**ORIGINAL RESEARCH PAPER**

# Predation of invasive red-necked longhorn beetle *Aromia bungii* (Coleoptera: Cerambycidae) eggs and hatchlings by native ants in Japan

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## Abstract

Success of an invasive organism depends on the level of biotic resistance from native species. Understanding the mechanism of biotic resistance is critical to the management of invasive species, but such case studies are sparse. Native to parts of Asia and Russia, the red-necked longhorn beetle *Aromia bungii* (Faldermann) (Coleoptera: Cerambycidae) recently invaded Germany, Italy, and Japan, causing serious damage to Rosaceae trees. To identify any specific mechanism of biotic resistance to *A. bungii*, we investigated whether Japanese native ants play a role in the biotic resistance through predation, because ants can impact many arthropods as generalist predators. In laboratory experiments, *A. bungii* eggs or hatchlings were offered to four ant species. Three species exhibited predatory behaviors toward the eggs within 30 min, and obvious damage was confirmed on the eggs within 2 days. Two ant species fatally damaged hatchlings within 30 min. In field surveys, we found a negative correlation between the number of *A. bungii* frass-ejection holes and the number of collected ants on tree trunks. These results suggested that Japanese native ant fauna can play a certain role in biotic resistance to *A. bungii*.

**Keywords** *Aromia bungii* · Biotic resistance · Cerambycidae · Formicidae · Predator

## Introduction

Invasive insect species have caused serious global problems, including biodiversity loss and economic damage (Renault et al. 2018; Ward and Masters 2007). Whether an invasive species can establish itself and reproduce in a new environment is affected by the level of biotic resistance from native species (Elton 1958). The mechanisms of biotic resistance are represented by competition, predation and parasitism, but are more diverse. In the case of invasive herbivorous insects, for instance, host plant resistance and host plant-parasitoid interaction also function as biotic resistance (Aluja et al. 2014; Brockerhoff and Liebhold 2017; Hopper and Mills 2016). An area with higher species diversity is thought to exhibit stronger biotic resistance, and indeed many field studies have shown a negative correlation between native species richness and success of invasive species (Levine and D'Antonio 1999). However, detailed case studies to specify the mechanism of biotic resistance have been relatively few (Levine et al. 2004); such knowledge is pivotal for the enhancement of biotic resistance and management of invasive species.

The red-necked longhorn beetle *Aromia bungii* (Faldermann) (Coleoptera: Cerambycidae) is native to China, Mongolia, Korean Peninsula, Vietnam, and Russia and is a major pest of peach orchards (Iwata 2018; Wang 2017). Since 2011, this species has been introduced into Germany, Italy, and Japan. In Japan, *A. bungii* has invaded multiple areas of Honshu and Shikoku islands and is typically present in orchards, urban house gardens, and parks. Its vigorous reproductive activity has led to mass infestation and death of Rosaceae trees such as the peach *Prunus persica*, Japanese apricot *P. mume*, and cherry *Cerasus* × *yedoensis* 'Somei-yoshino' (Shoda-Kagaya 2018). *Aromia bungii* adults are prevalent from June to August in Japan, and females lay eggs on the bark crevices of Rosaceae trees (Iwata 2018). Their eggs hatch 1 or 2 weeks later, and the hatchlings bore into the tree shortly thereafter and feed on the inner bark. In a heavily infested

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tree, larvae feed on large portions of cambium, and transportation of water and nutrient through xylem and phloem is inhibited. Then, the tree eventually dies. Because *A. bungii* spends most of its life in a larval stage under the bark, chemical control with pesticides is difficult, labor-intensive, and costly. Biotic resistance might be useful for the biological control and integrated pest management (IPM) of invasive *A. bungii*, but biotic resistance has not been studied in detail.

Natural enemies of longhorn beetles include hymenopteran and dipteran parasitoids, mites, predatory insect larvae, ants, woodpeckers, bacteria and nematodes (Hanks et al. 1992; Kenis and Hilszczanski 2007; Paine 2017). For *A. bungii*, bethylid wasps, bothriderid parasitoid beetles, nematodes, bacteria, and woodpeckers have been suggested as the natural enemies in the native range (Iwata 2018; Men et al. 2019). In most cases, however, the effects of these natural enemies on the density of longhorn beetles have not been assessed. Although ants are not specific predators of longhorn beetles, they are ubiquitously distributed with a large biomass, and thus, ants can affect the populations of many arthropods as generalist predators (Hölldobler and Wilson 1990). Field observations showed that a Japanese native ant *Monomorium intrudens* prey on up to 51.2% of Japanese pine sawyer *Monochamus alternatus* eggs on dead pine trees (Ochi and Katagiri 1979). Field manipulative studies also suggested that ants are significant predators of longhorn beetle eggs in some parts of the world (Muilenburg et al. 2008; Paine et al. 2001; Verble and Stephen 2009; Way et al. 1992). In a North American oak-hickory forest, a local ant species *Camponotus pennsylvanicus* among other ant species predate on red oak borer *Enaphalodes rufulus* eggs (Muilenburg et al. 2008; Verble and Stephen 2009). In an eucalyptus plantation in Portugal wherein eucalyptus borer *Phoracantha semipunctata* has invaded, an invasive ant species *Linepithema humile* and some native ant species predate on *P. semipunctata* eggs (Way et al. 1992). In the native range of *P. semipunctata* in Australia, ants and other predators reduce *P. semipunctata* density (Paine et al. 2001).

In this study, we used four species of Japanese native ants and examined if they can predate on *A. bungii* eggs and hatchlings in laboratory bioassays. We assumed *A. bungii* larvae were not accessible for ants, because the larvae live under the bark and plug up their tunnels with frass. *Aromia bungii* adults can avoid ant attacks easily with their wings and large body size. Therefore, only *A. bungii* eggs and hatchlings on the tree surface are vulnerable to ant predation. Thus, we focused on *A. bungii* eggs and hatchlings in our bioassays. We also conducted field surveys in *A. bungii* habitats to investigate native ant fauna and gain insight into its relationship with *A. bungii* density. We could not conduct field manipulative experiments, by law, the Invasive Alien Species Act in Japan.

# Materials and methods

## Laboratory experiments

Laboratory experiments examining ant predation on *A. bungii* eggs and hatchlings were conducted in August and September 2019. *Aromia bungii* eggs and hatchlings (both are ca. 1.7 mm in length: Urano and Shoda-Kagaya 2017) were obtained from female adults collected in Soka City (Saitama Prefecture), Tatebayashi City, and Oura Town (Gunma Prefecture). The female adults were reared in the laboratory and allowed to lay eggs on filter papers. Because the females laid their eggs firmly onto the filter papers with an adhesive substance, it was difficult to remove the eggs from the filter papers intact (Urano and Shoda-Kagaya 2017). Thus, the eggs and the filter papers were used for experiments. Eggs were used < 4 days post-oviposition. Active larvae < 3 days old were used.

Four species of ants were used for the predation tests: *Formica japonica* Motschoulsky (body length: 4.5–6 mm), *Lasius japonicus* Santschi (2.5–3.5 mm), *Camponotus quadrinotatus* Forel (4.5–6 mm), and *Crematogaster matsumurai* Forel (2–3.5 mm). They are all omnivorous species commonly seen on tree trunks through urban and rural areas of Japan (Harada et al. 2010; Nakamura et al. 2017; Terayama et al. 2014). *Formica japonica* nests underground but forages on trees. *Lasius japonicus* nests both underground and in trees. *Camponotus quadrinotatus* and *C. matsumurai* are arboreal species and nest in trees. The ants were collected in Tsukuba City, Ibaraki Prefecture. In egg predation tests and hatchling predation tests, 5 replicate trials were conducted for each ant species, respectively. Twenty worker ants from single nests were collected per trial, resulting in total 200 worker collection per ant species. As an exception, 10 workers of C. *quadrinotatus* were collected per trial, because its colonies were relatively small and collection of 20 workers per nest was difficult.

Predation tests were conducted in plastic cups (10 cm in diameter at the bottom and 5.5 cm high) at 25–28 °C. The bottom of each cup was covered with a solidified mixture of 25 g plaster and 25 g water. The inner wall of the cup was coated with talcum powder to prevent ants from escaping. The 20 or 10 worker ants were introduced into the cup immediately after field collection and were allowed to acclimate to the environment of the cup for approximately 3 h. After acclimation, a piece of filter paper (10–15 mm in length) with five *A. bungii* eggs was placed on the central part of the plaster floor and fixed with a stainless pin. Behavior of the ants was observed for 30 min after placing the eggs. The number of ant-egg contacts was counted. The responses of ants to eggs were

classified into the following four ranks based on the level of predatory behavior: 0 = ignore, 1 = investigate with antenna, 2 = open mandibles and get ready to attack, and 3 = attack by biting (Fig. 1). These ranks were modified from those for 'aggression test', which is often used to investigate inter-colony aggressiveness among ants (e.g., Roulston et al. 2003). The number of damaged eggs or those removed from filter paper was counted 1 and 2 days after. A small plastic cup (2 cm in diameter and 1 cm high) filled with aqueous sucrose solutions (25%) was added to the experimental cup 1 day after the start of the experiment to prevent death of the ants from starvation. Humidity, necessary for ant survival, was maintained by water evaporation from the bottom plaster.

For predation tests on *A. bungii* hatchlings, plastic cups with plaster were prepared in the same manner as described above. Ants were introduced into the cup immediately after field collection and were provided with an aqueous sucrose solution (25%). After 1 day of acclimation, an *A. bungii* hatchling was introduced into the cup. Ant behavior was observed for 30 min after introduction of the hatchling. The number of ant-hatchling contacts was counted. The responses of ants to hatchlings were classified into the following five ranks based on predatory behavior: 0 = ignore, 1 = investigate with antenna, 2 = open mandibles and get ready to attack, 3 = attack by biting, and 4 = carry away. The response of rank 4 represents that an ant recognized a hatchling as a food source and take back to its colony members (Fig. 1). The other ranks are also supposed to represent predatory behaviors, but instead they may represent aggressive behaviors toward enemies. Thus, the occurrence of rank 4 highly suggests ant predation on *A. bungii* hatchlings in the field compared to the other response ranks. During the 30-min observations of the aforementioned predation tests on eggs, a response of rank 4 was not recorded, probably because the adhesion of eggs to filter papers was too strong for ants to break up. After the 30-min observations, the hatchlings were moved to ant-free cups, and survival was checked 1 day later.

## Field survey

Field surveys to investigate native ant fauna and examine the correlation between ant and *A. bungii* densities were conducted on cherry trees *C. × yedoensis* planted on promenades or parks in Tondabayashi City (Osaka Prefecture, 34.51°N, 135.61°E), Fussa City (Tokyo Metropolis, 35.73°N, 139.32°E), and Tatebayashi City (Gunma Prefecture, 36.22°N, 139.60°E). All of these sites were adjacent to urban areas. *Aromia bungii* damaged the cherry trees of the
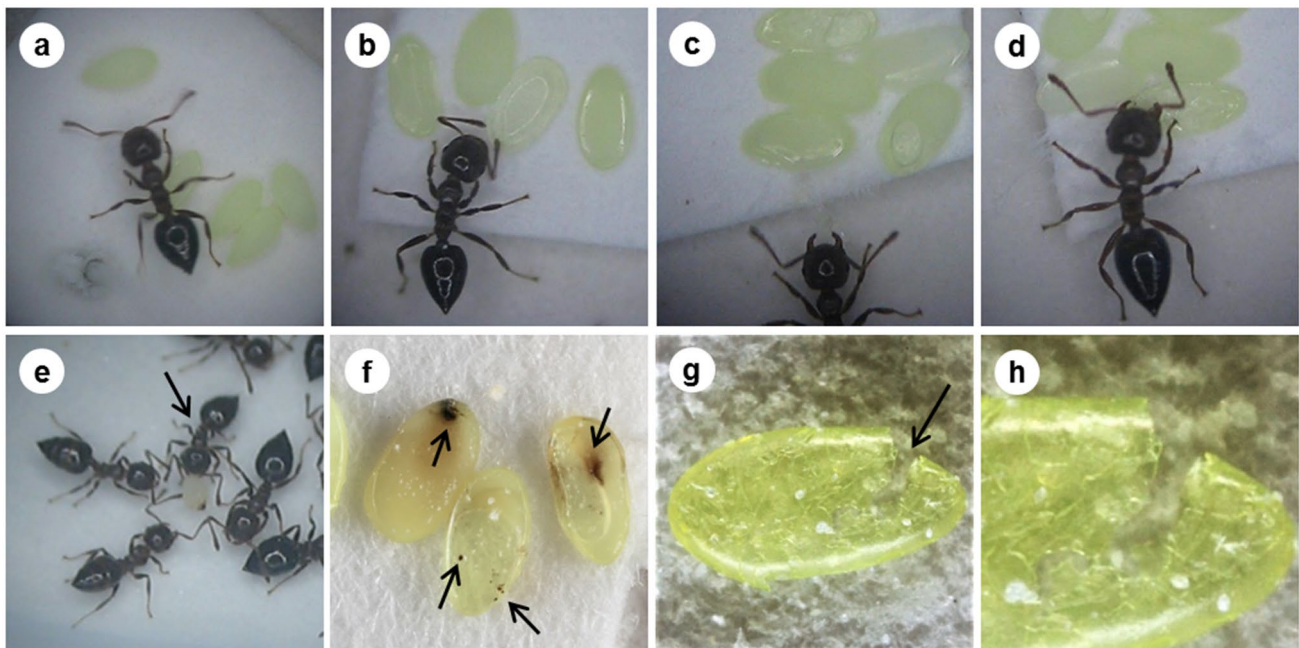


**Fig. 1** Examples of ant behavioral responses to *Aromia bungii* eggs and hatchlings. A *Crematogaster matsumurai* worker ant that is: **a** just ignoring and walking over the eggs (this corresponds to a rank 0 in the laboratory predation tests); **b** repeatedly tapping and investigating an egg with antennae (rank 1); **c** opening mandibles and getting ready to attack an egg immediately after contact with antenna (rank 2); **d** actually attacking an egg by biting (rank 3); or **e** bringing a hatchling to colony members (the worker ant bringing the hatchling is indicated by an arrow) (rank 4, only applied to hatchlings). **f** *Crematogaster matsumurai* made flaws on the eggs (indicated by arrows). **g** An egg broken by *Formica japonica* (the arrow indicates a large crack). **h** A close-up of the crack indicated in **g**

study sites for at least two generations, because there were emergence holes of *A. bungii* adults and frass-ejection holes in all sites. In Osaka and Gunma sites, the surveys were conducted in September 2019. In the Tokyo site, the survey was conducted in May 2019. The numbers of investigated trees were 50, 51, and 42 in Osaka, Tokyo, and Gunma sites, respectively.

Two bait tubes (15-mL polystyrene conical tube containing 2 g of canned tuna) were attached on each tree at a height of ca. 1 m above ground to evaluate the ant fauna and density on the tree trunk. Tuna was used to attract carnivorous and omnivorous ants, which may prey on *A. bungii*. After 2 h, the bait tubes were capped and ants inside the tubes were captured and brought back to the laboratory. Collected ants were counted and species identified via morphology using a stereoscope.

For each tree, the number of *A. bungii* frass-ejection holes at the height of ≤3 m was counted to evaluate *A. bungii* density on the tree trunk. This allowed us to detect the majority of *A. bungii* infestations, because *A. bungii* larvae mostly infest the lower parts of trees (< 2 m: Yamamoto and Ishikawa 2018). Indeed, there was no visible frass-ejection hole on the upper parts of the studied trees from the ground. In Japan, there are no other wood boring insects with similar signs. When more than 5 frass-ejection holes were present on a tree, however, it was difficult to identify and count frass-ejection holes accurately, because a large amount of frass was present on the tree surface. Thus, the number of holes > 5 was treated as 5. In this study, number of *A. bungii* eggs or hatchlings on the tree trunk was not used as the measure of *A. bungii* density, because the field surveys were conducted outside the *A. bungii* egg-laying period. Besides, even during the *A. bungii* reproductive period, it is hard to detect tiny *A. bungii* eggs in bark crevices and estimate the density accurately in a non-destructive way.

## Statistical analysis

Interspecific differences in the behavioral responses of ants to *A. bungii* eggs and hatchlings during the 30-min observations of laboratory predation tests were examined. ANOVAs were performed to test differences in contact frequency among ant species. Secondly and more importantly, two-way repeated ANOVAs were performed to test differences in predatory behavior among ant species. In these ANOVAs, the dependent variable was the rank of each response, and factors were ant species and the number of previous ant-egg (or ant-hatchling) contacts (i.e., ant colony's experience on eggs or hatchlings). If significant differences were detected in the behavioral responses among ant species in these ANOVAs, Games–Howell post hoc tests were performed to clarify which combinations of ant species were different. To test differences in the proportion of damaged eggs among ant species, a Kruskal–Wallis test was performed. Steel–Dwass tests were then performed to clarify which combinations of ant species were different.

To examine the relationship between *A. bungii* and ant densities measured in the field surveys, Spearman's rank correlations were tested for each site between the number of frass-ejection holes of *A. bungii* and the mean number of ant individuals (sum of all ant species) per bait tube on each tree.

All of the statistical analyses in this study were performed using R Version 3.6.0 (R Core Team 2019). *p* values of ≤0.05 were considered statistically significant.

## Results

### Ant predation tests on *A. bungii* eggs in the laboratory

There was at least 1 contact between ants and eggs in 18 of the 20 trials during the 30-min observations. In 14 trials, there were more than 10 contacts. The frequency of contact was not significantly different among ant species ($F_{3, 16} = 1.99$; $p = 0.16$; Table 1).

Ranks of behavioral response to eggs were significantly different among ant species ($F_{3, 931} = 22.29$; $p < 0.001$), although the effect of previous contacts on the behavioral ranks was not detected ($F_{1, 931} = 0.11$; $p = 0.73$). The observed ranks were higher in the order of *C. matsumurai*, *L. japonicus*, *F. japonica*, and *C. quadrinotatus* ($p < 0.01$ for

**Table 1** Behavioral response of ants to *Aromia bungii* eggs during the first 30-min observations of predation tests

| Ant species | Mean±SD number of contacts | Mean±SD rank occurrence (%)[a] | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| *Formica japonica* | 97±52 | 80±17 | 17±12 | 1.8±2.1 | 1.6±3.6 |
| *Lasius japonicus* | 24±27 | 68±20 | 19±14 | 13±13 | 0 |
| *Camponotus quadrinotatus* | 45±92 | 81±27 | 18±27 | 0.2±0.3 | 0 |
| *Crematogaster matsumurai* | 23±14 | 45±36 | 43±33 | 8.7±12 | 3.3±3.5 |

[a]Behavioral ranks: 0=ignoring; 1=examine with antenna; 2=opening mandibles and preparing to attack; 3=biting and attacking

every pair of ant species). Biting eggs (rank 3) was observed only in *C. matsumurai* and *F. japonica*.

*Formica japonica*, *C. quadrinotatus*, and *C. matsumurai* damaged or transported eggs within 2 days after provision of the eggs (Table 2). The proportions of damaged eggs on the second day were different among ant species ($\chi_3^2 = 10.12$, $p = 0.018$): *F. japonica* and *C. matsumurai* significantly damaged more eggs than *L. japonicus* did. *Formica japonica* and *C. quadrinotatus* took away > 40% of the eggs from filter papers, whereas smaller ant species, *C. matsumurai*, rarely did so (4%). Comparing 2 days after with 1 day after, the proportion of damaged eggs increased by 5 times in *C. quadrinotatus*, although damaged eggs did not increase much in *F. japonica* and *C. matsumurai*. Most of the eggs damaged by *F. japonica* were broken (Fig. 1). Eggs damaged by *C. matsumurai* had visible wounds and *C. matsumurai* workers were sucking the inner liquid from those wounds.

## Ant predation tests on *A. bungii* hatchlings in the laboratory

There were at least four contacts between ants and *A. bungii* hatchlings during the 30-min observations in all trials.

**Table 2** Fate of *Aromia bungii* eggs after 1 and 2 days of exposure to ants

| Ant species | 1 day | | 2 day | |
|---|---|---|---|---|
| | Damaged[a] | Removed[b] | Damaged | Removed |
| *Formica japonica* | 84 ± 36 | 80 ± 45 | 84 ± 36 | 80 ± 45 |
| *Lasius japonicus* | 0 | 0 | 0 | 0 |
| *Camponotus quadrinotatus* | 12 ± 27 | 4.0 ± 8.9 | 60 ± 55 | 44 ± 46 |
| *Crematogaster matsumurai* | 56 ± 17 | 0 | 76 ± 22 | 4.0 ± 8.9 |

[a]Mean ± SD percentage of eggs damaged

[b]Mean ± SD percentage of eggs removed from the deposited substance (filter paper). All of the eggs removed were destroyed

However, contacts varied among ant species ($F_{3, 16} = 7.64$; $p = 0.0022$; Table 3), and *F. japonica* had the most frequent contact with the hatchlings due to its large body size and activity levels.

Ranks of behavioral response to *A. bungii* hatchlings were different among ant species ($F_{3, 589} = 31.09$, $p < 0.001$), although effect of previous contacts on the behavioral ranks and interaction between ant species and previous contacts was not detected (previous contacts: $F_{1, 589} = 0.85$, $p = 0.36$; interaction: $F_{3, 589} = 1.24$, $p = 0.29$). The observed ranks were higher in the order of *C. matsumurai*, *L. japonicus*, *C. quadrinotatus*, and *F. japonica* ($p < 0.05$ for every pair of ant species). Carrying away hatchlings (rank 4) was observed in *F. japonica* (20% of 5 trials) and *C. matsumurai* (60%). Biting hatchlings (rank 3) was observed in *L. japonicus* (60% of 5 trials) and *C. matsumurai* (100%). All of the bitten hatchlings died by the next day, whereas the other hatchlings survived.

## Field survey

Frass ejection by *A. bungii* was confirmed in 72%, 35%, and 81% of the studied cherry trees in Osaka, Tokyo, and Gunma sites, respectively (Table 4). Five or more frass-ejection holes were observed on some of the trees. Meanwhile, ants were collected on 50%, 65%, and 48% of the studied trees in Osaka, Tokyo, and Gunma sites, respectively. Five or six ant species were collected in each site. They were all locally common, omnivorous species (Terayama et al. 2014). Both of tree-nesting species and ground-nesting species were collected in each site. The species composition was different among sites. *Camponotus vitiosus* and *C. matsumurai* were dominant in the Osaka site. Tokyo site included 3 species used in the laboratory predation tests (*F. japonica*, *C. matsumurai*, and *C. quadrinotatus*), and a closely related species (*Lasius sakagamii*). In this site, *F. japonica* and *L. sakagamii* were most frequently collected. *Temnothorax congruus* and *Monomorium intrudens* were dominant in Gunma site. The numbers of collected ants varied among sites and trees.

**Table 3** Behavioral response of ants to *Aromia bungii* hatchlings and fate of the hatchlings

| Ant species | Number of contacts[a] | Rank occurrence (%)[b] | | | | | Mortality (%)[c] |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | |
| *Formica japonica* | 67 ± 37 | 96 ± 5.8 | 3.0 ± 3.5 | 0.4 ± 0.8 | 0 | 0.7 ± 1.6 | 20 |
| *Lasius japonicus* | 18 ± 8.5 | 49 ± 31 | 23 ± 20 | 17 ± 8.3 | 12 ± 14 | 0 | 60 |
| *Camponotus quadrinotatus* | 11 ± 6.3 | 63 ± 38 | 10 ± 8.3 | 27 ± 42 | 0 | 0 | 0 |
| *Crematogaster matsumurai* | 24 ± 13 | 4.3 ± 4.9 | 37 ± 17 | 33 ± 12 | 22 ± 11 | 4.4 ± 5.5 | 100 |

[a]Mean ± SD number of ant-hatchling contacts during the first 30-min observations

[b]Mean ± SD frequency of the following behavioral ranks during the 30-min observations: 0 = ignoring; 1 = examine with antenna; 2 = opening mandibles and preparing to attack; 3 = biting and attacking; 4 = carry away

[c]Mortality of the hatchlings 1 day after the interactions

**Table 4** Results of *Aromia bungii* frass-ejection hole counts and ant sampling in three field study sites

| Site | Proportion of trees (%) | | Mean ± SD number of ants per tree | Frequency of ant species (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Frass present (≥ 5 frass[a]) | Ant collected | | Arboreal[b] | | | | | Arboreal/ground[c] | | | | | Ground[d] | |
| | | | | Cq | Cv | Cm | Ct | Tc | Ls | Og | Mi | Mc | Pf | Fj | Tt |
| Osaka | 72 (16) | 50 | 2.5 ± 6.6 | – | 20 | 18 | – | 8 | – | 12 | – | 6 | – | – | 4 |
| Tokyo | 35 (4) | 65 | 12 ± 41 | 10 | – | 10 | – | 4 | 22 | – | – | – | – | 37 | – |
| Gunma | 81 (24) | 48 | 33 ± 85 | – | – | – | 12 | 17 | 2 | – | 17 | – | 2 | – | – |

[a]≥ 5 frass-ejection holes of *A. bungii* were present

[b]Arboreal species nest in trees: *Cq* = *Camponotus quadrinotatus*; *Cv* = *Camponotus vitiosus*; *Cm* = *Crematogaster matsumurai*; *Ct* = *Cremato-gaster teranishii*; *Tc* = *Temnothorax congruous*

[c]Arboreal/ground species nest both in trees and underground: *Ls* = *Lasius sakagamii*; *Og* = *Ochetellus glaber*; *Mi* = *Monomorium intrudens*; *Mc* = *Monomorium chinense*; *Pf* = *Pheidole fervida*

[d]Ground species nest underground but can forage on trees: *Fj* = *Formica japonica*; *Tt* = *Tetramorium tsushimae*

A negative correlation between the numbers of frass-ejection holes and collected ant individuals was detected in the Osaka site ($r_s = -0.30$, $p = 0.03$) but not detected in the Tokyo and Gunma sites (Tokyo: $r_s = -0.05$, $p = 0.72$; Gunma: $r_s = -0.14$, $p = 0.36$) (Fig. 2). However, the number of collected ant individuals tended to be few on the trees with 5 or more frass-ejection holes in all sites.

## Discussion

In our laboratory experiments, all of the tested ant species showed predatory behaviors toward the *A. bungii* eggs or hatchlings. Some individuals of *F. japonica* and *C. matsumurai* attacked *A. bungii* eggs by biting during the first 30 min, although *F. japonica*, *L. japonicus*, and *C. quadrinotatus* frequently ignored the eggs. More explicit signs of predation such as egg destruction and consumption of egg content from the wounds, as well as removal of the eggs, were observed in *F. japonica*, *C. matsumurai*, and *C. quadrinotatus* within 2 days. *Formica japonica*, *C. matsumurai*, and *L. japonicus* fatally damaged *A. bungii* hatchlings during the 30-min observations. *Formica japonica* and *C. matsumurai* even transported the hunted hatchlings to the other members of the colony. These results showed that the ants recognized *A. bungii* eggs and hatchlings as prey.

The level of predatory behavior toward *A. bungii* differed among ant species and even differed within ant species depending on the *A. bungii* life stage (egg or hatchling). Differences among ant species in the preference for longhorn beetle eggs has also been previously reported (Muilenburg et al. 2008; Way et al. 1992). One of the most notable differences observed in this study was that *F. japonica* showed certain degrees of interest in *A. bungii* eggs in ca. 20% of contacts (Table 1), but this species rarely showed interest in the hatchlings (Table 3). This result was surprising, because unlike eggs, hatchlings are active and soft like caterpillars,
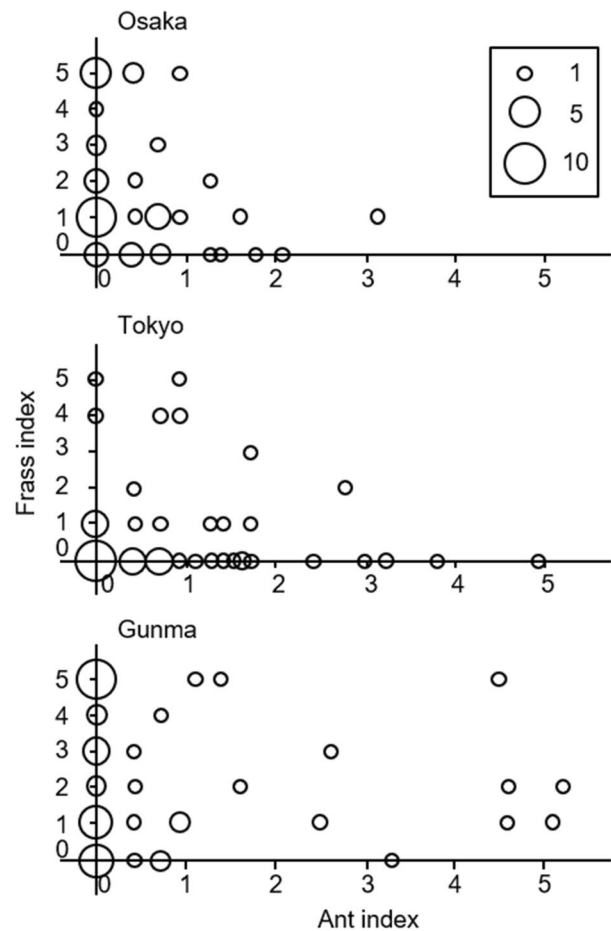


**Fig. 2** Relationship between ant density and *Aromia bungii* density in the field. Ant index and *A. bungii* frass index of individual trees are plotted for three study sites. Ant index: logarithmic value calculated for number of ants per bait tube plus 1. Frass index: number of frass-ejection holes; > 5 holes are all treated as 5. The size of plots indicates the numbers of individual trees, as exemplified in the upper-right box

and thus we expected ants were more likely to recognize hatchlings as prey than eggs. To date, knowledge is scarce on the mechanism of prey recognition in ants, but differences in surface substances (e.g., hydrocarbons: Hayashi et al. 2015) or color between *A. bungii* eggs and hatchlings may be reasons for the observed difference in ant response.

In contrast to the pattern observed in *F. japonica*, *L. japonicus* attacked *A. bungii* hatchlings but not eggs (Table 1, 3). *Lasius japonicus* took ready-to-attack postures (opening mandibles) toward *A. bungii* eggs more frequently than the other ant species (rank 2 in Table 1). However, *L. japonicus* did not actually attack the eggs, and the eggs remained intact (Table 1, 2). In the context of territorial fight between ants, the behavior of opening mandibles is considered a menacing behavior exhibited as the preliminary step of biting (e.g., Roulston et al. 2003). *Lasius japonicus* may have recognized and menaced *A. bungii* eggs as potential enemies rather than as prey. *Lasius japonicus* did not further attack the eggs as enemies, probably because the eggs were immobile and did not stimulate *L. japonicus*. The behavior of opening mandibles may not be related to predation.

In this study, predation tests were conducted with worker ants only, but not with whole colonies. Worker ants cannot ingest solid prey body or feed it to the larvae, and the latter can digest the prey body and can feed it back to the rest of the colony including queens and workers (Hölldobler and Wilson 1990). Experiments using whole colonies might induce more predatory behaviors than experiments with workers only, as the workers would be more likely to prey on *A. bungii* to bring back to the nest.

In our field survey, ant density negatively correlated with *A. bungii* density on tree trunks in Osaka site. In addition, ant density tended to be low on the trees with the highest level of *A. bungii* density in all sites. On the tree trunks with lower ant density, *A. bungii* reproductive success may have been higher because *A. bungii* received less ant predation on eggs and hatchlings. In addition to predation, ants might reduce the number of *A. bungii* eggs also by annoying and repelling *A. bungii* female adults from trees. To verify negative effects of ants on *A. bungii* density, following issues should be addressed. Firstly, ants collected in the study sites included species which were not used in the laboratory predation tests. It is not known whether these species prey on *A. bungii* eggs and hatchlings. Secondly, our field surveys were conducted outside the *A. bungii* egg-laying period. It is not clear if the number of frass-ejection holes correlates well with the number of hatchlings which initially bored into the tree. Factors such as host plant resistance may affect survival rate of *A. bungii* larvae in the tree. Thirdly, the relationship between ant and *A. bungii* density might have been correlative. Tree characteristics such as size and surface texture might influence both egg-laying site choice by *A. bungii* females and nesting/foraging site choice by

ants. Laboratory bioassays allowing *A. bungii* females to lay eggs on host plant logs and then providing ant colonies with the logs would give further insight into the interactions between ants and *A. bungii* under field condition.

In conclusion, this study showed that Japanese native ants recognize and attack eggs and hatchlings of invasive *A. bungii* as prey. In general, eggs were not very attractive to the ants and required some time to be preyed on, but it takes 1–2 weeks for *A. bungii* eggs to hatch (Iwata 2018). Some ant species attacked *A. bungii* hatchlings soon after contact. Thus, predation on *A. bungii* eggs and hatchlings by Japanese native ants is expected to occur in the field. Though weak, a negative correlation was detected between *A. bungii* and ant densities on tree trunks in our field survey. Therefore, these results suggested that native ant fauna can play at least a minor role in biotic resistance to *A. bungii*. Further field and laboratory studies are necessary to evaluate the level of ant predation and any other negative effect on *A. bungii* in the field. Approaches to enhance the level of biotic resistance of Japanese native ants should be also explored.

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