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.

3 August 1964

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* (Pseudomyrmicinae) (*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.

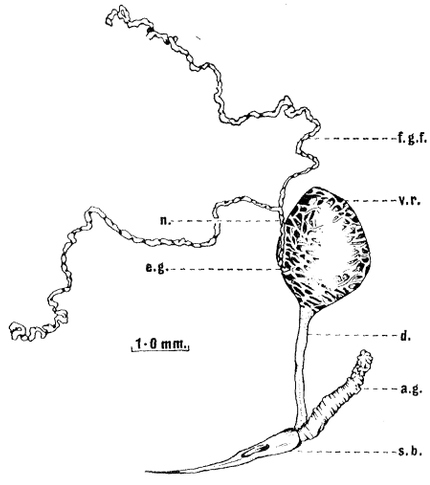

Fig. 1. Component parts of the venom apparatus of the bull ant, *Myrmecia gulosa* (Fabr.). Abbreviations: *a.g.*, accessory (Dufour's) gland; *d.*, venom duct; *e.g.*, point of entry of free venom gland between outer and inner layers of reservoir wall; *f.g.f.*, free gland filaments; *n.*, "neck" region of venom gland; *s.b.*, sting bulb; *v.r.*, venom reservoir.

The collection of sting-bearing ants in quantity and the extraction of their venom present many practical difficulties. These account in part for the paucity of knowledge of ant venoms (*7*), as distinct from anal and mandibular secretions. During the present study on *M. gulosa* collected in the Sydney area, the venom was obtained as a clear colorless fluid from the venom reservoir, which was dissected out of the gaster. The yield of dry venom was 0.3 mg per reservoir, or 0.35 percent of the body weight of the insect.

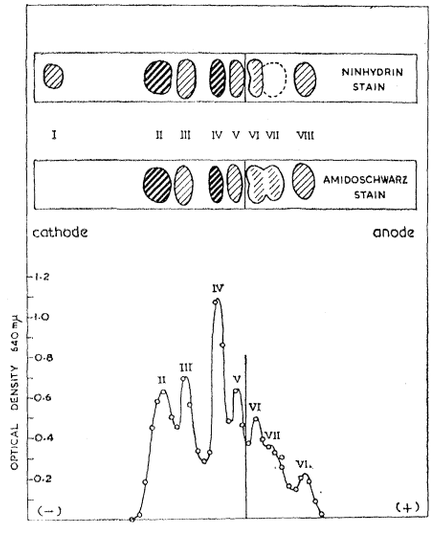

Fig. 2. Pherograms of paper electrophoretic separation (see *10*) of the venom of *M. gulosa* at *p*H 6.24, stained with ninhydrin, and with amidoschwarz 10B. The distribution curve for fractions II through VIII was determined by measurement of optical density at 640 mμ for the amidoschwarz-stained pherogram.

The venom, which was readily soluble in water but insoluble in organic solvents, gave a positive reaction to each of the following reagents: ninhydrin, alkaline cupric sulfate, Millon's reagent for phenolic amino acids, and Hopkins-Cole reagent for tryptophane. The ultraviolet spectrum showed absorptions at 204 mμ and at 277 to 282 mμ, the latter being characteristic of polypeptides containing aromatic aminoacids. Acid hydrolysis of the crude venom, followed by two dimensional paper chromatography of the hydrolysate (*8*), showed the presence of at least thirteen amino acids, including aspartic acid, glutamic acid, lysine, glycine, histidine, serine, alanine, proline, valine, leucine, and isoleucine.

When subjected to paper electrophoresis, with the horizontal technique (*9*), the venom was resolved into at least eight fractions (*10*). Each fraction gave a positive reaction with 0.2 percent ninhydrin; fractions II to VIII also reacted positively with the protein dyes bromophenol blue and amidoschwarz 10B. At *p*H 6.24, fractions I to V migrated to the cathode, and fractions VI to VIII to the anode. Pherograms, stained with ninhydrin and with amidoschwarz 10B, and the distribution curve for the separation of the fractions, are illustrated in Fig. 2. The venom was similarly resolved on starch gel (*11*), fractions II to VIII again being stained by amidoschwarz 10B.

Fraction I, ninhydrin-positive only, was shown by comparative paper electrophoresis and by paper chromatography in three solvent systems to correspond to histamine. A quantitative colorimetric estimation (*12*) of the dry venom showed 2 percent histamine, a value corresponding to that recently noted (*13*) for the venom of the bull ant, *M. pyriformis*. Fractions IV and V showed strong hyaluronidase activity, estimated by the turbimetric method of Tolksdorf *et al.* (*14*). This activity, which was stronger in fraction IV, was approximately one fifth that of a standard enzyme preparation (330 U.S.P. units per milligram) of hyaluronidase prepared from bovine testes. Fractions IV and V also showed a sustained kinin-like activity on guinea pig ileum and on rat uterus (*15*). Fraction VII contained a direct hemolytic factor, which was estimated by a modification of Neumann and Habermann's procedure (*16*). This hemolytic factor was heat labile. The venom did not appear to contain a cholinesterase, 5-nucleotidase, or proteinase.

This chemical investigation of the venom of *M. gulosa*, together with contemporary studies on *M. pyriformis* (*13*), establishes for the first time the nature of the components of a proteinaceous venom in the Formicoidea (see *7*). In terms of chemical taxonomy, the venom is more closely related to that of the wasps (Vespoidea) and bees (Apoidea) than to the venoms of higher ant genera. Morphologically, too, as a member of the Hymenoptera *Mymecia* would be regarded as a genus like *Vespa* and *Apis* whose venom apparatus is highly evolved. But *Myrmecia* is a basic and generalized ant genus, and within the Formicoidea the venom apparatus has evolved still further, so that there are structures such as those in the Formicinae and Dolichoderinae which are in part degenerate.

G. W. K. Cavill
Phyllis L. Robertson
F. B. Whitfield

School of Chemistry,
University of New South Wales,
Kensington, Australia

References and Notes

1. W. M. Wheeler, *Ants. Their Structure, Development and Behaviour* (Columbia Univ. Press, New York, 1910); W. L. Brown, Jr., *Insects Sociaux* **1**, 21 (1954).
2. J. Clark, *The Formicidae of Australia*, vol. 1, *Subfamily Myrmecinae* (C.S.I.R.O., Melbourne, 1951).
3. A. Forel, *Z. Wiss. Zool.* **30**, suppl., 28 (1878).
4. M. S. Blum and P. S. Callahan, *Psyche* **70**, 69 (1963).
5. P. S. Callahan, M. S. Blum, J. R. Walker, *Ann. Entomol. Soc. Am.* **52**, 573 (1959).
6. P. L. Robertson, unpublished work.
7. R. L. Beard, *Ann. Rev. Entomol.* **8**, 1 (1963).
8. F. G. Fischer and H. Dorfel, *Biochemische Z.* **324**, 465 (1953).
9. H. J. McDonald, *J. Chem. Educ.* **29**, 428 (1952); ———, M. C. Urbin, M. B. Williamson, *J. Colloid Sci.* **6**, 236 (1951).
10. An "Eel" electrophoresis unit was used with Schleicher and Schuell paper No. 2043, Sorenson's M/15 phosphate buffer (*p*H 6.24), and a current of 0.3 ma/cm was applied for 16 hours at 10°C.
11. O. Smithies, *Biochem. J.* **61**, 629 (1955).
12. S. Webster, *Farm. Revy* **46**, 749 (1957).
13. I. S. de la Lande, D. W. Thomas, M. J. Tyler, *Biochem. Pharmacol.* **12** (Suppl.), 187 (1963).
14. S. Tollesdorf, M. H. McCready, D. R. McCullagh, E. Schwenk, *J. Lab. Clin. Med.* **34**, 74 (1949).
15. We are indebted to Dr. I. S. de la Lande for conducting these tests.
16. W. Neumann and E. Habermann, *Arch. Exptl. Pathol. Pharmakol.* **222**, 1367 (1954).
17. Supported by USPHS grant EF00319 from the Division of Environmental Engineering and Food Protection. We thank P. J. Williams for technical assistance.

3 August 1964