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ORIGINAL RESEARCH PAPER

The presence of an alternative food source changes the tending behavior of the big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae) on *Dysmicoccus brevipes* (Homoptera: Pseudococcidae)

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Abstract
The mutualistic association between ants and hemipterans is often facultative and can be affected by the availability of other food sources. In the present study we tested whether the tending behavior of the big-headed ant, *Pheidole megacephala* (Fabricius), had a negative impact on the pink pineapple mealybug, *Dysmicoccus brevipes* (Cockerell), when alternative food sources: (1) sugar solution, (2) purified water + mealworms and (3) sugar solution + mealworms versus with purified water (control) were provided to the ant colonies. We found that the frequency of ant tending on *D. brevipes* decreased when ants were provided with alternative food sources. However, we did not see any aggressive behaviors and predation of ants on *D. brevipes*. Also, the survival of *D. brevipes* was not different among food condition treatments. These results suggest that the decreased tending frequency of ants can lead to the decline in ant protection service to mealybugs.

Keywords Mutualism · Alternative food sources · Big-headed ant · Pink pineapple mealybug · Survival

Introduction

Ants (Hymenoptera: Formicidae) can be beneficial biological control agents in integrated pest management (IPM) in agrosystems, because they are usually generalist predators (Way and Khoo 1992). However, ants are often regarded as pests, because they tend multiple taxa of honeydew-producing hemipterans (HPHs), such as Aphididae, Coccidae, Pseudococcidae, and Membracidae that damage plants (reviewed in e.g. Buckley 1987; Delabie 2001; Way 1963). By tending hemipterans ants receive energy-rich honeydew that can be essential for the ant's colony survival and growth (Brightwell and Silverman 2010; Fischer and Shingleton 2001; Yao and Akimoto 2002). In return, ant tending can enhance the survival and persistence of hemipteran populations, because ants protect the hemipterans against parasitoids and predators (Hölldobler and Wilson 1990; Nielsen et al. 2010; Way 1954, 1963). Controlling such mutualistic association between ants and hemipterans is a key issue in IPM in agroecosystems.

We focus on the fact that this mutualism between ants and hemipterans is often reported as facultative, and can be affected by the background environments, especially the availability of the other food resource. In some ants it is suggested that the ant-tending intensity is decreased when other sugar sources become abundant (Carabalí-Banguero et al. 2013; Katayama et al. 2013; Offenberg 2001; Schumacher and Platner 2009; Tena et al. 2013). Ant–hemipteran interactions may even shift from mutualism to predation depending on the nutritional condition of ants (Pontin 1958). For example, when an ant colony had excess sugar, the African weaver ant, *Oecophylla longinoda*, attacked their coccid partners (Way 1954). Likewise, *Lasius* ants stopped tending and started to prey on an aphid species, when ants found another more preferable mutualistic aphid species (Sakata 1995) and when they were provided with additional honey solution (Offenberg 2001). This sugar-based change in ant predaceous behavior can be utilized in biological control. However, the generality of this behavior is still to be examined.

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The pineapple mealybug, *D. brevipes* (Cockerell; Hemiptera: Pseudococcidae) is a primary pest of pineapple and attacks many other agricultural crops. This mealybug is associated with various ant species. The ant genera of *Pheidole* and *Solenopsis* are the most commonly associated with pineapple mealybugs throughout the world. Mutualism between ants and pineapple mealybugs causes increase population of mealybugs in the pineapple fields because ants protect mealybugs from their natural enemies (González-Hernández et al. 1999, 2005). A high density of mealybug populations can lead to the occurrence of wilt-associated viral diseases of pineapples which severely reduce yields. Although a wide variety of natural enemies prey on this mealybug, biological control to the mealybug remains unsuccessful. It might be due to the lack of appropriate ant management (Carter 1960; Rohrbach et al. 1988). This mealybug invaded southwestern Japan, including Okinawa and Ishigaki Islands, in the 1930s in association with imported pineapple plants. In Okinawa Island, invasive ants, especially the big-headed ant, and the Japanese white-footed ant, *Technomyrmex brunneus* are dominant ant species tending *D. brevipes* on pineapple plants (A.T. Win pers. obs.). The big-headed ant, *P. megacephala* (Fabricius; Hymenoptera: Formicidae), is known to protect many honeydew-producing hemipterans including *D. brevipes* with their ability to deter natural enemies (González-Hernández et al. 1999). However, it is still unclear whether the big-headed ants also have negative impact on *D. brevipes* when alternative food sources are present. In this study, we examined ant-tending behavior and predation when ants were provided additional sugar and protein sources.

Materials and methods

Stock culture

To obtain *D. brevipes* (PPM, hereafter) stock culture, colonies of PPM were collected from the pineapple fields at Okinawa Prefecture Forest Resources Research Center (OPFRC), Okinawa, Japan, in September 2015. We followed the methods of rearing PPM used by Glenn Taniguchi (2011). All Japanese pumpkins used in the experiments were obtained from grocery stores in Okinawa, washed with water and soap and air-dried for a night. The adult females and third instar mealybugs were transferred onto the terminal end of a pumpkin with a small paint brush. Then, an infested pumpkin was placed in a plastic box with the top rim (5–6-cm width) coated with Fluon (Asahi Glass Company, Tokyo, Japan) to prevent the crawlers escaping. The box was filled with vermiculite, covered with 400 nylon mesh and sealed by a lid with holes for ventilation. These mealybug-rearing boxes were maintained in a dark room at 22 ± 2 °C and 60–70% R.H. Stock colonies ($N = 8$) of the big-headed ant, *P. megacephala*, were collected during July–September 2015 in the campus of the University of the Ryukyus (Okinawa) and fed on honey and various, chopped, field-collected insects in the laboratory at 27 ± 2 °C, 60–70% R.H. and daily light condition, 16:8 h light-to-dark.

Experimental groups of mealybugs and ants

Prior to the experiment, a small fresh Japanese pumpkin (300–500 g) was washed with water and air-dried overnight. Exactly 30 adult female PPMs were randomly taken from the stock colony and transferred individually with a small paint brush (size 0) onto the terminal end of a pumpkin and allowed to settle 2 days before experimentation (a PPM colony, hereafter). The ant nest was made by using a glass test tube (1.4 cm in diameter and 15 cm in length). The wet cotton wool was placed to seal water at the bottom at about a 3-cm depth to maintain humidity. Two hundred and fifty minor workers and 50 larvae were placed into the glass test tube (an ant nest, hereafter), and covered by plastic red cellophane to maintain darkness like a natural ant nest. Ants were starved for 2 days before the experiment to obtain equal state of hunger. We made four ant nests from each of the stock colonies, because our experiment has four treatments as described below.

Experiments

A plastic tray (57-cm length × 38-cm width × 10-cm height) was used as an experimental arena (Fig. 1). We placed an ant nest in the center of one side of a plastic tray (at the timing described later). On the opposite side we placed 3 stages (12 cm apart from each other) that were connected to the ant nest by a fork-shaped cardboard bridge (5-mm width) coated with aluminum foil, so the ants could access the stages via the bridge. Double-sided adhesive tape was used to prevent ants going to the underside of the cardboard bridge. The inner wall of the plastic tray was brushed with Fluon to prevent the ants from escaping.

A PPM colony was always placed (Fig. 1, stage A) throughout the experiment. Additionally, we placed one of four sets of alternative food sources on the rest of the stages: (1) 5 ml of water (control; Fig. 1, stage C), W; (2) 40% water solution of caster sugar (Fig. 1, stage C), S; (3) water (Fig. 1, stage C) plus freshly chopped mealworm larvae, *Tenebrio molitor* L. (ca. 100 mg; Fig. 1, stage B), WM; (4) 40% sugar solution (Fig. 1, stage C) plus chopped mealworm (Fig. 1, stage B), SM. All those alternative foods were provided on a small plastic petri dish cover (40-mm diameter, 5-mm height).

Immediately after setting a PPM colony and a set of alternative food sources, we placed an ant nest in the

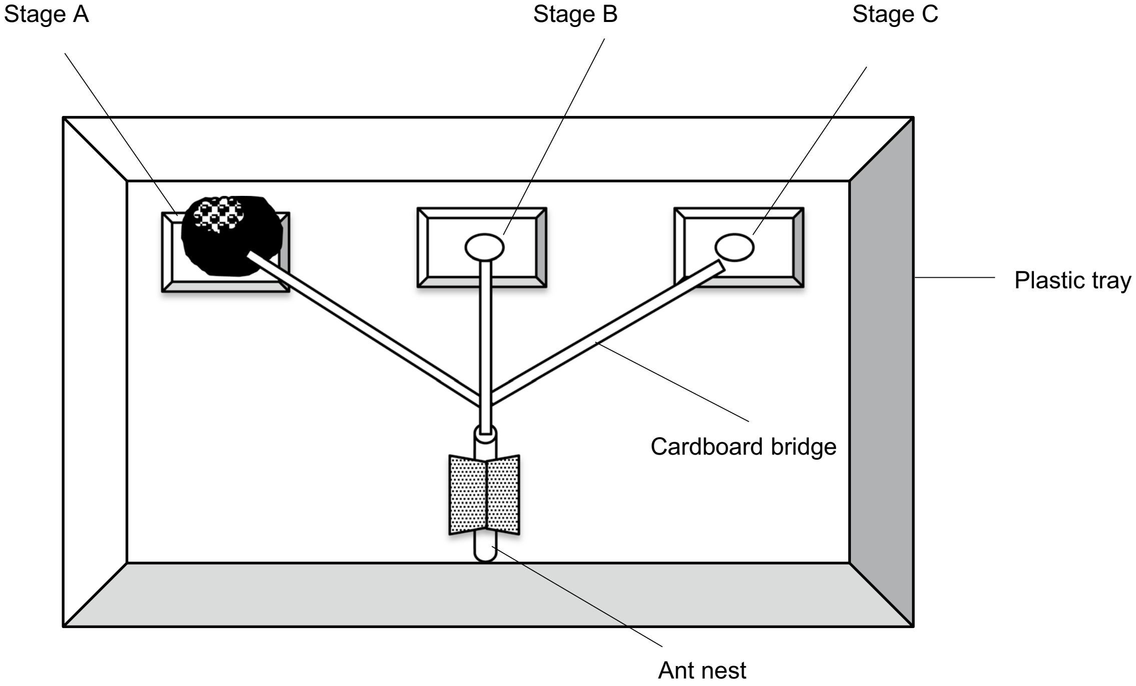

Fig. 1 Experimental setup. Stage A: *Dysmicoccus brevipes* colonies; stage B: chopped mealworms; stage C: purified water or sugar solution. Ant nests were connected to the three stages by cardboard bridges

experimental tray. The four experimental ant nests from each stock ant colonies were randomly assigned to the above four treatments (that means 4 treatments × 8 blocks, because we had 8 different stock ant colonies). One hour after placement of the ant nest, the numbers of ants on the pumpkins (where PPM existed) were counted 10 times at 1-h intervals. The experiment lasted for 7 consecutive days. The alternative foods were replaced with new ones every day before daily observation started. Occasionally we refilled with sugar solution after depletion by ants. At the end of experiments, the number of surviving PPM and ant workers for each treatment were counted. However, in this study, it is not certain whether ants attacked and killed the mealybugs. All experiments were carried out at 24 ± 1 °C under natural light condition. Daily observation was conducted during the day time.

Statistical analyses

All statistical analyses were conducted using R (v. 3.0.2) (R Development Core Team 2013). A generalized linear mixed model (GLMM) in package *lme4* was used to determine whether the number of ants in attendance on PPM in alternative food treatments (S, WM and SM) differed from that in the control (W) and each other among treatments, or not. The model included the interaction between treatment and day as fixed factors, and time of the day and the ant colony as random factors. The data were over-dispersed, due to excessive numbers of zero, so we ran this model with a negative binomial. Separate Kruskal–Wallis tests were used to determine differences in the number of ants tending on PPM in food sources and water (control) among different days. The post hoc (Nemenyi test) in the *PMCMR* package for multiple comparisons among treatments was used if the results of the Kruskal–Wallis test showed significant differences at the 0.05 significance level or not. One-way analysis of variance (ANOVA) was performed to test the survival of PPM and ant workers among treatments (W, S, WM, SM) because the data did not statistically significantly deviate from normal distribution and had similar variances.

Results

We found strong evidence that the frequency of ant tending on PPM decreased when ants were provided with alternative food sources (protein or sugar sources). The intensity of ant tending changed over the observational days. Overall, the ant-tending number on day 1 was significantly higher than other days (day 2–day 7). The treatment × day interaction was also statistically significant (Fig. 2 and Table S1). A significant treatment effect was found between S and SM on day 1 and between WM and SM on day 2. On day 1 in SM treatment, the number of ants tending was significantly lower than that in S treatment, while on day 2 the number of ants tending in SM treatment was significantly lower than that in WM treatment. However, there were no statistically

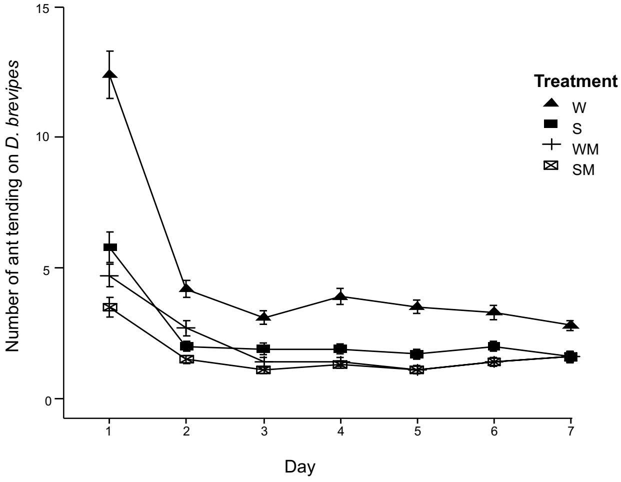

Fig. 2 Number of big-headed ant workers attending *Dysmicoccus brevipes* (mean ± SE) for the 7 days of observation period when provided with alternative food sources. Solid square: sugar solution (S); line cross: purified water + mealworms (WM); square with cross: sugar solution + mealworms (SM); and solid triangle: purified water (W) for control

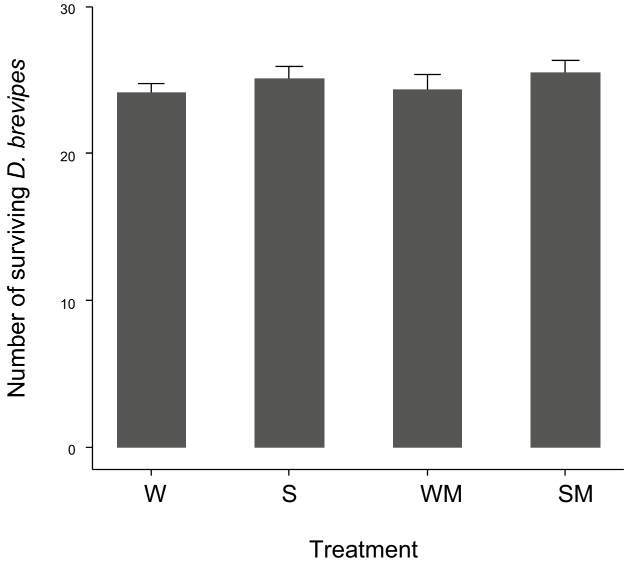

Fig. 3 Number of surviving *Dysmicoccus brevipes* (mean ± SE) when ant colonies were provided with alternative food sources. S: sugar solution, WM: purified water + mealworms and SM: sugar solution + mealworms, versus with purified water (W = control)

significant differences in the number of ants tending among S, WM, and SM on the other observation days (day 3–day 7; Table S2).

Although we did not continuously observed ant behavior, attack of PPM by ants was note observed throughout casual observation in the experiment. Moreover, the survival of PPM was not statistically significantly different among the treatments [W, S, WM, SM; one-way ANOVA: $F_{(3,\,28)} = 0.576$, $p = 0.636$; Fig. 3]. Also, there was no significant difference in ant worker survival among treatments [one-way ANOVA: $F_{(3,\,28)} = 0.214$, $p = 0.886$; data not shown]. Thus, the difference in ant-tending intensity among treatments was not due to the effect of the number of ant workers.

Discussion

In this study, we found that the intensity of ant tending on *D. brevipes* decreased when workers of the big-headed ant were provided with alternative food sources. However, we did not see any aggressive behaviors and predation by ants on *D. brevipes*. Our results resemble those of Carabalí-Banguero et al. (2013) in which colonies of the tropical fire ant, *Solenopsis geminata,* with access to a sugar source attended *D. brevipes* significantly less than those without additional sugar sources, but the ants did not kill the mealybug. This and our results slightly differ from Offenberg's (2001) in which the availability of alternative sugar sources led to decreased attendance of *Lasius niger* ants on the aphid partner, *Aphis fabae*, and caused increased ant predation on aphids. The absence of ant predation on *D. brevipes* even in our control treatment may suggest that *D. brevipes* is not a suitable protein source for the big-headed ant, or that honeydews of *D. brevipes* contain sugar mixed with rich amino acids important for ant colony growth and survival. However, the growth and survival of an ant colony has not yet to be empirically investigated for our particular scenario because our study only focused on ant-tending behavior with mealybugs when ant larvae are present in the colony.

There was another interesting difference in results between Offenberg's and ours; in our study ant attendance intensity decreased when alternative protein sources were provided, whereas such effect was not observed by Offenberg (2001). Those differences may reflect species specificity or other environmental differences. We noticed that colony composition differed between Offenburg's and our study; in our study colonies had broods, whereas no brood was present in Offenburg's. Generally, ant workers mainly need carbohydrates for their activity, while queens and larvae require protein for egg-laying and growth (Beattie 1985; Dussutour and Simpson 2009; Howard and Tschinkel 1981; Weeks et al. 2004). The presence of a brood in the colonies of our study might make the alternative protein source more attractive to the ants that can affect ant-tending behavior towards *D. brevipes*.

There is additional suggestive evidence in regard to the effect of the background nutritional condition on *D. brevipes* tending behavior of *P. megacephala*. The markedly statistically significant effect of the presence of alternative food (including the protein source) on the ant's *D. brevipes* attendance was observed only during the first 2 days. Hunger was likely related to this phenomenon, as ants were starved for 2 days before the experiment in this study. Admittedly, however, further study is needed on the relationship between the colonies' nutrition requirements and *D. brevipes* tending behavior in the big-headed ant.

The attack by natural enemies on myrmecophilous hemipterans is generally more successful when they are not tended by ants (e.g. Tanaka et al. 2011). The big-headed ant is a typical ant species that shows mutualistic association with Hemiptera. For example, in Hawaii, the presence of the big-headed ant reduced the predation success on *D. brevipes* by the coccinellid *Nephus bilucernarius* Mulsant, and the encyrtid wasp *Anagyrus ananatis* Gahan through behavioral interference (González-Hernández et al. 1999). Similar protection by the big-headed ant of other pest hemipterans from their natural enemies has been reported (Jahn 1992; Reimer et al. 1993). Importantly, our study showed that that big-headed ant decreased the tending frequency of *D. brevipes* when the ants were provided with alternative food sources although they did not predate on the mealybugs. We believe that the decreased tending frequency of ants can lead to the decline in ant protection of mealybugs. This can increase the opportunity for natural enemies to attack the mealybugs. Future experimental studies incorporating natural enemies of mealybugs into this system should examine if artificial provisioning of alternative food sources for ants in the field can improve a biological control program or not.

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