Entomological Science (2018) doi: 10.1111/ens.12314

SHORT COMMUNICATION

Effect of the Argentine ant on the specialist myrmecophilous cricket *Myrmecophilus kubotai* in western Japan

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Abstract

Effects of the Argentine ant on myrmecophilous animals living inside ant nests have been rarely studied to date. We investigated whether the "specialist" myrmecophilous cricket *Myrmecophilus kubotai* Maruyama that lives only in colonies of a Japanese native ant, *Tetramorium tsushimae* Emery, could live with the Argentine ant. In the field, the cricket was never found in nests of the Argentine ant. Our experiments showed that the cricket could not survive in artifical nests of the Argentine ant under laboratory conditions.

Key words: ant guest, host specificity, invasive ant, *Linepithema humile*, Myrmecophilidae, Orthoptera, symbiosis.

The Argentine ant *Linepithema humile* (Mayr) is known as one of the most harmful invasive species because of their remarkably negative effects on the native biota in areas where they are introduced (Holway *et al*. 2002). Its effects on native ants are particularly conspicuous: most native ants, except for small-sized and/or subterranean species, are replaced by the Argentine ant (e.g. Miyake *et al*. 2002). Therefore, myrmecophilous animals that depend on native ants should be considerably affected by Argentine ant invasion. However, investigation on the effects of the Argentine ant on myrmecophilous animals has been carried out for only a limited number of species (e.g. Suarez *et al*. 2000; Ness & Bronstein 2004; Glenn & Holway 2007; Ito *et al*. 2008; Touyama *et al*. 2008). To date, no studies have been undertaken on the effects of the Argentine ant on myrmecophilous species living inside nests of native ants.

In Japan, the Argentine ant is distributed from Tokyo to Yamaguchi Prefecture in Honshu Island, and Tokushima Prefecture in Shikoku Island (Okaue *et al*. 2007; Ikenaga & Ito 2012). In all cases, the Argentine ant is found in residential areas and/or factory districts. Miyake *et al*. (2002) and Touyama *et al*. (2003) showed that most native species, except for *Camponotus vitiosus* Smith, *Paraparatrechina sakurae* (Ito), *Temnothorax congruus* (Smith) and *T. spinosior* (Forel), are replaced by the Argentine ant. Of the native ants depopulated by the Argentine ant, *Tetramorium tsushimae* Emery often harbors the myrmecophilous cricket *Myrmecophilus kubotai* Maruyama in their nests. *Myrmecophilus kubotai* has two genetically different lineages: members of lineage I are "specialists" that are exclusively found in nests of *T. tsushimae*, whereas members of lineage II are found in 12 species of formicine and myrmicine ants (Komatsu *et al*. 2008, 2010, 2013; Maruyama *et al*. 2013). If the host is replaced by the invasive ants, host specialist crickets are naturally expected to disappear. However, the cricket's disappearance could be highly dependent on ant species. In this paper, to examine whether the host-specialist lineage of *M. kubotai* can live inside nests of the Argentine ant, we investigated the occurrence of the myrmecophilous cricket in nests of the Argentine ant in the field. Behavioral interactions between the myrmecophilous cricket and the Argentine ant were then observed in the laboratory.

Field survey: In 2009, the occurrence of *M. kubotai* in nests of the Argentine ant and the original host ant *T. tsushimae* was examined at 20 parks in a residential quarter of Hatsukaichi City and Hiroshima City (both Japan). Among the 20 parks, five harbored

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Received 19 December 2017; accepted 4 April 2018.

T. tsushimae but not the Argentine ant, five harbored both, and the Argentine ant, but not *T. tsushimae*, were found at the remaining ten. At each park, we dug out 1–14 nests of the Argentine ant and/or *T. tsushimae*, and checked for the occurrence of the myrmecophilous cricket. The difference in the frequency of the cricket occurrence was examined between nests of the two ant species, and between *T. tsushimae* nests in Argentine ant invaded and uninvaded parks by Fisher's exact probabilty test.

Laboratory observation: The Argentine ant was kept in artificial nests constructed of four small polystyrene boxes (33 mm × 33 mm × 12 mm) serving as nest chambers. The four nest boxes were arranged in a 2 × 2 grid fashion within a polystyrene container (138 mm × 220 mm × 50 mm) that served as a foraging arena. The four boxes were connected to each other with small holes, each box having two entrances (eight entrances in total in the nest chambers), because multiple entrances were found in natural nests. The bottoms of both the polystyrene container and the small chambers were covered with plaster of Paris for maintaining humidity. In each nest, three queens and 300–350 workers with some brood were released. Some pieces of mealworm and diluted honey water were given as prey. The same rearing condition was applied to *T. tsushimae*. In total, four nests of the Argentine ant and eight nests of *T. tsushimae* were used for observations.

Several individuals of *M. kubotai* were collected from nests of *T. tsushimae* in and around Takamatsu City in Shikoku Island. These *T. tsushimae* host colonies were not used for the experiment. The collected crickets were kept without host ants in a polystyrene container (138 mm × 220 mm × 50 mm) under the same conditions as ant rearing for a few days to

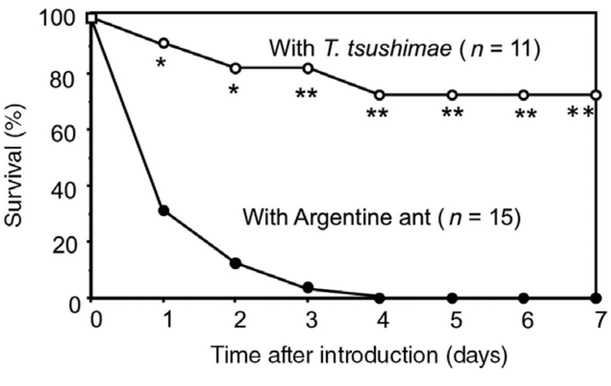

Figure 1 Survival of the myrmecophilous cricket *Myrmecophilus kubotai* in artificial nests of the Argentine ant and of *Tetramorium tushimae*. Fisher's exact probability test: $*P < 0.05$, $**P < 0.01$.

1 week before the experiment. We randomly chose the crickets and put them one by one into one of the Argentine ant or *T. tsushimae* nests. In total, 11 and 15 crickets were put in the nests of *T. tsushimae* and the Argentine ant, respectively. Each nest was used for this experiment two to five times. The survival of crickets was recorded every day for 1 week. Behavioral interactions between the ants and crickets were observed for 1 h per day until the sixth day. We counted the frequencies of three behavioral events (from the cricket side): (i) grooming ants; (ii) attacked by ants; and (iii) feeding on liquid food from ants (oral trophallaxis). The difference in survival of the crickets was examined between nests of the two ant species by Fisher's exact probability test. The frequencies per 1 h of each of the three behviors were compared between nests of the two ant species, for each day, by the two-sample test. All statistical tests were carried out using R (version 3.4.2) (R Development Core Team 2008).

In the five parks where the Argentine ant had not yet occurred, the myrmecophilous cricket was found in five of 20 *T. tsushimae* nests. In the ten parks harboring the Argentine ant but not *T. tsushimae,* we could not find any myrmecophilous crickets from 54 nests. In the five parks where the Argentine ant and *T. tsushimae* coexisted, two of 19 *T. tsushimae* nests harbored the myrmecophilous cricket, whereas no crickets were found in 15 nests of the Argentine ant. The frequency of the cricket occurrence in *T. tsushimae* nests was not statistically different between the parks with and without the Argentine ant (Fisher's exact probability test, $P = 0.41$). In total, seven of 39 *T. tsushimae* nests had the myrmecophilous cricket, whereas no crickets were found from 69 nests of the Argentine ant (Fisher's exact probability test, $P < 0.001$).

When we introduced *M. kubotai* into the nests of the Argentine ant, all crickets ($n = 15$) disappeared within 4 days, whereas 72% of the introduced crickets ($n = 11$) survived for 1 week in the nests of *T. tsushimae* (Fig. 1). In the nests of *T. tsushimae*, *M. kubotai* frequently showed grooming towards the ants and trophallaxis with the ant workers, whereas we never observed these behaviors in the nests of the Argentine ant (Fig. 2; two sample *t*-test: trophallaxis on the first day, $t = 2.425$, d.f. = 19, $P = 0.025$; grooming on the first day, $t = 3.42$, d.f. = 19, $P = 0.0029$). The frequency of aggression from the ants towards *M. kubotai* was also different between the two ant species: the Argentine ant more frequently attacked the crickets (two sample *t*-test: on the first day, $t = 2.66$, d.f. = 25, $P = 0.0029$). The significant differences in these behaviors were detected only on the first day (day 0); the sample size of crickets in nests of the Argentine ant

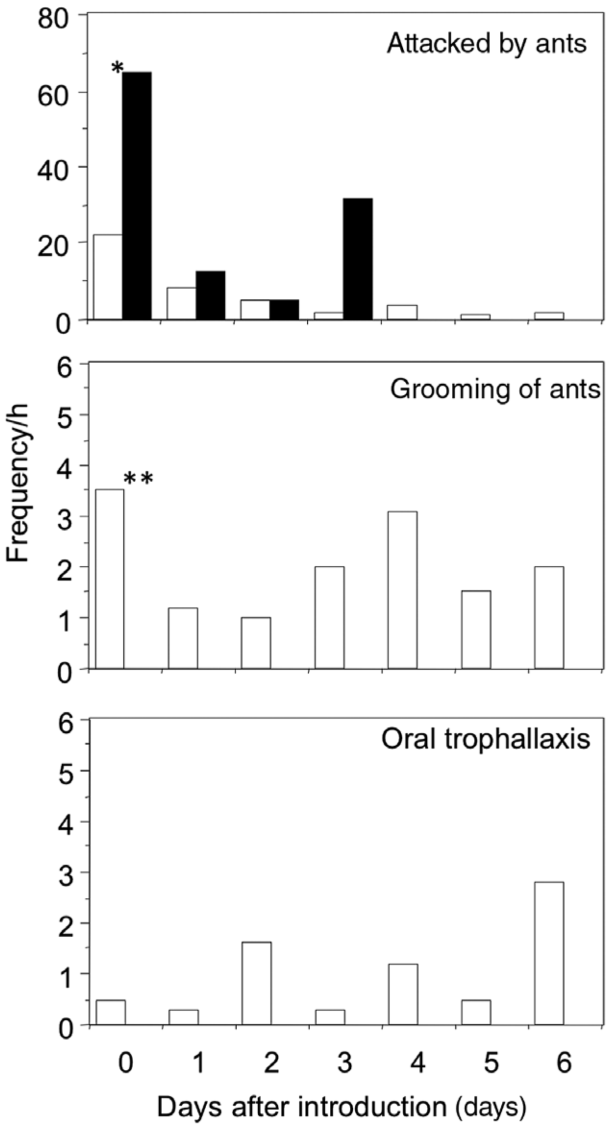

Figure 2 Frequencies of interactive behaviors between (□) *Tetramorium tsushimae* or (■) the Argentine ant and the myrmecophilous cricket *Myrmecophilus kubotai* in artificial nests. The frequencies of grooming, trophallaxis and attacking on the first day (day 0) were significantly different between nests of the two ant species (two sample *t*-test: $*P < 0.05$, $**P < 0.01$).

rapidly decreased due to their high mortality after the first day (Fig. 1), which should have lowered the statistical power.

To date, no studies have been carried out on interactions between the Argentine ants and myrmecophilous animals living in their nests. The present study indicates that the host-specific myrmecophilous species cannot survive in the Argentine ant nests. Although we did not directly observe predation on the crickets by ants in the experimental containers, the observed frequent aggression by the Argentine ant towards *M. kubotai* suggests that the ants attacked the crickets and consumed the cricket bodies; we could not find corpses of the crickets in the containers. *Myrmecophilus kubotai* is a species showing behaviors, such as grooming and trophallaxis, well integrated with its specific host ant, *T. tsushimae* (Komatsu *et al.* 2013), and they often approach the host ants. Such behavioral characteristics would make the cricket an easy prey for ants. Komatsu *et al.* (2009) showed that *Myrmecophilus albicinctus* Chopard, a specialist living in nests of *Anoplolepis gracilipes* (Smith), could not survive with *Diacamma* sp. *Myrmecophilus albicinctus* is similar to *M. kubotai* in showing frequent grooming to the host and oral trophallaxis (Komatsu *et al.* 2009). Host-generalist crickets such as *Myrmecophilus formosanus* Shiraki never shows such behaviors (Komatsu *et al.* 2009). For myrmecophilous animals to be highly integrated into host ant colonies, behavioral specialization might be necessary. In turn, however, such specialization could prevent them from surviving in colonies of other ant species.

In Japan, the Argentine ant is now found from Tokyo to Yamaguchi Prefecture in Honshu Island, and from Tokushima Prefecture in Shikoku Island (Okaue *et al.* 2007; Ikenaga & Ito 2012). Another host-specialist species distributed in this area, *Myrmecophilus tetramorii* Ichikawa, is also found exclusively in nests of *T. tsushimae* (Komatsu *et al.* 2013). Unlike *M. kubotai, M. tetramorii* does not show oral trophallaxis with the host ants, being more or less similar in behavior to host-generalist species like *M. formosanus* (Komatsu *et al.* 2013). Thus, it is uncertain whether *M. tetramorii* suffers such negative effects from the Argentine ant as shown here in *M. kubotai.* To investigate whether *M. tetramorii* can live in nests of the Argentine ant would shed more light on the behavioral mechanisms of specialization for myrmecophilous animals that live inside nests of their particular host ant species.

ACKNOWLEDGMENTS

We would like to sincerely thank T. Komatsu for providing us information on the biology of myrmecophilous crickets, N. Ikenaga for help during the field investigation and J. Billen for valuable suggestions on the manuscript. This research was supported by KAKENHI (C: 14540579, 22510246), Sumitomo Foundation and Ohshimo Foundation.

REFERENCES

Glenn S, Holway D (2007) Consumption of introduced prey by native predators: Argentine ants and pit-building ant lions. *Biological Invasions* **10**, 272–280.

Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ (2002) The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* **33**, 181–233.

Ikenaga N, Ito F (2012) Distribution of the Argentine ant, *Linepithema humile*, in harbor areas of Shikoku Isl., western Japan (Hymenoptera; Formicidae). *Kagawa Seibutsu* **39**, 63–69. (In Japanese with English abstract.)

Ito F, Okaue M, Ichikawa T (2008) A note on prey composition of the Japanese treefrog, *Hyla japonica,* in an area invaded by Argentine ants, *Linepithema humile*, in Hiroshima Prefecture, western Japan (Hymenoptera: Formicidae). *Myrmecological News* **12**, 35–39.

Komatsu T, Maruyama M, Ueda S, Itino T (2008) MtDNA phylogeny of Japanese ant crickets (Orthoptera: Myrmecophilidae): diversification in host specifity and habitat use. *Sociobiology* **52**, 553–565.

Komatsu T, Maruyama M, Itino T (2009) Behavioral differences between two ant cricket species in Nansei Islands: host-specialist versus host-generalist. *Insectes Sociaux* **56**, 389–396.

Komatsu T, Maruyama M, Itino T (2010) Differences in host specifity and behavior of two ant cricket species (Orthoptera: Myrmecophilidae) in Honshu, Japan. *Journal of Entomological Science* **45**, 227–238.

Komatsu T, Maruyama M, Itino T (2013) Nonintegrated host association of *Myrmecophilus tetramorii*, a specialist myrmecophilous ant cricket (Orthoptera: Myrmecophilidae). *Psyche* **2013**, Article ID 568536. https://doi.org/10.1155/2013/568536

Maruyama M, Komatsu S, Kudo S, Shimada T, Kinomura K (2013) *The Guests of Japanese Ants*. Tokai University Press, Hadano.

Miyake K, Kameyama T, Sugiyama T, Ito F (2002) Effect of Argentine ant invasions on Japanese ant fauna in Hiroshima Prefecture, western Japan: a preliminary report (Hymenoptera: Formicidae). *Sociobiology* **39**, 465–474.

Ness JH, Bronstein JL (2004) The effects of invasive ants on prospective ant mutualists. *Biological Invasions* **6**, 445–461.

Okaue M, Yamamoto N, Touyama Y *et al.* (2007) The distribution of the Argentine ant, *Linepithema humile*, along the Seto Inland Sea, western Japan: result of surveys in 2003 to 2005. *Entomological Science* **10**, 337–342.

R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available from URL: http://www.R-project.org

Suarez AV, Richmond JQ, Case TJ (2000) Prey selection in horned lizards following the invasion of Argentine ants in southern California. *Ecological Applications* **10**, 711–725.

Touyama Y, Ogata K, Sugiyama T (2003) The Argentine ant, *Linepithema humile*, in Japan: assessment of impact on species diversity of ant communities in urban environments. *Entomological Science* **6**, 57–62.

Touyama Y, Ihara Y, Ito F (2008) Argentine ant infestation affects the abundance of the native myrmecophagic jumping spider *Siler cupreus* Simon in Japan. *Insectes Sociaux* **55**, 144–146.