Sexual Calling Behavior in Primitive Ants

Abstract. *The wingless virgin females of the ponerine ant* Rhytidoponera metallica *attract males by the release of a pheromone from the tergal gland, a hitherto unrecognized exocrine gland located between the last two abdominal segments. This is the first evidence for sexual chemical communication in the large and primitive subfamily Ponerinae.*

Female sex attractants occur widely among insects, but within the ants only members of the subfamily Myrmicinae have been demonstrated to possess these pheromones. In each case, the myrmicine sex attractants have proved to originate either in the Dufour's gland or the poison gland (*1*). We report here the discovery of a specific sexual calling behavior in the primitive ant *Rhytidoponera metallica*, during which female reproductives release a chemical attractant from a hitherto unrecognized organ we propose to call the tergal gland.

Rhytidoponera is a large genus concentrated in Australia and New Guinea extending to Timor, the Moluccas, the Philippines, and the Solomon Islands (*2*). Many of the species are unusual in that normal alate females are either absent or rare and apparently do not play a role in colony reproduction. Instead a proportion of workers, externally morphologically indistinguishable from their fellows, possess functional spermathecae, are inseminated by males, and serve as multiple reproductives in the colony (*3*, *4*). Since none of these worker reproductives or "ergatoids" have wings to conduct nuptial flights, they have to employ other means to advertise their "readiness" for mating and to attract flying males.

For several years we have observed a remarkable behavior in these reproductive workers of the Australian *Rhytidoponera metallica* (*4*) that appears to be calling behavior: the ergatoids emerge from

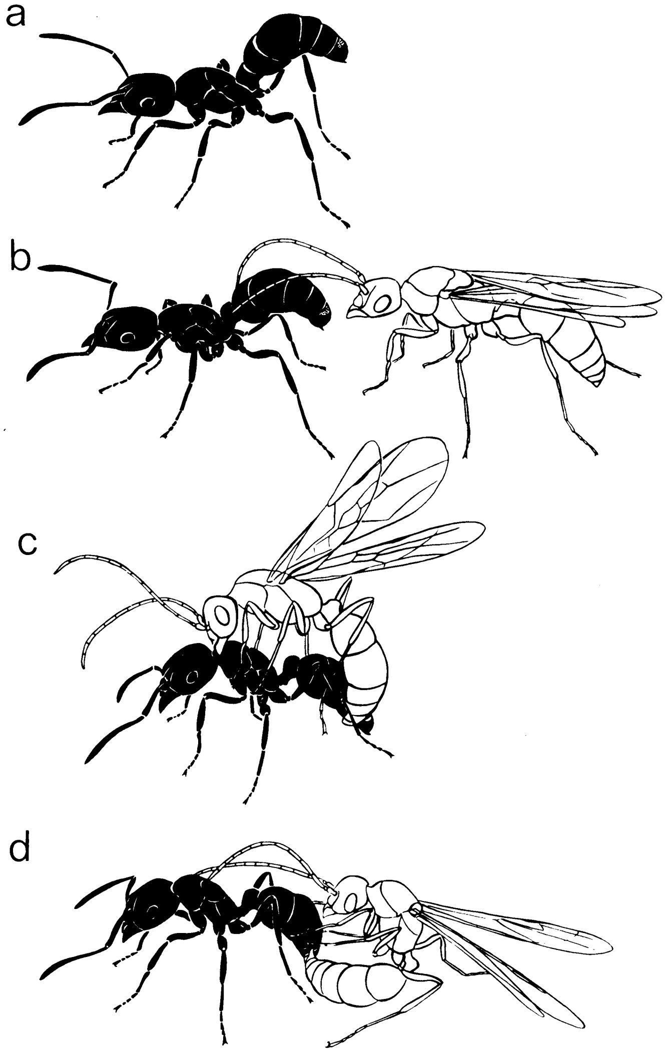

Fig. 1 (left). Mating in *Rhytidoponera metallica*. (a) Ergatoid female (black) in calling posture, during which the dorsal intersegmental membrane between the last two segments is extended. (b) A male (white) approaches a calling female, touching her with his antennae. (c) The male grasps the female at the thorax and mounts her. Simultaneously he extends his copulatory organ in a search for the female's genitals. (d) Copulation: the male has released his mandibular grip on the female's thorax. The drawings are based on photographs and observations.

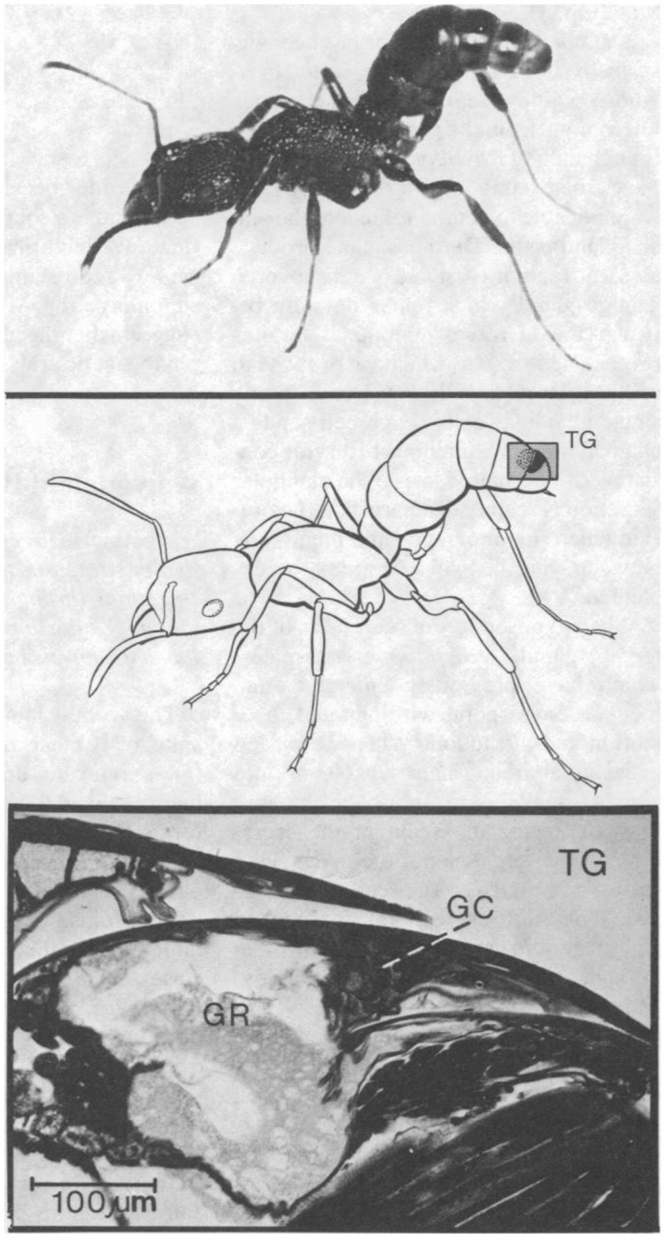

Fig. 2 (right). *Rhytidoponera metallica* female in calling posture (top). Note the expanded intersegmental membrane between the last two tergites, the location where the tergal gland (*TG*) opens to the outside (middle). Longitudinal section through the gaster of an *R. metallica* female, shows the tergal gland (*TG*) with its glandular cells (*GC*) and reservoir (*GR*) (bottom).

the nest and group quietly near their nest entrance, with the head and thorax lowered to the ground, the gaster raised and arched, and the intersegmental membrane between the last two segments dorsally extended (Fig. 1). Males flying out from other nests are attracted by these "calling" females. On drawing close to a female, the male first touches her with his antennae, then grasps the female's thorax with his mandibles. While riding on her back, he extends his copulatory apparatus in search of the female's genitalia. If she is ready to mate, she turns her abdomen slightly to the side, so that the male is able to couple. Then the male releases his mandibular grip on the female's thorax. With the pair in this position the copulation can last several minutes (Fig. 1).

A similar calling behavior has been described in *Harpagoxenus subleavis*, whose calling females release a sex pheromone from the poison gland (5). Although calling *Rhytidoponera* frequently have their sting slightly exposed, we were not able to demonstrate that the poison gland or the Dufour's gland produce a sex attractant. Instead, we discovered a large gland which opens dorsally between the last two abdominal tergites (Fig. 2). This organ, which we propose to call the tergal gland, consists of many single glandular cells. Each cell sends a channel into a voluminous reservoir consisting of two paired sacs. The glandular secretion is readily discharged to the outside when the intersegmental membrane between the last two tergites is expanded.

When exposed to the secretions of the tergal gland, *Rhytidoponera* males, which have previously emerged from their nests, respond with agitated locomotion and attraction. When we placed males in an arena (diameter, 60 cm) into which a weak air current (0.5 m/sec) was blown carrying the scent of the secretions of the tergal gland, the males were strongly attracted to the scented air current. They did not respond to a control air current simultaneously blown in through a second opening (Table 1). Several males exposed to tergal gland secretions attempted to mount one another. When a worker was made available, some males tried to mate with it, even though it was not "calling." These results suggest that some *Rhytidoponera metallica* workers produce a sex attractant in the tergal gland, which they discharge during sexual calling. This is the first evidence of sexual chemical communication in the primitive ant subfamily Ponerinae. A first histological survey of 11 ponerine species (6) indicates that the new gland is a common structure in Ponerinae. We have also found it in *Myrmecia vindex*, representing a second primitive subfamily, the Myrmeciinae. This fact suggests that the gland is a very primitive phylogenetic trait in ants generally. We suppose, however, that the primary function of the gland is not the secretion of sex pheromones, because we have found it to be present in workers of species that have no ergatoid reproductives.

Table 1. After males of *Rhytidoponera metallica* had left their nests, they were placed in an arena, which was then covered by a red glass plate. Weak air currents (0.5 m/sec) were next blown into the arena, one being first led over moist (H_2O) filter paper (control), the other over the secretion of a single tergal gland (test). A count was made of the number of males being attracted to either one of the air currents within a 5-minute interval. The openings through which the test current and the control current were led were alternated in position in successive replication. For each replication a new population of males was used.

Replication	Number of males tested	Number of males responding to	
		Tergal gland scent	Control
1	18	6	0
2	27	13	2
3	12	8	1
4	21	16	3
5	9	7	0
6	11	6	0
7	26	12	3

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References and Notes

1. B. Hölldobler, *J. Insect Physiol.* **17**, 1497 (1971); ——— and M. Wüst, *Z. Tierpsychol.* **32**, 1 (1973); A. Buschinger, Symposium of the International Union for the Study of Social Insects, Dijon, 1975.
2. W. L. Brown, Jr., *Bull. Mus. Comp. Zool. Harv. Univ.* **118**, 175 (1958); E. O. Wilson, *Evolution* **13**, 122 (1959).
3. R. M. Whelden, *Ann. Entomol. Soc. Am.* **53**, 793 (1960).
4. C. P. Haskins and R. M. Whelden, *Psyche* **72**, 87 (1965).
5. A. Buschinger, *Experientia* **24**, 297 (1968).
6. Ponerine ants found to possess the tergal gland include species of the genera *Amblyopone, Paraponera, Ectatomma, Odontomachus, Pachycondyla,* and *Platythyrea*. More detailed accounts of the sexual behavior of ponerine ants and the functioning of the tergal gland are being prepared.
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Amygdaloid Projections to Prefrontal and Motor Cortex

Abstract. *Direct projections from the amygdala to the entire frontal cortex were demonstrated in the cat using the retrograde transport of horseradish peroxidase. Injections throughout the prefrontal cortex labeled neurons in the ipsilateral basal magnocellular amygdaloid nucleus; injections in the premotor and motor cortices labeled neurons in the same nucleus plus a few cells in the anterior amygdaloid area.*

The frontal lobe of the brain has, in spite of a clear regional differentiation (1), a relevant unitary function in the neurobiological correlates of the highest forms of behavior (2). Stimulation of the amygdaloid complex, on the other hand, elicits certain motor and behavioral phenomena closely related to responses obtained by stimulation of the frontal lobe cortex (3). Such a relation instigated this search for a direct monosynaptic connection between the amygdala and the frontal cortex.

Studies of neocortical monosynaptic afferent connections are facilitated by using horseradish peroxidase (HRP) as a retrograde tracer [for example, see (4 and 5)]. In the present study we used HRP to trace systematically the afferent connections to the whole frontal cortex, including prefrontal area [gyrus proreus, comparable to the frontal granular cortex of primates (6)] and motor and premotor areas (gyrus sigmoideus and both banks of the sulcus cruciatus).

The entire frontal lobe was explored in a group of 34 adult cats, each of which received an intracortical injection of 0.3 to 1.5 μl of a 25 to 50 percent aqueous solution of HRP (Sigma VI). All injections were unilateral and most remained limited (Fig. 1). The animals were anesthetized 30 to 60 hours later and were perfused with a solution of paraformaldehyde and glutaraldehyde. In most cases perfusion commenced with 10 percent dextran. The brains were removed and processed according to a modified LaVail and LaVail method for revealing HRP (7).

Animals that received frontal lobe injections of HRP exhibited HRP-labeled cells in the amygdala; direct connections from this nucleus to the motor, pre-