

Sexual Calling by Workers Using the Metatibial Glands in the Ant, *Diacamma* sp., from Japan (Hymenoptera: Formicidae)

Kensuke Nakata,^{1,4} Kazuki Tsuji,² Bert Hölldobler,³ and Akio Taki¹

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Several species of the ant genus Diacamma reproduce through mated workers (gamergates). Such gamergates have no wings and therefore are unable to conduct nuptial flight. Instead they perform a sexual calling behavior by standing outside the nest and rubbing the tibiae of their hindleg over the surface of the arched gaster. In a series of exclusion experiments we demonstrate that secretions from the metatibial gland are the most important component in making the virgin female attractive to the males.

KEY WORDS: metatibial gland; calling behavior; sexual pheromone; ponerine; *Diacamma*.

INTRODUCTION

In most ant species only the queen caste reproduces and the worker caste remains sterile. However, in some species of the "primitive" subfamily Ponerinae, a queen caste has disappeared in the course of evolution, and instead one or a few workers in each colony mate and reproduce (Peeters, 1991). Mated reproductive workers are called gamergates (Peeters and Crewe, 1984). As ant workers have no wings and therefore are unable to conduct nuptial flights, mating takes place in the vicinity of or inside the nest (Hölldobler and Haskins, 1977; Peeters and Crewe, 1984). In the queenless colonies of the ponerine species *Rhytido-*

¹Department of Zoology, Faculty of Science, Kyoto University, Kitashirakawa-Oiwakecho, Sakyou-ku, Kyoto City, 606, Japan.

²Department of Biology, Faculty of Science, Toyama University, Toyama, 930, Japan.

³Zoologie II, Biozentrum der Universität Würzburg, Am Hubland, D-97074, Würzburg, Germany.

⁴To whom correspondence should be addressed.

ponera metallica, workers exhibiting sexual calling behavior arch their gaster upward and expose the opening of the pygidial gland. Experiments demonstrated that at least part of the calling pheromone originates from the pygidial gland (Hölldobler and Haskins, 1977). Such behavior is known also from some ant species with queenright colonies, where the virgin queens exhibit similar sexual calling behavior (Hölldobler and Bartz, 1985), but the anatomical source of the pheromone is known from only a few species (Hölldobler and Wilson, 1990).

Among the queenless ponerine ants, species of the genus *Diacamma*, i.e., *Diacamma* spp. from Japan and Malaysia and *D. australe*, have a special mode of regulating the reproductive division of labor (Fukumoto *et al.*, 1989; Peeters and Higashi, 1989; Sommer *et al.*, 1993). In these species all workers eclose with a pair of tiny bladder-like organs attached to the mesothorax, termed "gemmae" (Peeters and Billen, 1991). Immediately after eclosion, however, young workers lose their gemmae, which are bitten off by the gamergate or other adult workers. When the gamergate of a colony dies, one of the newly eclosing virgin workers is not mutilated. This individual eventually mates with a winged male from another colony and subsequently becomes the new gamergate. As a consequence, each colony usually has a single gamergate (monogynous); it is the only individual that remains unmutilated, i.e., it retains intact gemmae.

The function of the gemmae is not clear. Because they contain glandular cells with pore openings in the cuticle (Peeters and Billen, 1991) it has been hypothesized that they might be involved in sexual calling behavior (Peeters *et al.*, 1992). On the other hand, in *Diacamma vagans*, Peeters *et al.* (1992) report a calling behavior during which the worker repeatedly rubs the tibiae of the hindlegs over the arched gaster. Furthermore, a recent study revealed the external and internal structure of the well-developed metatibial gland in *D. vagans* and demonstrated that the pore plate opening of the gland is wiped over the surface of the gaster during calling behavior (Hölldobler *et al.*, 1996). These observations suggest that the calling pheromone originates from the metatibial gland, and not from the gemmae.

Diacamma vagans might be a special case, however, because in this species all workers retain their gemmae (Peeters *et al.*, 1992). The metatibial gland display may therefore be typical in sexually calling females of *D. vagans* but not of the other *Diacamma* species. Although the metatibial gland exists in a number of ant species, including all *Diacamma* studied (Bolton 1990; Hölldobler *et al.*, 1996), this does not necessarily imply that it plays the same role in all those species. An alternative possibility is that in the other *Diacamma* species, in which only the dominant virgin worker retains its gemmae, the sexual calling pheromone emanates from the gemma glands.

In this paper we report our investigations of the sexual calling and mating behavior in Japanese *Diacamma*, a species in which only one dominant reproductive worker in each colony retains its gemmae. Furthermore, we present evidence

that demonstrates that the metatibial gland is the major source of the sexual calling pheromone in *Diacamma*.

MATERIALS AND METHODS

Thirty-eight colonies of *Diacamma* sp., previously called *D. rugosum* by Fukumoto *et al.* (1989), were collected in Shuri, on the main island of Okinawa, the subtropical region in southwestern Japan, during 1990–1996. It is the only *Diacamma* species found in Japan. They were housed in plastic containers (27.5 × 20.0 × 5.0 cm), which served as foraging arenas and contained a small nest box which was kept moist with wet cotton. In the laboratory we maintained a constant temperature (25°C) and a natural L:D cycle. The ants were fed with mealworms (*Tenebrio molitor*) and honey water.

Observation of Mating Behavior

To obtain virgin unmutated workers (VUW), we removed the gamergates from the colonies. Shortly thereafter young workers eclosed and retained their gemmae. Males from other colonies were released into the foraging arena of each colony. The behavior was recorded with a Sony Hi-8 Video Camera Recorder (CCD TR-1000) or by direct observations until mating occurred. In this way we were able to follow the mating behavior 20 times.

Experimental Procedures

In order to examine the role of the metatibial gland, two tests were carried out as follows.

Gland-Coating Test

In VUWs (8–10 days after eclosion), the ventral and interior sides of their hindleg tibiae were coated with vinyl lacquer (Dai-Nihon-Toryo Co.). For this procedure five colonies were available, and only one VUW of each colony was used. Twelve additional colonies were the source for the control VUWs, which were treated in different ways: in four individuals we applied vinyl lacquer on the dorsal petiole, in three other VUWs we coated the gemmae, in three VUWs we coated the tibiae of the midlegs, and in four VUWs we coated the edge between the sixth and the seventh abdominal tergites (the opening of the pygidial gland). Coating was conducted only after the VUWs had begun to exhibit the specific calling behavior described below. Though hindleg-coated and gaster-coated workers sometimes entangled their hindlegs due to the coating material, which seemed to constrain the ants' movements somewhat, all VUWs were observed to conduct calling behavior after coating. Males from alien colonies were introduced into the respective arenas of the treated virgin workers. The behavior was

recorded by direct observations. When a male came within 1 cm of the VUW, we recorded the male's courtship behavior, which we subsequently divided into the following five rank categories: (1) ignoring, (2) touching, (3) chasing, (4) mounting, and (5) copulation. Differences in average rank between manipulated and control VUWs were tested by Mann–Whitney *U* test.

Choice Test

Each of 28 males tested was subsequently placed together with a metatibia-coated or with a petiole-coated VUW in a laboratory dish (diameter, 5.6 cm) for a period of 5 min. The sequence of differently treated females introduced to each male was randomly assigned. Each male confined with one type of treated female was observed for a period of 5 min. We noted whether or not the male showed intentions to mount. However, before copulation behavior began, we removed the female, replaced it with another type, and observed the behavior of the male for an additional 5 min. When a male did not respond to either individual of a test pair, another pair was offered. The difference in mounting rates exhibited by the males toward the differently treated VUWs was tested by the Wilcoxon signed-rank test.

RESULTS

Mating Behavior

Virgin unmutated workers (VUW) remained inside the nest for at least 7 days after eclosion. No oviposition was observed during this period. At the age of 7–9 days the VUWs began to explore the foraging arena until they began the calling behavior. They lowered their head and thorax and arched their gaster. In this position they rubbed the tibiae of their hindlegs along the pleural and dorsal surface of the gaster, 5–15 times during each episode (Fig. 1). This became the most frequent behavior during a daily period stretching from 1400 to 2200, and it occurred both inside and outside the nest. During the same period the males, 3–4 days old, flew around the foraging arena. When a male encountered a VUW, he approached her from behind irrespective of whether or not she currently conducted the arching and rubbing behavior. He antennated the female's thorax and gaster and chased behind her when she ran away. When she stopped walking, he stretched his antennae forward while bending his gaster toward the female and attempted to mount her, simultaneously grabbing her gaster. The male's antennae were placed between the female's antennae, and by swinging his antennae sideways he repeatedly touched hers. The female often responded by sliding the sting aside, which subsequently led to copulation with the male. On some occasions, however, mating was unsuccessful because the VUW refused the male and escaped into the nest. When a male attempted to follow her, mutilated workers

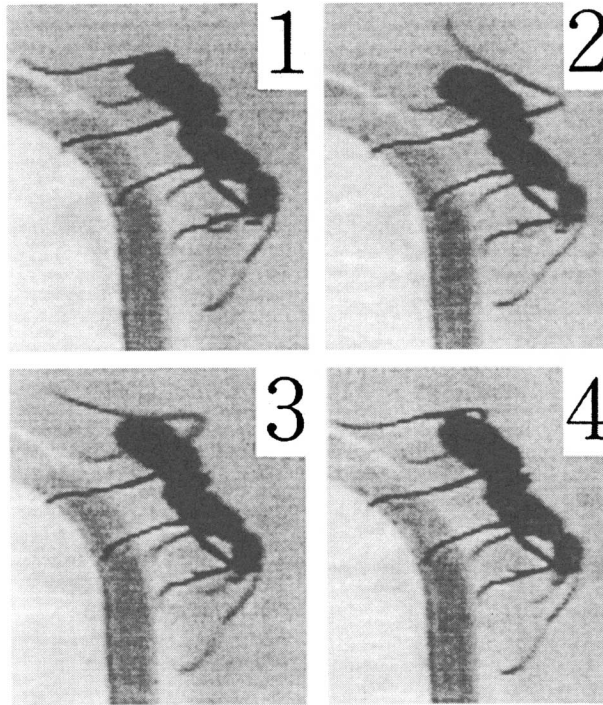


Fig. 1. Video shot of calling behavior of virgin unmutated worker in *Diacamma* sp. She raises her left hindleg (2) and brings it down and rubs off her gaster (3, 4).

attacked and expelled him and, sometimes, even killed the male. Once copulation was complete, the female moved into the nest, dragging the motionless male in copula along. Some of the nest workers gathered around the pair, and by attacking the male they severed its head, wings, legs, and thorax. The gaster remained connected to the female for 20–24 h. After a completed copulation only a few gamergates left the nest again to repeat the calling behavior. Such behavior was observed during 1 or 2 days after the copulation. We introduced males into the foraging arena again, but no second copulation occurred. The gamergates behaved timidly and escaped into the nest when males approached. Afterward, the gamergates were never observed to call males throughout their lives, suggesting that most gamergates mate only once in their lives.

Gland-Coating Test

Table I shows the results of gland-coating tests. Females with coated hindleg tibiae were significantly less attractive to males than the control female (Mann–

Table 1. The Difference in Males' Responses to Control Unmutated Virgin Workers and Manipulated Unmutated Workers Whose Hindleg Tibiae Were Coated with Vinyl Lacquer

Manipulation	Coated region	Male behavior					Average rank	
		Approach	Ignoring (1) ^a	Touching (2)	Chasing (3)	Mounting (4)		Copulation (5)
Control	Hindlegs	13 ^b	11	2	0	0	0	1.15
	Hindlegs	7	7	0	0	0	0	1.00
	Hindlegs	11	7	3	0	1	0	1.55
	Hindlegs	13	12	1	0	0	0	1.08
	Hindlegs	28	13	7	3	4	1	2.04
	Midlegs	1	0	0	0	0	1	5.00
	Midlegs	6	0	0	0	5	1	4.17
	Midlegs	4	2	0	0	1	1	2.75
	Petiole	15	1	1	3	9	1	3.53
	Petiole	8	2	2	1	2	1	2.75
	Petiole	1	0	0	0	0	1	5.00
	Petiole	2	0	0	0	1	1	4.50
	Tergite	16	4	1	2	9	0	3.00
	Tergite	15	2	1	2	10	0	3.33
	Tergite	8	2	1	0	5	0	3.00
Tergite	9	1	1	0	7	0	3.44	

^aThe number in parentheses after each category of response is the rank order to quantify the strength of the male response.

^bNumerals show the stage to which mating behavior proceeded.

Table II. Results of the Choice Experiment

Pair No.	No. males mounted		
	Only on control female	Only on tibia-coated female	On both females
1	7 ^a	0	0
2	4	0	1
3	4	0	0
4	3	1	1
5	3	0	1
6	3	0	0

^aNumber of males which mounted on one or both types of paired females is shown.

Whitney U test, $U = 0$, $P < 0.01$). In 63 of 72 approaches by males to a test female, the males did not proceed in chasing her. All petiole-coated ($n = 4$) and midleg-coated ($n = 3$) control workers successfully copulated, whereas only one of five hindtibia-coated workers succeeded. None of the gaster-coated control workers ($n = 4$) mated successfully. However, in these cases males usually pursued the female and tried to grab her on the tergites, but they failed, apparently because the coating material made the female gaster surface slippery. Females whose gemmae were coated ($n = 3$) did not leave the nest. When these females were individually presented to a male in an experimental dish as in the choice tests, males (3 of 7, 3 of 7, and 1 of 2 for each female) tried to mount them, but this did not lead to copulation.

Choice Test

The choice tests brought similar results. The mounting rate by males was significantly higher when encountering control females (petiole-coated) than when meeting a test female (metatibia-coated) (Table II) (Wilcoxon signed-rank test, $P = 0.031$).

DISCUSSION

Virgin, un mutilated workers of *Diacamma* sp. from Japan perform a stereotyped sexual calling behavior during which they rub the hindleg tibiae over the arched gaster. This kind of calling behavior, which was first described for *D. vagans*, is obviously more common in the genus *Diacamma*. It had been suggested that during this hindleg tibia rubbing, the female releases a sexual calling pheromone from the metatibial gland. The metatibial gland of *Diacamma* sp. from Japan consists of a layer of cylindrical secretory cells, located ventrally in the hindtibia, beginning immediately behind the metatibial spur and extend-

ing approximately one-fifth of the length of the tibia. Externally the pore plate can be covered by thick bristles, some of them spatula-shaped, whereas in other specimens a small bald patch of the pore plate can be seen (Hölldobler *et al.*, 1996).

Our observations and our experiments with females, whose hindleg tibiae were coated with vinyl lacquer, thus plugging the opening of the metatibia glands, indicate that secretions of this gland serve as sexual calling pheromones. We did not find evidence that secretions emanating from the gemmae play a significant role in making unmutated workers sexually attractive to males. Gland-coating tests showed that virgin unmutated workers with coated gemmae were still sexually attractive to males. Likewise the plugging of the pygidial gland opening itself apparently did not affect the female's sexual attractiveness. Most males approaching pygidial gland-coated VUWs proceeded with mounting. This is the first identification of a function of the metatibia gland in an ant species, but we expect that there are other functions because this gland was found in several ant genera in which reproduction by mated workers does not occur (Hölldobler *et al.*, 1996).

In this study we could not entirely resolve the role, if any, of the gemmae during mating behavior. The behavior of the females with coated gemmae was clearly affected, because such females did not exhibit calling behavior; in fact they did not even leave the nest. In this context one additional observation is of interest. We carefully removed the coating on the gemmae of one female. This female resumed normal calling behavior. Although this is only one incidental observation, it points to the behavior modulating function of the gemmae in some *Diacamma* species (see Gronenberg and Peeters, 1993). It is unlikely, however, that the secretions of the gemma glands play a major role in attracting the males during mating behavior. This function is obviously reserved for the metatibia gland secretions.

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