INTRODUCTION

Seeds of many plant species bear external appendages called elaiosomes (Beattie & Culver, 1981; Handel et al., 1981; Beattie, 1985; Gaddy, 1986; Servigne & Detrain, 2008; Lengyel et al., 2010). Foraging ants attracted by elaiosomes bring back the seeds to their nests. Although elaiosomes are consumed as food, the main parts of seeds are discarded inside or outside the nests (Hughes & Westoby, 1992a; Gómez et al., 2005). Consequently, mutualism between ants and plants is achieved. Specifically, ants can obtain essential nutrition such as lipids and nitrogen-rich amino acids. In return, seeds are dispersed from the parent plants, which remove the seeds from the competition with other plants (Higashi et al., 1989; Lanza et al., 1992; Gamman et al., 2005; Boulay et al., 2006; Fischer et al., 2008).

Stick insects are well-known for their twig-like forms of plant mimicry. The shapes of stick-insect eggs also resemble those of plant seeds. Furthermore, in some stick insects, appendages, called capitula, are attached to the opercula of the eggs (Clark, 1976). Several studies have revealed that ants transfer stick-insect eggs with capitula (Compton & Ware, 1991; Hughes & Westoby, 1992b; Windsor et al., 1996; Stanton et al., 2015). Ants are less attracted to eggs from which capitula are artificially removed or covered by paint (Windsor et al., 1996; Stanton et al., 2015). Furthermore, when capitula removed from Eurycnema goliath (Gray) eggs are attached to polystyrene balls, workers of Rhytidoponera
metallica (Smith) intensively collect the balls (Stanton et al., 2015). These studies show clearly that capitula of stick-insect eggs are attractive to ants, as are the elaiosomes of plant seeds. Those results suggest that the transportation of eggs by ants is an adaptive strategy used by slow-moving stick insects to disperse their progeny (Compton & Ware, 1991; Hughes & Westoby, 1992b; Windsor et al., 1996).

Ant nests can protect stick-insect eggs from egg parasitoids and fire. However, ant nests are not safe for stick insect hatchlings because of the predation risk by ants. Compton and Ware (1991) reported that workers of Acantholepis capensis Mayr ignored first instar nymphs of Bacillus coccyx Westwood emerged in their nests. Nevertheless, it remains unclear whether or not workers ignore hatchlings in the other combinations of ants and stick insects.

Nest-moving habits of ants are important factors affecting stick insect survival. Hughs and Westoby (1992b) reported that the predation risk of stick-insect nymphs is mitigated by frequent nest moving in many ant species. Specifically, even if eggs are stored in the nests, the long embryonic period of stick insects enables nymphs to hatch after the nests have already been moved elsewhere.

Even without ants, hatching success decreases when stick-insect eggs are buried deep into the soil. When eggs of Podacanthus wilkinsoni Macleay are buried at 6 cm or less in the soil, nymphs emerge successfully from 98% of the eggs (Hughes & Westoby, 1992b). However, nymphs reach the surface from only 11% of the eggs buried at 12 cm. Consequently, ant species that transport eggs deep into the soil cannot be good partners for stick insects.

The mortality of hatchlings can decrease if the eggs of stick insects are not stored in the ant nests. Eggs of Calynda bicuspis Stål are not stored in the nests of Ectatomma ruidum Roger (Windsor et al., 1996). Specifically, many eggs are soon discarded outside of the ant nests. The risk of parasitism by egg parasitoids is decreased even though ants do not protect eggs in the nests. Thus, we can infer that the horizontal transfer of eggs from the original places is effective in environments where forest fires are rare (Windsor et al., 1996).

Pristomyrmex punctatus Smith, widely distributed in Southeast Asia, is a widely dispersed ant species in western Japan (Japanese Ant Database Group, 2003). This species transports seeds of plants such as Thesium chinense Turcz and Sciaphila secundiflora Thwaites ex Bentham (Suetsugu, 2015; Suetsugu et al., 2017). Pristomyrmex punctatus is queenless, and workers produce additional workers by thelytoky (Mizutani, 1980, 1984; Itow et al., 1984; Tsuji, 1988a). A large colony can comprise several tens of thousands to several hundred thousand workers. Nests are established under stones or inside decayed trees (Japanese Ant Database Group, 2003). However, permanent nests are not constructed: P. punctatus relocates its colony frequently (Mizutani, 1982; Tsuji, 1988b).

Phraortes illepidus (Brunner von Wattenwyl), a bisexual stick insect, is widely distributed in Japan’s Honshu, Shikoku, and Kyushu islands (Okada, 1999). Capitula are attached to the opercula of P. illepidus eggs (Fig 1). It is expected that capitula attract ants and that they disperse stick-insect eggs. For this study, we conducted rearing experiments in the laboratory to examine 1) whether or not P. punctatus are attracted by P. illepidus eggs, and 2) where and how deep stick-insect eggs are transferred by ants. We first examined the response of P. punctatus to P. illepidus eggs. We presented stick-insect eggs to ants in small rearing containers and counted the capitula of eggs removed by ants. Next, we examined the destination of P. illepidus eggs placed near the artificial nests of P. punctatus in large rearing containers. When eggs were buried in the sand, the burial depth of each egg was measured. The results demonstrated an interspecific relation between P. punctatus and P. illepidus.

**MATERIALS AND METHODS**

**Collection and rearing of ants**

Colonies of P. punctatus were collected in Okayama City, Japan (34.7 °N, 133.9 °E) during the spring-summer of 2011. Small colonies were maintained in plastic containers with two levels. The bottom of each lower container (140 × 65 mm, 35 mm deep) was covered with plaster. Dried

*Fig 1. Photograph of a Phraortes illepidus egg, which bears a capitulum.*
bloodworm and insect jelly, an artificial diet for adult beetles (Fujikon Co. Ltd., Nose, Osaka, Japan), was placed on the upper containers (70 × 40 mm, 20 mm deep) once every week. A proper quantity of water was also provided through the plaster.

Large colonies were maintained in large plastic containers (31 × 17 cm, 22 cm in height). Bottoms of the containers were filled with sand of approximately 2 mm maximum particle size, and the sand depth was 8-9 cm. A square plaster plate (approximately 10 × 10 cm, 2 cm in height), under which ants can construct a nest, was placed on the sandy surface of each container. In addition, a sufficient amount of dried bloodworm and insect jelly were provided once a week. Water was provided through the plaster plate every day. The colonies were maintained under room conditions at approximately 20-25 °C.

Rearing of stick insects and collection of eggs

First or second instar nymphs of *P. illepidus* were collected in Okayama City, Japan, in late April 2011. Insects were reared in plastic containers (35 × 30 × 38 cm height) under room conditions at 20-25 °C. The rearing density of early instar nymphs was approximately 30 per container. After that, the respective densities of fourth and fifth instar nymphs and adults decreased to approximately 10 per container. Insects were supplied with fresh leaves of cherry, *Prunus yedoensis* Matsumura, twice a week. Eggs laid by adults were collected every other day and were used for the experiments.

Capitulum removal by ants

Experiments were performed with the two-level containers, which were used for the maintenance of the ants. In the first experiment, three colonies of *P. punctatus* were kept under room conditions at approximately 20-25 °C. Ants were provided a sufficient food source in addition to stick-insect eggs. Specifically, dried bloodworm and insect jelly were provided on the upper containers once a week. Water was also provided once a week. In every ant colony, 50-100 eggs of *P. illepidus* within 48 h of oviposition were placed. One week later, all the eggs were collected; eggs without capitula and those on the lower containers were counted.

For the next experiment, five colonies of *P. punctatus* were used. The experimental procedure was fundamentally identical to that of the experiment described above. No food source other than stick-insect eggs were provided to ants, and an adequate amount of water was provided once a week. In the upper part of the containers, several tens of *P. illepidus* eggs were placed within 48 h of oviposition. One week later, eggs without capitula and those on the lower containers were counted. Then, all the eggs were removed from the containers. Newly laid eggs were placed in the upper containers. The experimental treatment described above was applied repeatedly. The placing and removal of eggs were made twice: the total number of eggs was more than 100 for every colony.

The fate of stick-insect eggs transported by ant workers

Five large colonies of *P. punctatus* were used in this study. Experiments were performed in large plastic containers (31 × 17 cm, 22 cm in height), which were used to maintain ants. Throughout the experimental period, dried bloodworm and insect jelly were provided once a week. Water was provided through the plaster plates every day.

Three times a week, 40-80 eggs of *P. illepidus* were placed on a square aluminum foil of approximately 5 × 5 cm. They were placed on the sandy surface of each container. The aluminum foil square was located 5 cm away from the short wall of the container, on the opposite side of the plaster plate under which ants constructed a nest. The distance between the nearest edges of the plaster plate and aluminum foil was approximately 5 cm. One week later, new eggs were added to the aluminum foil square. All the eggs placed earlier were left as they were. Egg-placeing lasted until the cumulative egg number became more than 1000 per colony, except for one colony for which the egg number did not reach 1000 because of the death of stick insects. Then, *P. punctatus* colonies were retained for an additional month. After that, all the eggs found in the containers were collected; the localities of the eggs were divided into five categories: sandy surface, under the plaster plate, less than 3 cm in the sand, 3-6 cm in the sand, and deeper than 6 cm in the sand.

Data analysis

Percentage data were evaluated using chi-square tests or Fisher’s exact probability tests.

RESULTS

Capitulum removal by ants

Stick-insect eggs were presented to *P. punctatus* that were kept in containers with two levels. When ants were provided diet, capitula were removed from more than 99% of the eggs (Fig 2). When ants were not provided diet, capitula were removed from many eggs (81.0%). A significant difference was found in the percentages of eggs without capitula between the experimental treatments with and without food (*p* < 0.001, Fisher’s exact probability test).

The percentage of eggs found in the upper containers was 90.5% in the experimental treatment with dried bloodworm and insect jelly in addition to stick-insect eggs. As for the experimental treatment without food, we found 74.9% of eggs, which was significantly lower than in the experimental treatment with food (*p* < 0.001, Fisher’s exact probability test).
The fate of stick-insect eggs transported by ant workers

We presented 462-1178 stick-insect eggs to large colonies of *P. punctatus* in this experiment. When workers carried the stick-insect eggs, they bit the capitula (Fig 3). Some eggs were transported into the nests under the plaster plates. However, we also observed that many eggs were not brought into the ant nests; capitula were removed from the eggs on the sandy surface.

As a whole, only 50 of 4926 eggs were lost a month after the final egg placement (Table 1). Furthermore, the number of dead eggs (i.e., rotted eggs and debris of eaten eggs) was also small. The total percentage of eggs without a capitulum was 98.9% of the 4855 intact eggs. The results were almost identical among colonies.

### DISCUSSION

#### Interspecific relation between ants and stick insects

Workers of ants are attracted by capitula of stick-insect eggs, and transfer the eggs to nest structures (Compton & Ware, 1991; Windsor et al., 1996; Stanton et al., 2015). The results obtained from this experiment reveal that workers of *P. punctatus* intensively remove capitula of *P. illepidus* eggs (Table 1, Fig 2). Furthermore, ants transported stick-insect eggs from their place of origin (Table 2). From these results, it can be inferred that *P. punctatus* responses to *P. illepidus* eggs are similar to those of many ant species to elaiosome-bearing plant seeds and capitula of several other stick insects.

Adults of *P. illepidus* have no wings, similar to many other stick insects, and they do not move often. Eggs are scattered on the ground by female adults on trees. Consequently, egg transportation by ants can be an adaptive strategy for slow-moving *P. illepidus* to disperse progeny, as seems to be true also for other stick insects (Compton & Ware, 1991; Hughes & Westoby, 1992b; Windsor et al., 1996).

#### Capitula as a diet for ants

From elaiosomes of plant seeds, ants can obtain essential nutrition such as lipids and nitrogen-rich amino acids (Higashi et al., 1989; Lanza et al., 1992; Gamman et al., 2005; Boulay et al., 2006; Fischer et al., 2008). Capitula of stick-insect eggs also present nutrients such as several fatty acids. (Stanton et al., 2015). The present study shows that ants removed capitula from *P. illepidus* eggs even when a sufficient food source other than eggs was provided (Fig 2). This result suggests that *P. punctatus* can obtain necessary nutrition from the capitula of *P. illepidus* eggs.

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**Table 1.** Capitula removal of *Phraortes illepidus* eggs provided for *Pristomyrmex punctatus.*

<table>
<thead>
<tr>
<th>Colony number</th>
<th>Initial number of eggs</th>
<th>Number of lost eggs</th>
<th>Number of rotted eggs</th>
<th>Number of eggs eaten by ants</th>
<th>Number of intact eggs</th>
<th>Number of intact eggs without capitula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony 1</td>
<td>1085</td>
<td>25</td>
<td>4</td>
<td>1</td>
<td>1055</td>
<td>96.1</td>
</tr>
<tr>
<td>Colony 2</td>
<td>1178</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>1166</td>
<td>99.7</td>
</tr>
<tr>
<td>Colony 3</td>
<td>1099</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>1089</td>
<td>100.0</td>
</tr>
<tr>
<td>Colony 4</td>
<td>1102</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>1094</td>
<td>99.4</td>
</tr>
<tr>
<td>Colony 5</td>
<td>462</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>451</td>
<td>99.3</td>
</tr>
<tr>
<td>Total</td>
<td>4926</td>
<td>50</td>
<td>10</td>
<td>11</td>
<td>4855</td>
<td>98.9</td>
</tr>
</tbody>
</table>
The percentage of eggs from which capitula were removed by ants was higher with supplemented than without supplemented food (Fig 2). We expected the contrary, that starved ants would remove capitula more intensively. The average numbers of eggs per colony were, respectively, 77 and 170 in the experimental treatment with and without supplemented food. The colony sizes of the two experimental treatments were similar. The difference in egg numbers might affect the percentage of capitulum removal.

A significant difference in the percentage of eggs transported to lower containers was found when ants were with and without food. Specifically, ants not provided dried bloodworm and insect jelly intensively transported eggs to the lower containers. Consequently, it might be possible that starved ants tend to store capitulum-bearing eggs of stick insects as a future diet.

**Possible effects of P. punctatus nest-moving habits**

For plants, the nest-moving habits of ants affect the germination and survival of the progeny. The frequent relocation of ant nest entrances might result in the lack of nutritional enrichment, although it enables seeds to reduce competition between establishing seedlings (Higashi et al., 1989; Hughes, 1991). By contrast, stick-insect nymphs that emerge in active nests might be in danger from the ants, and the danger is mitigated by the nest moving habits of many ant species (Hughes & Westoby, 1992b). Specifically, stick insects are not in active ant nests at the time of hatching.

This study revealed some eggs of *P. illepidus* in the nests of *P. punctatus* constructed under plaster plates (Table 2). This result suggests that the survival rate of nymphs decreases if hatching occurs in the nests. However, *P. punctatus* has no permanent nest, and it moves its nest frequently (Mizutani, 1982, 1984; Tsuji, 1988b). Furthermore, *P. illepidus* has a long embryonic period that may last from seven months to more than one year according to the timing of oviposition (S. Matsumoto, personal communication, March 19, 2019). Therefore, it is likely that the first-instar nymphs of *P. illepidus* from the eggs stored in the nests can safely reach the ground surface after *P. punctatus* has already moved its nest to another location.

**Depth of *P. illepidus* eggs buried by *P. punctatus***

If eggs are buried deep in ant nests, first instar nymphs of stick insects can have difficulty reaching the ground surface. Hughes and Westoby (1992b) showed that first instar nymphs of *P. wilkinsoni* could reach the surface from only 11% of eggs experimentally buried at 12 cm depth. Consequently, ant species that do not bury eggs deep in the soil can be effective partners for stick insects.

In the present study, the percentage of eggs buried in the sand was small (Table 2). Furthermore, most of the buried eggs were found near the surface, and only one of the 246 buried eggs was found at a depth greater than 6 cm from the surface. Although we did not observe stick insect hatching, nymphs are likely to reach the host plants from *P. punctatus* eggs buried near the soil surface.

**Horizontal transfer of stick-insect eggs on the ground surface**

Germination of elaiosome-bearing seeds in the ant nets is regarded as adaptive for plants. Specifically, nests can prevent predators from finding seeds and can protect seeds from ants.
from fire (O’Dowd & Hay, 1980; Heithaus, 1981; Beattie & Culver 1981; Bond & Slingsby, 1984; Bond & Breytenbach, 1985; Hughes & Westoby, 1992a; Ohkawara & Higashi, 1994; Espadaler & Gómez, 1997). Germination rates increase because of high available nutrients and aeration within the ant nests (Horvitz, 1981; Zettler et al., 2001; Passos & Oliveira, 2002). For stick insects, however, hatching in the ant nests is not adaptive, as discussed above. In C. bicuspid, many eggs are not stored in the nests but are instead transported horizontally on the ground surface (Windsor et al., 1996). Consequently, first instar nymphs that hatched out from eggs outside the ant nests can decrease the predation risk by ants. Furthermore, the ground surface transfer of eggs decreases the risks of parasitism by egg parasitoids (Windsor et al., 1996).

Most P. illepidus eggs were not found in ant nests under plaster plates in the study described herein. Specifically, 82% of the intact eggs were found on the sandy surface (Table 2). Consequently, P. punctatus transfers eggs mainly horizontally on the surface, enabling first instar nymphs of P. illepidus to reach host plants safely without being attacked by ant workers. Furthermore, eggs can escape from egg parasitoids by transfer from the original location. Results suggest that aboveground transfer by P. punctatus is favorable also for P. illepidus eggs.

**CONCLUSION**

The present study revealed that P. punctatus are attracted by capitula of P. illepidus eggs and transport the eggs from their place of origin. Ants transport eggs mainly horizontally on the surface, which can decrease the predation risk of stick-insect hatchlings. Although some eggs were stored in the ant nests or buried in the sand, first-instar nymphs can presumably reach the host trees safely because of the nest-moving habits of P. punctatus and the depth of buried eggs. Based on the results, we infer that P. punctatus can be a good partner of P. illepidus: ants disperse stink-insect eggs safely, probably in exchange for some nutrition from the capitula of eggs.

**COMPETING INTERESTS**

The authors declare no conflict of interest related to this report or the study it describes.

**AUTHORS’ CONTRIBUTIONS**

KN: conceptualization, methodology, writing
YT: conceptualization, methodology, investigation, writing
IK: investigation

**REFERENCES**


Yoshiyuki Toyama, Izuru Kuroki, Keiji Nakamura – Dispersal of Phasmida eggs by ant workers


