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Morphological differences between sexes in the ponerine ant *Diacamma* sp.

(Formicidae: Ponerinae)

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Running title: Differences between sexes in *Diacamma* sp.

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We report herein detailed morphological differences between sexes in the ponerine ant _Diacamma_ sp. The female individuals of this species show monomorphism, making them particularly suitable among the social hymenopterans for comparing adult sexual traits and developmental processes. Our observations revealed some intriguing sex-specific characteristics. For examples, antennal sensilla are gender-specialized in these ants; males possess sensilla coeloconica, thought to respond to air conditions, while females possess a particular type of sensilla basiconica that is a putative contact chemoreceptor. The antennal cleaners in the foretibiae of the legs are also sex-specific, in addition to the trichome patterns on the spurs in the mid- and hindtibiae.

Furthermore, only male tarsal claws have a denticle inside the claw, and the male posterior has a hook-like curved spine at the edge of the eighth tergite. This spine is thought to facilitate the tight connection of a mating pair. Based on these findings, we hypothesized that most of the sexually dimorphic traits are morphologically specialized to and thus function in the different gender life strategies, such as flight ability, lifespan, and male suicidal copulation.
INTRODUCTION

Differences between sexes often represent adaptation for successful reproduction in both sexes (Reynold and Harvey 1994). Among animal species, the order Hymenoptera exhibits particularly conspicuous sexual differences (Stubblefield and Seger 1994). The social and parasitic nature of hymenoptera may have derived distinct life history strategies between the sexes, resulting in these significant morphological differences (Stubblefield and Seger 1994).

The molecular basis underlying haplodiploid sex determination in hymenoptera is poorly understood, although the primary sex determination gene \textit{csd} (complementary sex determiner) was recently identified in the honeybee \textit{Apis mellifera} (Beye et al. 2003). Ongoing studies into hymenopteran sex determination will hopefully provide a model of the haplodiploid sex determination that exists in 20% of all animal species (Bull 1983). However, the association between primary sex determination and expression of sex-specific morphological traits remains unknown. Careful and extensive examination of the morphological differences between sexes is necessary to address this question.

Ant societies are characterized by their sophisticated polymorphism, such as winged queens males and wingless workers, and these differences are sometimes subdivided into major and minor (Wilson 1953, Nijhout 1999). The female polymorphisms that manifest as social castes make it difficult to simply compare the developmental process between males and females. In the ant genus \textit{Diacamma}, the winged queen has been lost, with some worker females acting as functional queens, so
that all female individuals are monomorphic (Fukumoto et al. 1989, Peeters and Higashi 1989). In addition, adult males of *Diacamma*, which are bee-like and red in color, are largely different from the females macroscopically. In these aspects, *Diacamma* is a suitable model species for the study of sexual differences and expression of such sexual traits. In this study, we conducted a detailed morphological analysis of both sexes in adult *Diacamma*. 
MATERIALS AND METHODS

Ants

In Japan, Diacamma sp. is the only known species of the genus Diacamma. Diacamma sp. is thought to be the closest species to D. indicum (Viginier et al. 2004), although this is still under taxonomic revision. We collected 30 colonies from two populations (Nakijin, Kenmin no Mori) on the main island of Okinawa in 2005. The colonies were maintained in artificial nests made of plastic containers paved with wet plaster. The ants were fed honey water, mealworms, termites, and crickets three times a week. Males were collected mainly from gamergate-removed “orphan” colonies, and females were collected from gamergate-right colonies.

Morphological observations

Morphological characteristics of male and female ants were assessed using a scanning electron microscope and a binocular light microscope. For scanning electron microscopy, specimens were dehydrated through a graded series of ethanol solutions and finally in acetone. After being air-dried, the samples were coated with gold using an ion sputter coater (E1010 Ion sputter, Hitachi, Tokyo) for imaging in a scanning electron microscope (JSM-5510LV, JEOL, Tokyo). A light microscope (SZH, Olympus, Tokyo) and digital camera (C-7070, Olympus, Tokyo) were used to capture microscopic images for low-resolution observation.
RESULTS AND DISCUSSION

*General description and body features*

The head, trunk, petiole, and dorsal abdomen of the female ant were boldly “striated” (Fig. 1a), while males lacked these striations (Fig. 1b). The female cuticle appeared thick and wavy across the striations (Fig. 1a, inset), unlike the smooth and thin male cuticle (Fig. 1b, inset). The exoskeleton was bright red in males, and black to greenish-black in females. Males had a small head with large compound eyes (approx. 650 µm along major axis) and three oceli (approx. 150 µm along major axis), while females possessed smaller compound eyes only (approx. 500 µm along major axis).

The male thorax had two pairs of membranous wings (Fig. 1b) while females had a pair of gemmae, which are vestigial forewings characteristic of female *Diacamma* (Fig. 1a). The male thorax was well-developed with flight muscles (Fig. 1b).

*Antennae*

Male antennae were straighter and longer than those of the female, which were L-shaped (Fig. 2a, b). Both sexes possessed more than three types of hair-shaped long sensilla that might be categorized into sensilla tricoda or sensilla chaetica (Fig. 2c, e). In males, these sensilla were considerably uniform in size and shape (Fig. 2c), while female antennae showed curved, hair-shaped sensilla. Since we did not use silver staining to visualize the sensilla pores (Navasero and Elzen 1991), we cannot speculate as to the function of these hair-shaped sensilla. However, based on previous work in hymenopterans (Dumpert 1972, Martini and Schmidt 1984, Renthal et al.)
2003, Hashimoto 1990), they may function either as volatile/pheromone receptors (pored sensilla) or mechanoreceptors (nonpored sensilla). We also noted distinct sexual dimorphisms in the distribution of the sensilla coeloconica and one particular type of sensilla basiconica. A detailed description of these sensilla is presented below.

**Sensilla coeloconica**

Sensilla that are set on the bottom of relatively shallow depressions or pits are called 'sensilla coeloconica' (Zacharuk 1985). This type of sensillum was only observed in male antennae in *Diacamma* (Fig. 2e, f), and showed a dense distribution at the antenna tip (13th to 10th antennomer). In workers of the genus *Atta*, sensing of atmospheric parameters such as temperature, humidity, and CO₂ is conducted not by hair-shaped sensilla, but by pit-shaped sensilla (i.e., sensilla coeloconica and ampullacea). Sensilla coeloconica can respond to temperature stimuli (Kleineidam and Tautz 1996), as well as humidity and chemicals in ants (Jaisson 1969). Together with our finding, these results suggest that sensilla coeloconica in male antennae function as air-condition sensors. *Diacamma* sp. males leave their nest in the evening to find their mates, and a high density of these sensilla in male antennae may be valuable in timing flights according to the atmospheric parameters.

**Sensilla basiconica**

Peg-like short sensilla are categorized as sensilla basiconica (Zacharuk 1985). One particular type of sensilla basiconica, appearing as conical pegs with round smooth tips, was specific to female *Diacamma* (Fig. 2b, c; arrowheads). They were observed
in whole antennal segments excluding the scape (1st antennomer). At the tip, the sensilla basiconica were more densely distributed at the inner and ventral sides, which females frequently use for antennation. In males, the antennal sensilla tips were sharp, and males seemed to lack the homologous sensilla to basiconica.

The morphology of the sensilla basiconica observed in this study was almost identical to that reported for the *Camponotus* worker; in these ants the sensilla basiconica act as contact chemoreceptors to recognize cuticular hydrocarbons on nest mates (Ozaki et al. 2005). The sensilla basiconica functioning as contact chemoreceptor is also suggested in *Solenopsis* workers (Renthal et al. 2003). Judging from these studies and the pattern of distribution observed here, the sensilla basiconica in *Diacamma* are likely to function in contact chemoreception. As is typical of many ant species (Howard and Blomquist 1982), *Diacamma* use cuticular hydrocarbon as a tool of chemical communication (Cuvillier-Hot et al. 2001). Female *Diacamma* ants may therefore possess a high density of these structures to complement their long and complicated social life that requires multiple and varied chemical communications.

The sexually biased distribution patterns of sensilla basiconica and coeloconica in *Diacamma* (Ponerinae) were consistent with those in *Solenopsis* (Myrmicinae) (Renthal et al. 2003), and it is interesting that these two phylogenetically distant groups (Corrie et al. 2006) share similar sex-specific morphological features.

*Mouthparts*

Female ants can retract their labiomaxillary complex inside the stipes (Gotwald 1969, Paul et al. 2002). A stipe is a plate-like structure located at the basal part of maxilla
Males tended to extend their long maxillary and labial pulps, with the entire labiomaxillary complex not retracted inside the stipes. Male mandibles were short and diminutive (Fig. 3c). Since *Diacamma* males rarely feed and do not take part in foraging, male mouthparts are un retractable and fragile.

**Leg structures**

Trichomes on the male femurs were uniform in size (Fig. 4d, f), while a few large setae were found on all female limbs (Fig. 4e, g; arrowheads). Both sexes possessed comb-like structures on the inner (ventral) foretibiae (Fig. 4a, b; arrowheads), which is also known as the 'comb of the notch' (Schönitzer and Lawitzky 1987). Together with the comb of the foretibial spur (Fig. 4a, b; S), these structures constitute an “antenna cleaner” (Schönitzer and Lawitzky 1987). The female comb of the notch consists of two rows of combs, while only a single comb is present in males. Little is known about this sexual dimorphism in the ant antenna cleaner. In females, extensive cleaning of the L-shaped antennae by the female-specific combs is probably required to maintain the long and complicated social life. Male mid- and hindtibial spurs were covered with a number of hairs, compared to female corresponding spurs (Fig. 4d-g).

In hindtibiae, comb-like structures was observed at the inside (i.e., posterior) spur in both sexes (Fig. 4f, g).

There was a small denticle inside each claw exclusively on the male pretarsi (Fig. 5a, b). Relatively short trichomes were also widely distributed all over the claws in males (Fig. 5a), while females had short trichomes only at the claw base where there was also a long setae in parallel with each tarsal claw, known as 'tarsal claw
macrosetae' (Oriver et al. 2001). These structures were consistently present in all the
legs of respective sexes. The male claws play an important role in sticking to vertical
places, especially to rough surfaces (Betz 2002). While Diacamma females are ground
dwelling and usually inhabit horizontal areas, winged males fly and behave in
three-dimensional space. In addition, Diacamma males often adhered upside-down to
the artificial nest ceiling. Therefore, the denticles in the male claws may help males to
cling to such surfaces.

**Petiole**

Females possessed two sharp spines on the dorsal side of the petiole (Fig. 6a), which
were not observed in males (Fig. 6b). In ants, these body spines, especially on the
thorax and petiole are thought to work in defense against predators (Hunt 1983,
Buschinger and Maschwitz 1984). Since males have a shorter lifespan and are not
ground dwelling, they may not require such defensive structures. However, two small
spines were apparent at the ventral side of petiole in both sexes (Fig. 6a, b).

**Abdomen**

Comparison of abdominal structures between sexes revealed a hook-like spine at the
last tergite in males (Fig. 7b, c), but not in females (Fig. 7a). This dorsal spine was
curved ventrally, and covered with hair-like sensilla emerging from small dents (Fig.
7c). During copulation, the spine touched the female abdominal sternite (Fig. 7e, f).
The male abdomen also possessed cerci and a gap between the last tergite and the last
sternite (Fig. 7b, c). The male sternites were also covered with numerous hair-like
sensilla at a higher density than observed in females (Fig. 7a, b). Mating in *Diacamma* is suicidal for males, and lasts extraordinarily longer than in typical ant species (Wheeler and Chapman 1922, Fukumoto et al. 1989, Allard et al. 2002). After a successful copulation, the male head and thorax are mutilated from the abdomen by workers, and the remnant abdomen attaches to the female abdomen for approximately one day (Fukumoto et al. 1989). This prolonged copulation process in ants are regarded as mating plugs to ensure fatherhood (Monnin and Peters 1998, Allard et al. 2002). Allard et al. (2002) proposed a mechanism that supports the prolonged copulation in *Diacamma*, whereby the inflation of the male penis in the female genital chamber strengthens the mating connection. However, the function of the hook-like spine had been unknown. In addition to the inflated pressure from inside, it is suggested that the hook-like male spine presses the female abdomen from the outside to support the solid connection of the pair. The hook-like dorsal spine might therefore facilitate the irreversible suicidal copulation in this species. To our knowledge, the existence of this male-specific abdominal structure is a common and unique feature in the genus *Diacamma* (Wheeler and Chapman 1922).

**CONCLUSION**

Flight ability is a major life-history difference between males and females in *Diacamma*, and thus underlies several sexual differences. The macroscopically visible male features such as wings, large compound eyes, and oceli are distinct sexually dimorphic characteristics in *Diacamma* and are undoubtedly related to flight ability. The microscopic features such as claw structure, distribution of antennal sensilla, and
cuticle structure also seem to reflect the flight ability and three-dimensional behavior in the male life. Another major difference between the sexes in *Diacamma* is lifespan, which is approximately 200 days in female workers and 580 days in gamergates (Tsuji et al. 1996), while males live only for a few weeks and die immediately after the suicidal copulation (Allard et al. 2002). The thin, soft cuticle and lack of the petiole spine in males may reflect an efficiency in morphological costs in males because of their short life.

In addition to the sexual dimorphism, many ant species have intrasexual polymorphism (i.e., caste polymorphism among females; Hölldobler and Wilson 1990). In this aspect, ants seem to have the potential to generate large morphological variation even within a single species (Stubblefield and Segar 1994). Both in sexual dimorphism and caste polymorphism, various morphological characteristics exist with the same set of genome information, although sexual expression is triggered by the haplodiploid sex determination. It is known that developmental mechanisms, such as the hormonal regulation controlling sexual dimorphism, also play a role in the regulation of intrasexual polymorphism in horned beetles (Emlen and Nijhout 1999, 2001, Emlen et al. 2005). Similarly, mechanisms producing caste polymorphism can be co-opted in generating sexual dimorphism and vice versa.

The sexual dimorphism in *Diacamma* seems to be distinctive among ants. Unfortunately, precise descriptions of male traits are scarce in extant ant taxa (Boomsma et al. 2005, Stubblefield and Segar 1994), making comparative study difficult. From a developmental point of view, the expression of sexual traits follows primary sex determination (Beye et al. 2003). Identifying regulatory mechanisms of
sexual trait expression in hymenoptera will therefore improve our understanding of
the developmental basis of sexual dimorphism.

Acknowledgments

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**FIGURE LEGENDS**

Figure 1. Whole images of body trunk in female (a) and male (b, F) *Diacamma* sp. Insets indicate the dissected cuticle of mesopluron. *M*: male, *F*: female ants.

Figure 2. Antennae of male and female *Diacamma* sp.; (a) female antenna, (b) male antenna. (c) Magnified view of female antennal tip, where arrowheads indicate sensilla basiconica. (d) Sensilla basiconica of female antenna. (e) Magnified view of the male antennal tip where arrowheads indicate sensilla coeloconica. (f) Sensilla coeloconica of male antenna. *M*: male, *F*: female ants.

Figure 3. Mouthparts of male and female *Diacamma* sp. (a) Female mouth parts when retracted inside stipes. (b) Female mouthparts when extruded. (c) Male mouthparts. Note that maxillary pulps (mp) elongates out of the head. (d) Dissected female mouthparts. (e) Dissected male mouthparts. ga: galea, lb: labium, lp: labial pulp, Mn: mandible, mp: maxillary pulp, st: stipe. *M*: male, *F*: female ants.

Figure 4. Leg structures around the junction between tibia and tarsus in *Diacamma* sp. There are antenna cleaners at foretibiae both in male (a, arrowhead) and female (b, arrowhead). Note that the female tibia has two rows of combs (c, arrowheads). Trichomes on mid- and hindtibial spurs (S) are dense in male (d, f), but sparse in female (e, g). *Mfl*: male foreleg, *Ffl*: female foreleg, *Mml*: male midleg, *Fml*: female midleg, *Mhl*: male hindleg, *Fhl*: female hindleg. *M*: male, *F*: female ants.

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Figure 5. Pretarsal claws of *Diacamma* sp. There is a small denticle inside each claw in males (a, b). Female claws lack these denticles inside (c, d). Photos show pretarsal claws in midlegs. *M*: male, *F*: female ants.

Figure 6. Female (a) and male (b) petiole of *Diacamma* sp. In males, two sharp dorsal spines are missing.

Figure 7. Terminal structures of male and female abdomens of *Diacamma* sp. (a) Lateral view of female abdominal tip (upper: dorsal). (b) Lateral view of male abdominal tip (upper: dorsal). (c) Ventral view of male terminal abdomen. (d) Magnified image of male-specific terminal structure. Note that males have hook-like structure at the tip of the last tergite (b, c, d). Lots of dents and trichomes can be seen on the hook-like structure (d). The hook touches female sternite in copulation (e, f). *ce*: cercus, *d*: dorsal, *v*: ventral. *M*: male, *F*: female ants.
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Figure legends

Fig1. Whole images of male and female *Diacamma* sp.
female (b) male, insets indicate the dissected cuticle of mesoplonur.

Fig2. Antennae of Male and Female Diacamma sp.

(a) female antenna, (b) male antenna, (c) expanded view of tip of female antenna, arrowheads indicate s. basiconica, (d) s. basiconica of female antenna (e) expanded view of the tip of male antenna, arrowheads indicate s. coeloconica (f) s. coeloconica of male antenna

Fig3. Mouthparts of Male and Female Diacamma sp.

Female mouth parts (retracted) (b) Female mouthparts (extruded) (c) Male mouthparts. Note that maxillary pulp (mp) elongates out from head. (d) Dissected mouthparts of female (e) Dissected mouthparts of male. st:stipe, lb:labrum, mp:maxillary pulp, lp:labial pulp, ga:galea, Mn:mandible

Fig4. Tibia and tarsus of Diacamma sp.

Foreleg have antenna cleaner both in male (a, arrowhead) and female (b, arrowhead). Note that female has two rows of combs (c, arrowheads). Trichomes on midtibial spurs (S) are obvious in male (d), but obscure in female (e).
Trichomes on hindtibial spurs are also clearer in male (c) than female (f).

Fig 5. Claws of *Diacamma* sp.

Male claw has spine near the tip of claw (a,b). Females lack these spines of claw (c,d).

Midleg claw were shown.

Fig 6. Petiole of *Diacamma* sp.

female (b) male. Males lack two sharp dorsal spines.

Fig 7. Abdomen of *Diacamma* sp.

(a) Female abdomen, lateral view, the upper side is dorsal (b) Male abdomen, lateral view, the upper side is dorsal. (c) Male, ventral view, (d) Male, expanded view. Note that males have hook-like structure at the tip of the last targite (b,c,d). (e). The hook-like structure has dents and trichomes (d). The hook touches female sternite in copula (f,g). ce: circus, d: dorsal, v: ventral.