ASIAN MYRMECOLOGY Volume 8, 1 – 6, 2016
ISSN 1985-1944 © Fuminori Ito, Kentaro Taniguchi and Johan Billen

DOI: 10.20362/am.008014
Published online ahead of print

Defensive function of petiole spines in queens and workers of the formicine ant *Polyrhachis lamellidens* (Hymenoptera: Formicidae) against an ant predator, the Japanese tree frog *Hyla japonica*

Fuminori Ito[1,4], Kentaro Taniguchi[1,2] and Johan Billen[3]

[1]Laboratory of Entomology, Faculty of Agriculture, Kagawa University, Ikenobe, Miki 761-0795, Japan.
[2]Present address, Kurata, Okayama 703-8265, Japan.
[3]Zoological Institute, KU Leuven, Naamsestraat 59, box 2466, 3000 Leuven, Belgium.

[4]Corresponding author's email: ito@ag.kagawa-u.ac.jp

ABSTRACT. The defensive function of petiole spines in queens and workers of the formicine ant *Polyrhachis lamellidens* was investigated using the ant predating tree frog *Hyla japonica.* Ant workers have hook-like large spines on their petiole while the queen petiole has only small slightly curved spines. Intact workers of *P. lamellidens* are unpalatable while workers without spines and intact queens are palatable, indicating that the spines of workers provide an effective defense against the tree frogs. Caste differences of spine morphology therefore affect the defense potential of the ants.

Keywords: ants, defense, predation, spines, treefrogs

INTRODUCTION

Ants are social insects that live together with nestmates, the majority of which are wingless workers. Such insects may form an easy prey for insectivorous animals, and therefore must have an efficient defense system (Redford 1987, Peeters & Ito 2015). An effective defense system might contribute to the current success of ants in the terrestrial ecosystem (Buschinger & Maschwitz 1984, Peeters & Ito 2015). However, investigations on the defense of ants by using ant predators have been rarely carried out to date, except for our studies using tree frogs and chicks (Ito et al. 2004, Taniguchi et al. 2005a).

The formicine genus *Polyrhachis* is one of the biggest ant genera (Wilson 1976), including 697 species (Bolton 2015). Many species of *Polyrhachis* are characterized by large spines on the petiole and/or alitrunk (Dorow 1995). Spines are one of the typical defense devices in animals and plants (e.g. Mikolajewski & Rolff 2004, Inbar & Lev-Yadum 2005, Hanley et al. 2007). Hook-like spines on the petiole of the workers in some subgenera of *Polyrhachis* seem to be a very powerful defense apparatus (Fig. 1). The function of the spines in *Polyrhachis* is supposed to protect them against vertebrate predators (Buschinger & Maschwitz 1984), however, no experimental evidence exists so far. Such remarkable spines may function as a visual signal against predators. As shown in aquatic firefly larvae, a conspicuous visual signal in conjunction with deterring substances functions as an effective multimodal aposematic anti-predator defense (Fu et al. 2007). Interestingly, such large spines were found in workers only whereas the queen's petiole has only small spines (Kohout 2014).

We compared the defensive function of the spines of *P. lamellidens* Fr. Smith between queens and workers, by using the Japanese tree frog, *Hyla japonica* (Günther), which is a common generalist predator occurring in western Japan (Hirai & Matsui 2000), and which feeds on many species of ants (Taniguchi et al. 2005a,b, Ito et al. 2009). First, we compared the petiolar spines between queens and workers. The fine structure of the spine surface was also observed by SEM to check whether there are openings for injecting eventual chemical substances from the spine. Then, we investigated whether the frogs are able to feed on *P. lamellidens* workers and queens. To verify the defensive function of spines of workers, we offered ablated workers without spines to the frogs. Furthermore, to check whether spines of workers function as a visual signal for predators, we offered workers without spines to frogs that had previously experienced workers with spines.

MATERIALS AND METHODS

Ants

Polyrhachis lamellidens is an uncommon ant in western Japan. Colonies nest in large holes of standing trees in woodland. Recently this species was assigned as an endangered species by the Japan Ministry of the Environment (2014). The nuptial flight occurs in October to November. Mated dealated queens thereafter enter the nest of *Camponotus japonicus* or *C. obscuripes* (Kohriba 1963, Furukawa et al. 2013) or hibernate in dead wood, and enter the host ant nest the next spring. The founding queens show temporary social parasitism (Kohriba 1963): the *P. lamellidens* queen kills a host queen, and subsequently the parasite workers replace the host workers. Colony size is relatively large, reaching more than 10,000 workers.

Morphology of petiole spines

The petiole was removed from 10 workers and 10 queens, and its width and height were measured using Motic Images Plus 2.1 after digital photography. The Welch-test in the statistical Package R v3.0.2 (R Development Core Team 2013) was used for statistical analysis of average petiole size between queens and workers, because the test for homogeneity of variance can be omitted. A worker petiole for scanning microscopy was detached from the body, gold coated in a Bal-Tec Sputter Coater SCD 050 and examined in a JEOL JSM-6360 scanning electron microscope.

Behavior of frogs against ants

We collected in total 105 mature frogs (length of snout tip to cloacal opening > 3.00 cm) in and around rice fields in Miki-cho, Kagawa Prefecture, western Japan, from April to August. They were kept in the laboratory in glass containers. Mealworms were given as prey. Before the experiment, a frog was transferred into a small cylindrical container (ø 120 mm x height 75 mm). The bottom of the container was covered with plaster to provide humidity. Each frog was subjected to food deprivation during three days prior to the experiment. A colony fragment of *P. lamellidens* including workers, alate queens and broods was kept in a large polypropylene container in the laboratory.

We offered an intact worker, a worker with petiole spines experimentally ablated (Fig. 1C), or a queen to a frog in the cylindrical container. The number of frogs examined in this experiment was 60 frogs for intact workers, 30 frogs for workers without spines, and 15 frogs for queens. All frogs were used for this experiment only once. Just before each experiment, the petiole spines of workers were cut off with clippers, while the wings of alate queens were removed with forceps. According to former reports on frog predation behavior by using tree frogs (Taniguchi et al., 2005a) and toads (Brower et al., 1960), frog behavior against prey animals can be divided into the following three categories: ignore, attack but not eating, and attack followed by eating. In our study, the second category was further divided: prey is rejected just after the frog touched it, or is spit out after the frog had put it in the mouth. For each frog, feeding behavior was recorded as follows: (1) The frog attacked or ignored the ant. If a frog ignored the ant during 10 min, we stopped the experiment. (2) If the frog attacked the ant, we recorded whether the ant was taken in the mouth or whether the ant was rejected just after the frog touched it. (3) For the frogs that took the ant in their mouth, we recorded whether they fed on the ant or whether they spit it out. Furthermore, to check whether frogs consider the spines as a visual signal, we offered a worker with or without spines to frogs (both N = 10), that

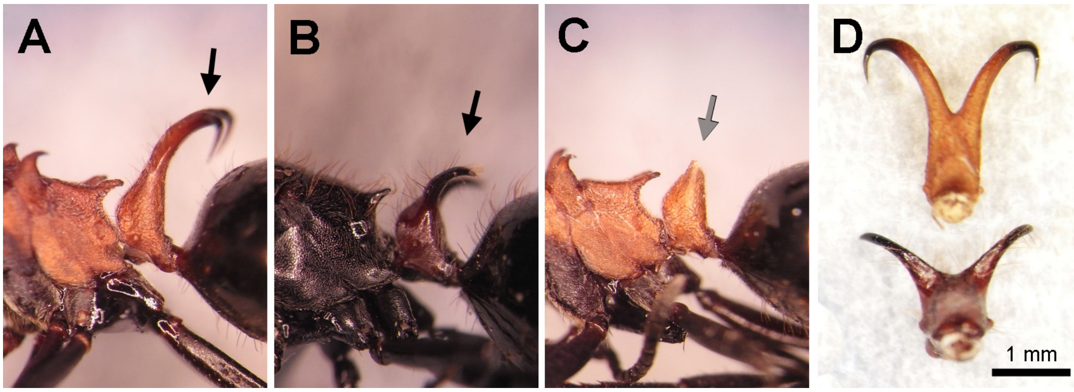

Fig. 1. Petiole profiles of intact worker (A), queen (B), and ablated worker (C), and frontal view of petiole of worker (D top) and queen (D bottom) of *Polyrhachis lamellidens*. Black arrows indicate petiolar spine, grey arrow indicates ablated spine of worker petiole.

had experienced an ant with spines and spit it out the day before. For this experiment, we recorded whether a frog attacked or not. When the frogs ignored or refused ants, we gave them a mealworm or a small cockroach to check whether they were hungry or not. Frog behavior was analyzed by a contingency table test as in Brower et al. (1960) and Taniguchi et al. (2006). For comparisons of the behavior against each type of ants, a pairwise comparison with a Fisher exact test in R v3.0.2 (R Development Core Team 2013) was used.

RESULTS

Morphology of spines

The morphological characteristics of the petiole are markedly different between workers and queens (Fig. 1). Workers have a pair of large hook-like spines whereas the queen's petiole has a pair of short slightly curved spines. Spine width of workers is slightly wider than that of queens ($t = -3.2$, $df = 13.9$, $P = 0.006$), but the height is remarkably different between the two castes ($t = -19.3$, $df = 15.2$, $P < 0.001$). SEM observation of the hook of the worker's petiolar spine (Fig. 2) shows that there are many pores with a diameter around 1 μm on its surface. The tip of the hook of the worker's spine has no such pores, and there were no major openings for emitting eventual chemical substances.

Behavior of frogs against ants

In total, 75 of 105 frogs that had no prior experience with ants in the laboratory attacked the ant (Table 1). The proportion of frogs that ignored ants was not statistically different among intact workers, mutilated workers, and queens. All 30 frogs that ignored ants fed on mealworms or cockroaches, indicating that the frogs avoid the ants as prey. Among the 75 frogs that attacked the ant, 15 frogs that attacked an intact worker and 8 frogs that attacked an ablated worker refused the ant: they stopped attacking after their tongue touched the ant. These frogs fed on mealworms or cockroaches just after the experiment, indicating that they were hungry but chemical substances or physical properties of the cuticular surface including spines have some defensive function. The proportion of refusing frogs was not statistically different with respect to their refusing of intact workers, ablated workers, and intact queens. The remaining 52 frogs took ants in their mouth (Table 1). The majority of frogs (27 out of 30) that put an intact worker into the mouth spat it out. When the frogs took an intact worker, the spines often stuck in their mouth. Four frogs spent time and effort to remove the ant from their mouth. All ants vomited by the frogs died. In contrast, all but one frog fed on an ablated worker ($N = 14$). All frogs that took an intact queen in their mouth ($N = 8$) ate it without vomiting. The feeding ratio of the intact workers was significantly lower than that of both the ablated workers and queens (pairwise comparison with a Fisher test,

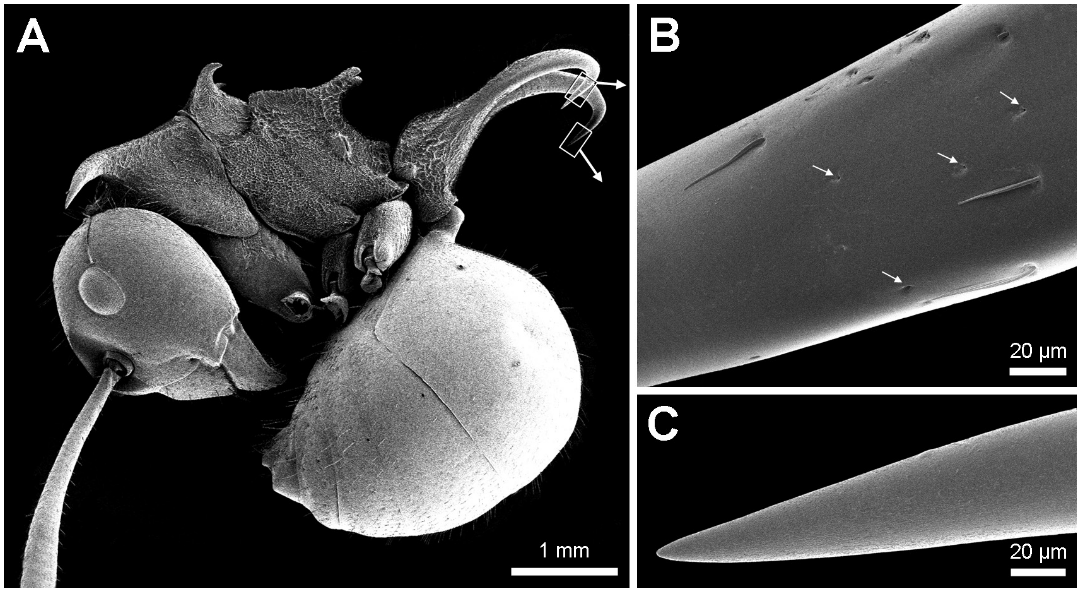

Fig. 2. SEM appearance of worker petiole in *Polyrhachis lamellidens*. Small arrows in B indicate pore openings of subepithelial gland ducts.

both P < 0.001), indicating that the spines of *P. lamellidens* workers do function as an effective defense against the predators.

Among 20 frogs that spat out the intact worker in the first experiment mentioned above (Table 1), seven of 10 frogs who were offered the intact workers, and nine of 10 frogs who were offered ablated workers ignored the ant (lower part of Table 1). The behavioral response by frogs who experienced intact workers previously was significantly different from the frogs before the experience (against intact workers, P = 0.04, against ablated workers, P = 0.0016), indicating that the frogs learned to recognize unpalatable prey. In this experiment, both ablated ants and intact ants were ignored in a similar ratio, indicating that the spines have no function as signals of bad prey for frogs.

DISCUSSION

The huge spines on the petiole of *P. lamellidens* workers have a strong defensive effect against tree frogs, and that the frogs can learn to recognize unpalatable prey. To our knowledge, this is the first experimental evidence on the defensive function of ant spines against predators. Defensive spines in insects sometimes can introduce toxins into other animals, e.g. larvae of Limacodidae (Rothschild et al. 1970, Murphy et al. 2010). SEM observation of the petiole indicated that there are many pores, which probably correspond with the openings of subepithelial glands. These glands are distributed over the whole body in several ant species (Gobin et al. 2003). It is unlikely that these tiny pores on the surface of the petiole spines of *P. lamellidens* emit toxic substances for defense, as they represent single cell openings that cannot emit sufficiently large amounts of substance. Although we do not yet know the function of these glands, the defensive function of the spines seems based on their mere physical characteristics.

Spines of queens are small and have no defensive effect against the tree frogs. Huge spines seem to be a hindrance for flying: generally ant queens have smaller spines if compared to conspecific workers (Kohout 2014, Peeters & Ito 2015). Furthermore, activity outside nests by queens is generally limited to just before and after the nuptial flight. Thus, such strong defensive apparatus against predators is not important in comparison to workers.

Table 1. Behavioral responses of the Japanese tree frogs against queens and two types of *Polyrhachis lamellidens* workers. The different letters in each column refer to a significant difference (pairwise comparison with a Fisher exact test).

				from attacked ants:			from ants that were put in mouth:		
	Ignored	Attacked	Total	Refused	Put in mouth	Total	Fed on	Spit	Total
Frogs without experience:									
intact workers	15	45	60[a]	15	30	45[a]	3	27	30[a]
ablated workers	8	22	30[a,c]	8	14	22[a]	13	1	14[b]
queens	7	8	15[a,b]	0	8	8[a]	8	0	8[b]
Total	30	75	105	23	52	75	24	28	52
Frogs that spit out intact workers:									
intact workers	7	3	10[b,c]						
ablated workers	9	1	10[b]						
Total	16	4	20						

Our observations indicate that the tree frogs learn to recognize the unsuitable prey and avoid it as shown by Taniguchi et al. (2005a). However, the frogs that experienced the intact worker subsequently ignored not only intact workers but also the ablated workers (see lower part of Table 1), which indicates that the ant spines alone do not seem sufficient for the frogs to recognize unpalatable prey. How the tree frogs recognize such unpalatable prey is still unknown and will be studied in a future project.

The occurrence of such spines in *Polyrhachis* might contribute to the current prevalence of this genus. The defense function may be especially important for arboreal life where many vertebrate predators like tree lizards and birds forage. In contrast, workers of *Tetramorium tsushimae* Emery, *Crematogaster osakensis* Forel, and *Pheidole fervida* Fr. Smith which have small propodeal spines, are frequently eaten by tree frogs (Taniguchi et al. 2005b, Ito et al. 2009). At least against tree frogs, small spines are not functional as a defensive apparatus, though it is possible that small spines are effective against the other predators.

ACKNOWLEDGMENTS

We thank H. Izawa and H. Tanaka for their preliminary experiments, and anonymous referees for comments on an earlier version of the manuscript. This research was supported by grants for scientific research from JSPS [Nos. 14405036(B) and 18570022 (C)].

REFERENCES

Bolton B, 2015. AntCat. An online catalog of the ants of the world. Http//www.antcat.org.

Brower LP, Brower JVZ and Westcott PW, 1960. Experimental studies of mimicry. 5. The reactions of toads (*Bufo terrestris*) to bumblebees (*Bombus americanorum*) and their robberfly mimics (*Mallophora bomboides*), with a discussion of aggressive mimicry. *The American Naturalist* 94:343 – 355.

Buschinger A and Maschwitz U, 1984. Defensive behavior and defensive mechanisms in ants. In: *Defensive Mechanisms in Social Insects* (H.R. Hermann, Ed), Praeger Scientific, New York, pp 95 – 150

Dorow WHO, 1995. Revision of the ant genus *Polyrhachis* Smith, 1857 (Hymenoptera: Formicidae: Formicinae) on subgenus level with keys, checklist of species and Bibliography. *Courier Forschungsinstitut Senckenberg* 185: 1 – 113.

Furukawa K, Ohbe T and Ito F, 2012. Collection of a *Polyrhachis lamellidens* queen from a nest of *Camponotus obscuripes* (Hymenoptera: Formicidae). *Kagawa Seibutsu* 39: 61 – 62. In Japanese with English abstract.

Gobin B, Ito F and Billen J, 2003. The subepithelial gland in ants: a novel exocrine gland closely associated with the cuticle surface. *Acta Zoologica* 84: 285 – 291

Hanley ME, Lamont BB, Fairbanks MM and Rafferty CM, 2007. Plant structural traits and their role in anti-herbivore defence. *Perspectives of Plant Ecology Evolution and Systematics* 8*:* 157–178.

Hirai T and Matsui M, 2000. Feeding habits of the Japanese treefrog, *Hyla japonica*, in the reproductive season. *Zoological Science* 17: 977 – 982

Hölldobler B and Wilson EO, 1990. *The Ants*. Harvard University Press, Cambridge, MA. 732 pp

Inbar M and Lev-Yadun M, 2005. Conspicuous and aposematic spines in the animal kingdom. *Naturwissenschaften* 92: 170 – 172.

Ito F, Hashim R, Yek SH, Kaufmann E, Akino T and Billen J, 2004. Spectacular Batesian mimicry in ants. *Naturwissenschaften* 91: 481 – 484

Ito F, Okaue M and Ichikawa T, 2009. 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.

Kohout R, 2014. A review of the subgenus *Polyrhachis (Polyrhachis)* Fr. Smith (Hymenoptera: Formicidae: Formicinae) with keys and description of a new species. *Asian Myrmecology* 6: 1 – 31.

Kohriba O, 1963. A parasitic life of *Polyrhachis lamellidens* F. Smith (Hymenoptera, Formicidae), first report. *Kontyu* 31: 200 – 209. In Japanese with English summary.

Mikolajewski DJ and Rolff J, 2004. Benefits of morphological defence demonstrated by direct manipulation in larval dragonflies. *Evolutionary Ecology Research* 6: 619 – 626.

Murphy SM, Leahy SM, Williams LS and Lill JT, 2010. Stinging spines protect slug caterpillars (Limacodidae) from multiple generalist predators *Behavioral Ecology* 21: 153 – 160.

Peeters C and Ito F, 2015. Wingless and dwarf workers underlie the ecological success of ants (Hymenoptera, Formicidae). *Myrmecological News* 21: 117 – 130.

R Development Core Team, 2013. The statistical Package R v3.0.2.

Redford KH, 1987. Ants and termites as food. Patterns of mammalian myrmecophagy. In: Current Mammalogy (Genoways HH, ed), Springer, pp349 – 399.

Rothschild M, Reichstein T, von Euw J, Alpin R and Harman RRM, 1970. Toxic Lepidoptera. *Toxicon* 8: 293 – 296.

Taniguchi K, Maruyama M, Ichikawa T and Ito F, 2005a. A case of Batesian mimicry between myrmecophilous staphylinid beetle, *Pella comes*, and its host ant, *Lasius (Dendrolasius) spathepus*: an experiment using the Japanese treefrog *Hyla japonica* as a real predator. *Insectes Sociaux* 52:320 – 322.

Taniguchi K, Tanaka H and Ito F, 2005b. Ants as prey of the Japanese treefrog *Hyla japonica* in an urban area of western Japan. *Sociobiology* 45: 495 – 502.

Wilson EO, 1976. Which are the most prevalent ant genera? *Studia Entomologia* 19: 187-200.