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SHORT COMMUNICATION

Liquid exchange via stomodeal trophallaxis in the ponerine ant *Diacamma* sp. from Japan

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Abstract

Trophallaxis plays a major role in the sharing of food in colonies of many social insects, and two modes of this are known: stomodeal (oral) and proctodeal (abdominal) trophallaxis. In social Hymenoptera, only a small proportion of colony members perform the task of food collection, and oral trophallaxis is predominant in their social sharing of food. Typically, foragers distribute liquid food stored in their crop to nestmates via oral trophallaxis. Similar to bees, some ants (Formicidae) forage for liquid food from plant secretions (nectars) and insect exudates (honeydew). While regurgitation is common in ants, it has been documented in only two species of the Ponerinae. Here, we report the ability of *Diacamma* sp. from Japan to perform trophallaxis. After thirsty ants had been paired with ants provided with colored water, the abdomens of both groups of ants were dissected. The digestive organ was colored red in half of the receivers. In addition, we observed mouth-to-mouth interactions in the laboratory, not "social bucket" behavior (i.e., exchange of liquid held between mandibles). Our results suggest that *Diacamma* sp. can exchange liquid by true oral trophallaxis and shed new light on social organization via liquid exchange.

Keywords Hymenoptera · Oral trophallaxis · Social bucket · Mouth-to-mouth interaction

Introduction

Trophallaxis is the interaction comprising food exchange between social insects (Wilson and Eisner 1957). It includes the proctodeal (abdominal) transfer of material (Nalepa and Jones 1991; Machida et al. 2001; Jaffe et al. 2001) and the stomodeal (oral) transfer of liquid (Hölldobler and Wilson 1990). Social insect colonies comprise tens to millions of individuals, and the different tasks performed by their workers include foraging, brood care, and nest defense (Oster and Wilson 1979; Duarte et al. 2011). When foragers find liquid food outside the nest, they store it in their crop then share it among nestmates or the brood by regurgitation. Stomodeal trophallaxis plays an important role in the regulation of colony nutrition, brood development, hydrocarbon exchange for nestmate recognition, and social immunity in ants and bees (Kukuk and Crozier 1990; Cassill and Tschinkel 1996; Boulay et al. 2000; Provecho and Josens 2009; Hamilton et al. 2011).

Although stomodeal trophallaxis is common in ants, to our knowledge, it has been documented in only two species of ponerine ants (Ponerinae), *Hypoponera* sp. (Hashimoto et al. 1995) and *Ponera coarctata* (Liebig et al. 1997). Eisner (1957) assessed the structure of the proventriculus, which is an important organ in trophallaxis, by making a comparison between eight subfamilies, the Myrmicinae, Pseudomyrmecinae, Aneuretinae, Dolichoderinae, Formicinae, Ponerinae, Cerapachyinae, and Dorylinae, and reported a high degree of anatomical variation across the taxa. The proventriculus is a muscular valve that forms a tight constriction between the crop and the midgut. Food in the crop is considered part of the communal supply, whereas the food in the midgut, where it is digested, is considered the personal supply (Hölldobler and Wilson 1990; Cook and Davidson 2006; Davidson et al. 2004). While Myrmicinae,

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Pseudomyrmecinae, and Ponerinae present morphological similarities, the proventriculus of *Camponotus* is one of the most developed within the ants; it dams the efflux of the crop contents, allowing for liquid to accumulate in the crop and facilitate stomodeal trophallaxis (Eisner 1957; Cook and Davidson 2006). Trophallaxis was previously considered a derived form of behavior, and was believed to be absent in the ant subfamilies retaining "primitive" social behavior, i.e., Ponerinae (Hölldobler and Wilson 1990). Subsequently, Hölldobler (1985) identified the "social bucket" system in the ponerine ant *Pachycondyla villosa.* The social bucket, sometimes also called "pseudo-trophallaxis," involves the transmission of liquid food held by surface tension in the mandible with no actual regurgitation, and is practiced in various ponerine species (Hermann 1975; Corbara et al. 1989; Lachaud and Dejean 1991). Ponerine ants are unable to store food in the crop owing to the morphology of the proventriculus.

Most ponerine ants are generalist arthropod predators and scavengers. Some species have been observed to collect liquid food, such as floral nectar and aphid and coccid honeydew (Dejean and Suzzoni 1997; Peeters 1997). Similar to *P. villosa*, *Diacamma* sp. exhibits social bucket behavior (Fig. S1). Interestingly, however, we also observed mouth-to-mouth interactions in *Diacamma* sp. in the laboratory without liquid held between their mandibles(Fig. 1). Up until now, it has been unclear whether this mouth-to-mouth interaction involves true trophallaxis (i.e., regurgitation). In this study, we conducted an experiment using colored water to investigate the existence of actual liquid transfer by regurgitation in *Diacamma* sp. Here, we report the ability of Japanese *Diacamma* to perform trophallaxis.

Fig. 1 Mouth-to-mouth interaction between two workers of *Diacamma* sp. from Japan (body length approximately 1 cm). *Left* Donor with expanded abdomen, *right* starved receiver. Photo credit: Taku Shimada (AntRoom)

Materials and methods

Ant collection and rearing

Colonies of *Diacamma* sp. (the only species of this genus in Japan) were collected from Kenmin-no mori (Onna), Okinawa, Japan, in July 2016. The focal species is likely closest to *Diacamma indicum* from India, but this is under discussion (Viginier et al. 2004; Terayama et al. 2014). The colonies, each of them containing a mated gemma-possessing female (i.e., functional queen, the gamergate), 150–200 workers, and brood, were kept in plastic artificial nests filled with moistened plaster (9 cm diameter × 1.5 cm height). The artificial nests were placed in a plastic arena ($17 \times 24 \times 7.5$ cm^3). Nests were maintained at 25 °C under a 16-h light/8-h dark regime (light phase: 0800–2400 hours). Reared colonies were fed chopped mealworms and chopped frozen crickets three times per week and were provided water ad libitum. Although larvae receive food via trophallaxis in many ant species, *Diacamma* sp. larvae were observed to feed on the insect prey directly (H. F. and Y. O., personal observation). We therefore focused on worker-to-worker trophallaxis.

Experiment

To investigate whether *Diacamma* sp. can exchange water by mouth-to-mouth interaction via regurgitation, we collected foragers (approximately 1 year old) from outside the nest and established two groups. One group was given colored water [less than 0.001% pigment including 85% dextrin and 15% Food Red no. 102 (Kyoritsu Foods, Japan)]; the other group was not given the colored water. We did not observe avoidance behavior with respect to the colored water. We used nutrient-free water because viscosity may affect the ability of ants to regurgitate. Each group comprised 18 ants (total $n = 36$), and were kept in Petri dishes (diameter = 9 cm) without water and food for 6 h, after which one group was provided with water containing the red dye for 24 h (Fig. S2). Ants in the two groups were marked using different colored paint (Tamiya, Japan). After supplying the colored water, two ants, one from each group, were selected and kept in a Petri dish (diameter 6 cm). We confirmed that there was no liquid in the ants' mandibles before this. After 24–48 h, the abdomen of each ant, including the crop, midgut, hindgut, and rectum, was dissected in 1 × phosphate-buffered saline. The color of the digestive organ was observed and photographed under a microscope.

Table 1 Presence of dye in the digestive organ of *Diacamma* sp. from Japan

	Crop, midgut, and rectum (donor)	Crop only (donor)	Crop, midgut, and rectum (receiver)	Crop only (receiver)
Alive	18/18	0/18	6/13	1/13
Dead	0/0	0/0	0/5	2/5

Numerator indicates the number of individuals that had a colored digestive organ; *denominator* indicates the total number of individuals (dead or alive)

Results and discussion

The digestive organ, including the crop, midgut, hindgut, and rectum, was red in all donors (Table 1; Figs. 2a, c, S2, S3, S4), suggesting that all 18 ants had ingested the colored water. Since receivers were not given water and food for about 78 h, five ants died before dissection (Table 1; Fig. S5). The digestive organs were red in nine out of 18 receivers (Table 1; Figs. 2b, d, S3a–f, S5a, b). As these ants did not have access to the red-colored water, their digestive organs could not have been red without liquid exchange between ants by regurgitation. As previously mentioned, we also observed mouth-to-mouth interaction between ants that were not holding liquid in their mandibles when placed in the Petri dish (Fig. 1).

Trophallaxis is generally considered to be absent in ponerine ants, with the exception of two species, because the morphology of the crop prevents food exchange in this taxon (Hashimoto et al. 1995; Liebig et al. 1997). The proventriculus of our focal ant, *Diacamma* sp., was not

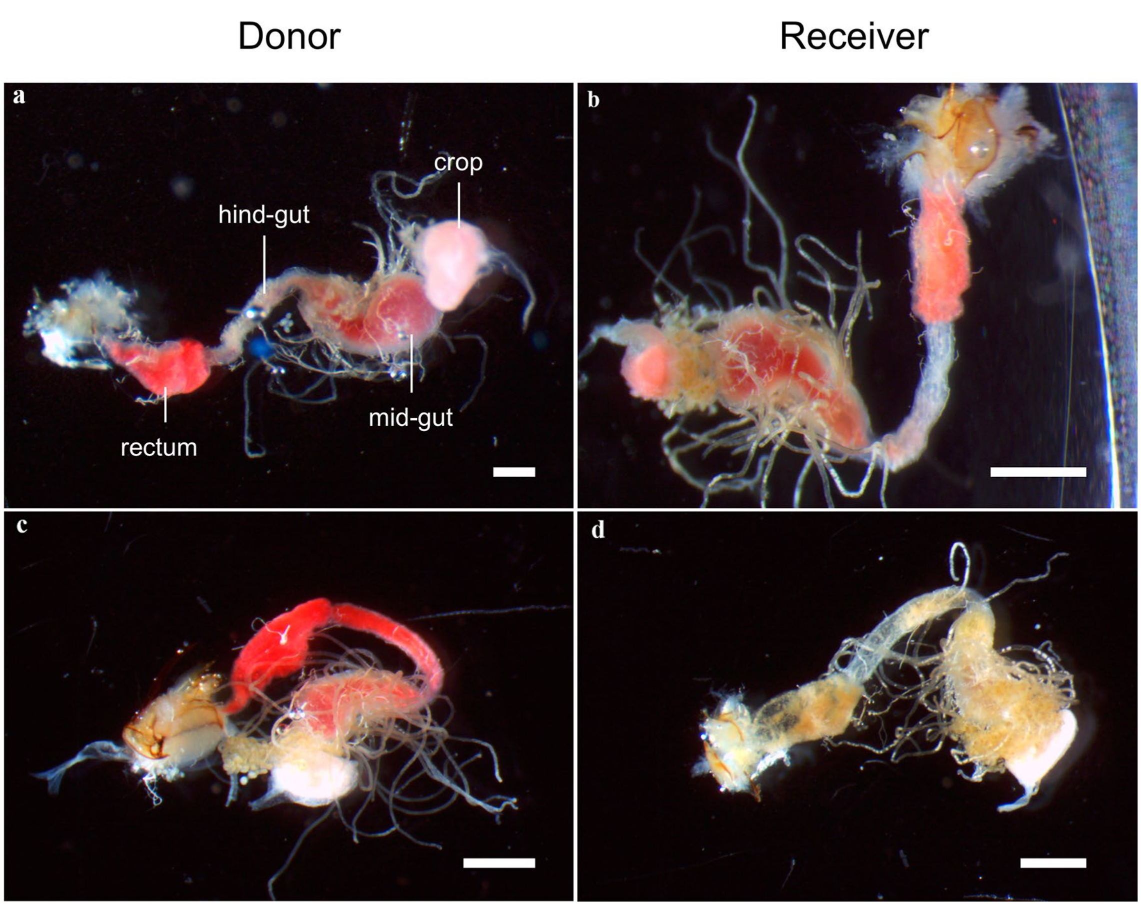

Fig. 2a–d Digestive organ in *Diacamma* sp. **a**, **c** Donors from the group that received red-colored water; **b**, **d** receivers from the group that did not receive colored water. Dissections were conducted 24 h after providing colored water. **b** Digestive organs are *red* showing that the ant received colored water via stomodeal trophallaxis. *White scale bars* indicate 1 mm

shaped like the valve of *Camponotus* sp. (Fig. 2, Eisner 1957; Eisner and Aneshansley 2008). However, regardless of the structure of the proventriculus, stomodeal trophallaxis was performed between *Diacamma* sp. workers, thus stomodeal trophallaxis may not be as rare as previously thought in ponerine ants. We restricted access to food and water for 30–78 h during the experiment, and half of the workers did not receive the colored water. The amount of water inside the digestive organ varied among donors (Fig. S3, S4, S5), and some donors may not have donated an adequate amount of water to the thirsty ants. However, we cannot be certain of this, as we did not measure the amount of water inside the donors' digestive organs. *Diacamma* sp. is a generalist arthropod predator and scavenger (Win et al. 2018). It is therefore reasonable to consider that the frequency of liquid exchange is lower and the amount of liquid exchanged smaller in this taxon than in other ponerine taxa where liquid exchange occurs.

Similar to *Diacamma* sp., major workers of *Pheidole magacephala* perform both stomodeal trophallaxis and social bucket behavior (Dejean et al. 2005). In the laboratory, when our focal ants found liquid food, they sometimes performed social bucket behavior. It is possible that the distance of food from the nest and its quality (e.g., solution concentrate) affect whether *Diacamma* sp. perform these behaviors. Social bucket behavior can be used to transfer a large amount of liquid food, but the liquid food is carried in an exposed state. When the distance between a food patch and the nest increases, social bucket behavior might be risky.

Diacamma sp. from Japan has the ability to exchange liquid by true trophallaxis. Stomodeal trophallaxis involves food searching, hydrocarbon exchange for nestmate recognition and information transfer and plays a role in social immunity (Boulay et al. 2000; Provecho and Josens 2009; Hamilton et al. 2011), which are important colony organizational traits in all ants. The results of the present study indicate that the classic thought on trophallaxis in ponerine ants needs to be reconsidered. In the ponerine ant *Hypoponera* sp., the frequency of liquid exchange was not equal between castes (Hashimoto et al. 1995). Although we only observed trophallaxis between foragers of *Diacamma* sp., the frequency and the amount of liquid exchanged might depend on their behavioral roles (e.g., forager or nurse). Thus, our next step will be to observe trophallaxis in the entire colony.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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