

Table 2. Activity of extracts of clams kept in a bath with a flow of water maintained at different temperatures. Before being placed in the bath the clams had been kept in an outdoor pool, approximate temperature 5°C, for 90 days. The activity of extracts obtained from clams after removal from the pool was 346 units.

Temperature of bath (°C)	Time in bath (days)	Activity (retine units/kg)
15.5	7	300-500
21.0	7	400-500
15.5	14	1355
15.5	14	1122
21.0	14	1400
15.5	26	2981
15.5	47	2900-3000

the high summer values can be restored by keeping the clams in warm water for 4 weeks before preparing the extracts.

ANDREW HEGYELI

*Institute for Muscle Research,
Marine Biological Laboratory,
Woods Hole, Massachusetts*

References and Notes

1. A. Szent-Györgyi, A. Hegyeli, J. A. McLaughlin, *Science* **140**, 1391 (1963).
2. A. Hegyeli, J. A. McLaughlin, A. Szent-Györgyi, *ibid.* **142**, 1571 (1963).
3. M. R. Schmeer, *ibid.* **144**, 413 (1964).
4. Aquacide II, (C grade, Calbiochem).
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Chromosome Complements of Two Species of Primates: *Cynopithecus niger* and *Presbytis entellus*

Abstract. *Forty-two chromosomes were counted from expanded cultured cells of four Celebes apes, Cynopithecus niger. Mitotic cells from three Indian langurs, Presbytis entellus, contained 44 chromosomes. These counts support cytogenetically the taxonomical classification of these animals according to Simpson.*

At least part of the confusion that still attends the classification of primates has been created by those students "who had no experience in taxonomy and who were completely incompetent to enter this field" (1). New species have been commonly named entirely on the basis of gross morphology, coat color-pattern, or the geographical location of a single specimen. Modern classification, however, is based on representatives of a population and on the integration of

social behavior, ecology, geography, gross anatomy and physiology, and paleontology; species designation based upon such broad biological lines has greater validity. In recent years blood typing (2), immunologic properties (3), and chromosome analysis (4, 5) have also become indispensable criteria for animal classification.

In this report we present the chromosome counts of two species of primates from the colony of the Oregon Regional Primate Research Center. This information supports the present classification according to Simpson's taxonomic scheme.

Tissues from four specimens of *Cynopithecus niger* (two males and two females) were examined. Monolayer cultures of kidneys and lungs were grown in vitro (Melnick, 6). Bone marrow taken from the femur with a heparinized syringe was dispensed into a flask containing medium 109 (7) and 15 percent fetal calf serum and incubated until there was a sufficient growth of cells for chromosome preparations. The metaphasic cells were expanded according to the methods of Tjio and Puck (8) and Rothfels and Siminovitch (9).

Ninety percent of well-expanded chromosomes of the cells prepared from bone marrow, kidney, and lung cultures exhibited a diploid number $2n = 42$ (Fig. 1A). Analysis of chromosomes based on the centromere position (10) showed 11 pairs of metacentric and 9 pairs of subterminal chromosomes. Of the sex chromosomes, the X chromosome was subterminal and the Y chromosome appeared acrocentric. The satellite pair was considered to be in the metacentric group and was generally the fourth to fifth longest.

These animals, commonly called Celebes apes, are found in the Celebes Islands and several other islands east of Wallace's Line. In general, fauna east of the Line has been more isolated from continental Asia than that west of the Line. Male and female adult *Cynopithecus* have a characteristic crest of hair at the top of the head and darkly pigmented skin, and are covered with heavy, black body-hair. Although tailless, the body-build, facial architecture, and locomotor pattern of these animals place them closer to the macaques than to any other form of primate in Southeast Asia.

As further evidence of this relationship, the chromosome number of 42 is the same for *Cynopithecus* and all known *Macaca* (9, 11), although the

centromeres are in somewhat different positions. The African counterpart of these animals, the baboon (*Papio*), also has 42 chromosomes, with ten pairs each of metacentric and subterminal autosomes, and X and Y metacentric sex chromosomes (11). Our karyograms for two male Guinea baboons are in close agreement with those previously described by Chu and Giles (11), except that we considered the Y chromosomes to be acrocentric rather than metacentric. The satellite pair was considered metacentric and was about the fifth largest in that group. These fundamental cytological similarities support Simpson's classification of the Celebes ape in the subfamily Cercopithecinae with the macaque and baboon.

We also examined cultured tissues from the kidneys and bone marrow of three Indian langurs (*Presbytis entellus*). At least 90 percent of the countable metaphasic cells examined had a diploid number of 44 chromosomes (Fig. 1B). There were 14 metacentric and six subterminal pairs and one acrocentric pair of autosomes, and subterminal X and Y chromosomes. The satellite pair was considered metacentric and was nearly always the smallest in size. The count of 44 chromosomes in our specimens does not agree with that of Makino (12), who reported 50. Although Makino studied cells from only one animal, the discrepancy in these findings may be attributed to today's improved techniques. For another species of Colobinae from West Africa, *Colobus polykomos*, Bender and Chu (4) reported a count of 44 chromosomes, with 28 metacentric, 14 subterminal, 1 metacentric X, and 1 acrocentric Y. One of the cytogenetic differences noted between these two species is the position of the male sex chromosome.

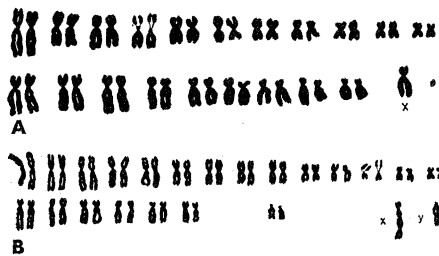


Fig. 1. (A) Representative karyotype of a male *Cynopithecus niger*. From a cultured bone marrow cell with a count of 42 chromosomes. (B) Representative karyotype of a male *Presbytis entellus*. From a cultured kidney cell with a count of 44 chromosomes.

While the specific origin of the Colobinae used in this study is unknown, their coat-color and facial characteristics distinguish them as Indian langurs.

RICHARD N. USHIJIMA
F. STUART SHININGER
THEODORE I. GRAND

Oregon Regional Primate Research
Center, Beaverton, Oregon

References and Notes

1. G. G. Simpson, *Bull. Am. Museum Nat. Hist.* **85**, 181 (1945).
2. D. Franks, in *The Primates, Proceedings of a Symposium held in London, April 1962*, J. Napier and N. A. Barnicot, Eds. (Zoological Society, London, 1963), vol. 10, p. 243.
3. E. Zuckerkandl, in *Classification and Human Evolution*, S. L. Washburn, Ed. (Aldine, Chicago, 1963), p. 243.
4. M. A. Bender and E. H. Y. Chu, in *Evolutionary and Genetic Biology of Primates*, J. Buettner-Janusch, Ed. (Academic Press, New York, 1963), vol. 1, p. 261.
5. J. L. Hammerton, in *The Primates, Proceedings of a Symposium held in London, April 1962*, J. Napier and N. A. Barnicot, Eds. (Zoological Society, London, 1963), vol. 10, p. 211.
6. J. L. Melnick, in *Diagnostic Procedure for Viral and Rickettsial Diseases* (American Public Health Association, New York, ed. 2, 1956), p. 142.
7. Microbiological Associates, Inc.
8. J. H. Tjio and T. T. Puck, *J. Exptl. Med.* **108**, 259 (1958).
9. K. H. Rothfels and L. Siminovitch, *Chromosoma* **9**, 163 (1958).
10. J. H. Tjio and A. Levan, *Hereditas* **42**, 1 (1956).
11. E. H. Y. Chu and N. H. Giles, *Am. Naturalist* **91**, 273 (1957).
12. S. Makino, *Cytologia, Tokyo* **16**, 288 (1952).
13. We thank Beverly Cole for her technical assistance. This is Oregon Regional Primate Research Center Publ. No. 43.

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Venom and Venom Apparatus of the Bull Ant, *Myrmecia gulosa* (Fabr.)

Abstract. *The venom of Myrmecia gulosa (Myrmeciinae, Formicidae) is of a proteinaceous type and is separated by electrophoresis into eight components. The venom contains histamine, a hyaluronidase, and a direct hemolytic factor. It also shows kinin-like activity. In structure the venom apparatus is found to be massively formed internally as well as externally. It is distinguished from the apparatus of representatives of other ant subfamilies by characteristics of the free gland filaments, the reservoir wall, the intra-reservoir glandular area, and the accessory gland.*

The bull ant, *Myrmecia gulosa* (Fabr.), was chosen as a subject for studies on venom and the venom apparatus because it belongs to the most primitive subfamily of Formicidae known (1), the Myrmeciinae, a group whose distribution is restricted to the Australian region (2).

Reduction of the sting in higher ants such as the Dolichoderinae and Formicinae (3), coupled with the loss of stinging function as an aspect of behavior, has long been recognized. On the other hand the sting is well developed in the Myrmeciinae, where it fulfills a major function in the general pattern of behavior. In *M. gulosa* workers, the sting is used both defensively, for the protection of the nest, and offensively, in solitary foraging for insects used as larval food. Stinging activity is related directly to temperature. At 18° to 20°C insect prey introduced into laboratory colonies are killed only after an interval, if at all. At 25° to 30°C insects introduced are killed immediately. This temperature relationship has been confirmed by observation of the seasonal behavior of *M. gulosa* in the field.

The stimulus to sting appears to be based on visual or sound perception, but in nest defense, which follows a complex behavior pattern that includes stinging, a pheromone may also be involved.

In *M. gulosa*, as in other Hymenoptera, the venom apparatus of which the sting is the external part, is made up of (i) a pair of gland filaments lying free in the body cavity which are united in the form of a Y; (ii) a spheroidal venom reservoir into which the base of the latter is inserted, and from which a duct supplies the bulb of the sting; (iii) an accessory gland, also running into the sting bulb; (iv) the sting mechanism itself, which is supported on each side by interconnected chitinous plates modified from the last three abdominal segments.

In the larger workers the terminal part of the gaster containing the venom apparatus is approximately 4.0 to 5.0 mm in length by 3.5 mm in width, while the reservoir is 1.8 to 2.0 mm by 1.4 to 1.5 mm. The sting shaft, normally retracted under the gaster, is three-fifths as long as the latter,

and the two venom gland filaments coiled within the gaster are twice its length. The lateral plates of the sting are up to 2.7 mm by 1.9 mm in extent, and they enclose strongly developed muscles which fill the end of the gaster and activate the sting lancets.

Several characteristics distinguish the venom apparatus of *M. gulosa* (Fig. 1) from that of representatives of other ant subfamilies. (i) The free gland filaments are considerably longer, but more slender than in other forms; compare *Pseudomyrmex* (Pseudomyrmecinae) (4), *Solenopsis* (5), *Myrmica* (Myrmicinae) (3), *Bothriomyrmex* (Dolichoderinae) (3), *Formica* (Formicinae) (3). (ii) The basal section uniting the filaments (Fig. 1, n) has been found free only in *Pseudomyrmex* and in two genera of Ponerinae (6), in addition to *Myrmecia*. In higher subfamilies the glands penetrate the wall of the reservoir at their point of union. (iii) The basal gland section enters the wall of the venom reservoir at about one third the length of the reservoir from its posterior end. In *Formica* the point of entry is at the posterior end, in *Solenopsis* it is about the center, and in *Myrmica* and *Bothriomyrmex* it is at the apex. (iv) The wall of the venom reservoir consists of an outer network of heavy muscle strands and an inner, tightly folded cuticular layer. A similar muscle layer is reported to occur in *Formica*, but has not been reported in other genera. (v) After penetrating the wall of the reservoir the venom gland passes forward between its inner and outer layers, only the terminal enlargement being invaginated into the cavity of the reservoir. In *Formica* the invaginated region is relatively much larger, while in *Myrmica* and *Bothriomyrmex* the gland is completely invaginated from its point of entry into the wall. (vi) The central duct of the venom gland enlarges terminally and opens straight into the cavity of the reservoir, as in *Myrmica* and *Bothriomyrmex*. In *Formica*, on the other hand, it remains narrow and is elongated and convoluted. (vii) The accessory gland is a tubelike sac of more or less uniform diameter as in other groups of Hymenoptera, in contrast to the bulbous form in higher ants such as *Formica* and *Myrmica*. Further data on the morphology of the venom apparatus of *M. gulosa* will be reported in detail elsewhere.