts to study the chromosomes of *Cercopithecus neglectus* conducted in the pre-banding era have led to conflicting chromosome counts (1, 8). Only recently has this species been reinvestigated by Dutrillaux et al. (4) who reported an R-banded karyotype. Q-banding is a fluorescent method which stains positively in the areas that respond negatively by R-banding. Therefore, Q-banding is complementary to R-banding and provides unique information about the Q-positive regions. Q-banding has not been reported for *C. neglectus* nor for most of the other 25-30 species in the superfamily Cercopithecoidae. Those few species that have been published include *Miopithecus talapoin*, *Cercopithecus aethiops* (3), and *Erythrocebus patas* (7).

Methods

Peripheral blood was obtained from four males maintained by the Psychology Department at Indiana University, Bloomington. Four lymphocyte cultures were prepared for each specimen using 10ml of Chromosome Medium 1A (GIBCO). Cultures were incubated at 37°C for 72 hours. During the last 90 minutes of incubation, the cells were exposed to .001mg of Colcemid (GIBCO) to arrest the cells at metaphase. This was followed by treatment with hypotonic solution (.075M KCl) for 10 minutes and then fixation using three changes of methanol: acetic acid (3:1). Slides were prepared, stained in 4% Giemsa (Harleco) and scanned for chromosome spreads. Photos were taken at 1200X on 35mm Kodak Technical Pan film. Slides were destained then stained with 0.5mg/ml quinacrine mustard dihydrochloride (Sigma) in McIvaine's buffer (pH 6.5) for 20 minutes. Following a 10 minute wash in distilled water, slides were mounted in buffer and observed under epifluorescent illumination with an Olympus Vanox microscope. Photographs were taken using Kodak Tri-X film.

Results and Discussion

Tappen (8) reports a diploid number of 60 for *C. neglectus* based on one male specimen. Efforts by Chiarelli (1) to karyotype the species resulted in different conclusions from each of two specimens. He observed a diploid number of 58 and 62 and noted differences in the chromosome morphology as well. More recently Dutrillaux et al. (4) proposed an R-banded karyotype with $2n = 62$, although the number of specimens they used is not clearly specified. The authors claim that their analyses utilized either one or two animals for each species reported. Using four unrelated male specimens, we report a diploid number of 62 in each. Previous studies which failed to find 62 chromosomes may have been caused by incorrect species identification, chromosomal rearrangements unique to that specimen, or previously unrecognized subspecies. Since *C. neglectus* is distinctively identifiable by the white beard and orange crest on the forehead, thereby minimizing the likeli-

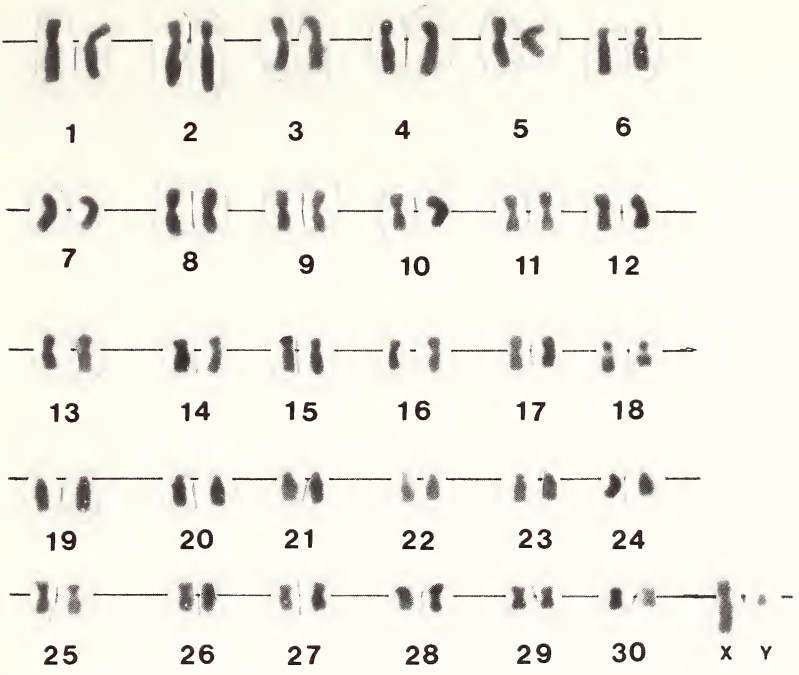

FIGURE 1. *Giemsa stained karyotype of Cercopithecus neglectus.*

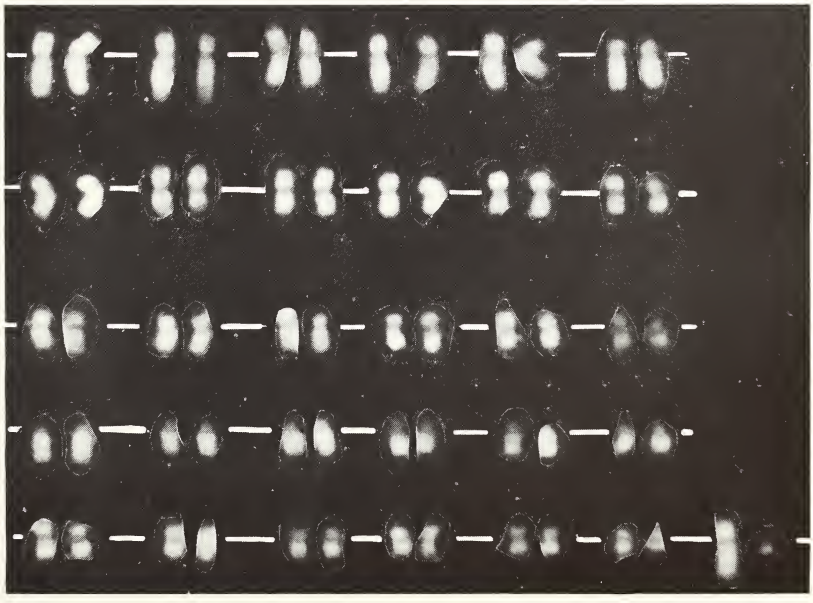

FIGURE 2. *Q-banded karyotype of the same chromosome spread shown in Figure 1.*

hood of missidentification, confirmation of the species integrity is aided by chromosome reports from diverse sources.

The relative size and centromeric position are most readily defined on Giemsa stained preparations (Figure 1). The karyotype consists of seven large submetacentrics [1-7], five medium metacentrics (7-12], eleven medium and small submetacentrics [13-17, 25-30], seven acrocentrics [18-24] and the sex chromosomes.

Giemsa staining delineates the chromosome morphology, but does not enable complete chromosome identification. Therefore, sequential staining was used to yield a Q-banded karyotype (Figure 2). Positive bands are located in the AT rich regions of the chromosome and can display region to region variability of intensity. The X chromosome is comparable in size, centromeric position, and staining pattern to the X chromosome of other primates. However, the fluorescent pattern of the Y chromosome has not been reported for this genus. The small short arm is nonfluorescent and there is a broad band of medium intensity located medially in the long arm.

The marker chromosome 18 is most distinctive due to the unstained secondary constriction observed when Giemsa stained and is found in all members of the genus studied. In several other genera of old world monkeys Henderson et al. (6) demonstrated that this corresponds to the rDNA genes and represents the nucleolar organizing region (NOR). Positive silver staining in *C. aethiops* by Estop et al. (5) implicates this as the NOR for all Cercopithecoides. Among six specimens observed by Estop and coworkers, one marker chromosome lacked a secondary constriction and two others revealed size related heteromorphic variation. The four unrelated specimens observed in the present study did not display size heteromorphisms.

The NOR regions of human chromosomes are located on the short arm of the D and G group acrocentrics and are often positioned close together, a phenomenon known as association. Among 44 *C. neglectus* spreads sampled from the four males, 14 (32%) were laterally associated such that the NOR were in contact and 9 (20%) located within two chromosome lengths of each other. Investigation of human chromosomes by Warburton et al. (9) demonstrated that the number of rDNA gene copies in a chromosome is positively correlated with the frequency of satellite association. Since strong association is also demonstrated in *Cercopithecus*, it would be of interest to test this observation with heteromorphic chromosomes from old world monkeys as well.

Acknowledgments

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Literature Cited

1. CHIARELLI, B. Chromosome polymorphism in the species of the genus Cercopithecus. *Cytologia* (Tokyo) *33*:1-16, 1968.
2. CHIARELLI, B. Chromosomes and evolution of the old world primates. *Caryologia 26*:507-512, 1973.
3. DUTRILLAUX, B., E. VIEGAS-PEQUIGNOT, J. COUTURIER, and G. CHAUVIER. Identity of euchromatic bands from man to cercopithecidae. *Hum. Genet. 45*:283-296, 1978.

4. Dutrillaux, B., J. Couturier, and G. Chauvier. Chromosomal evolution of 19 species of sub-species of cercopithecinae. *Ann. Genet. 23*:133-143, 1980.

5. Estop, A., J. J. Garver, and P. L. Pearson. Further studies on the comparative karyology of the African Green and Rhesus monkeys. *Genetica 49*:131-138, 1978.

6. Henderson, A.S., D. Warburton, S. Megraw-Ripley, and K. C. Atwood. The chromosomal location of rDNA in selected lower primates. *Cytogenet. Cell Genet. 19*:281-302, 1977.

7. Ponsa, M., R. Miro, A. M. Estop, and J. Egozcue. Banding patterns of the chromosomes of Erythrocebus patas (Schreber 1774) compared to other primate species. *Genetica 56*:39-45, 1981.

8. Tappen, N. C. Genetics and systematics in the study of Primate evolution. *Symposia of the Zoological Society of London 10*:276, 1960.

9. Warburton, D., K. C. Atwood, A. S. Henderson. Variation in the number of genes for rRNA among human acrocentric chromosomes; correlation with frequency of satellite association. *Cytogenet. Cell Genet. 17*:221-230, 1976.