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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)

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Abstract

Prey composition of the Japanese treefrog, *Hyla japonica* GÜNTHER, 1859, was investigated in an area invaded by the Argentine ant, *Linepithema humile* (MAYR, 1868). Ants were the most common taxa among the prey animals in young treefrogs, constituting 82.4% of the numeric proportion and 49.9% of the volumetric proportion of all prey items in the frog stomachs. Among ants, Argentine ants were most abundant; 333 workers (52.2% of all ants) were found in 36 frogs (85.7% of frogs examined), indicating that Argentine ants are palatable to the treefrogs.

Key words: Argentine ant, *Linepithema humile*, treefrog, *Hyla japonica*, predation, Japan.

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Introduction

The Argentine ant, *Linepithema humile* (MAYR, 1868), is an invasive tramp species damaging biological communities in invaded areas (MAJER 1994, PASSERA 1994, HOLWAY & al. 2002). In areas invaded by Argentine ants, many native ants are displaced. Furthermore, the change of the ant fauna affects the distribution of plants and ant-eating vertebrates (CHRISTIAN 2001, HOLWAY & al. 2002). SUAREZ and co-workers (2000, 2002) reported that populations of horned lizards *Phrynosoma coronatum* (HARLAN, 1825) in California are declining due to the invasion of Argentine ants because their main prey, harvester ants (*Messor* spp. and *Pogonomyrmex* spp.), are eliminated by Argentine ants. Investigations on such bottom-up effects of the invasive ants are important to understand the effects of invasive ants on biological communities. However, detailed studies are limited to these coastal lizards.

Argentine ants have invaded Hatsukaichi City, Hiroshima Prefecture, at least since 1993, and they are now widespread in and around this city (SUGIYAMA 2000, MIYAKE & al. 2002, TOUYAMA & al. 2003, OKAUE & al. 2007). Vertebrates specialized on ants in this area are not known; however, some species of Japanese frogs eat ants as prey (e.g., HIRAI & MATSUI 1999, 2000a, b, c, TANIGUCHI & al. 2005a, b). As part of our comprehensive research on the effects of Argentine ants on Japanese biodiversity, we investigated prey composition of the Japanese treefrog, *Hyla japonica* GÜNTHER, 1859, in an area invaded by Argentine ants. Predation on ants by frogs is well documented by examination of stomach contents of several frog species, but most reports identified the ants preyed upon only at family level (e.g., OPLINGER 1967, TOFT 1980, CALDWELL 1996, HIRAI & MATSUI, 1999, 2000a, b, c). Recently we reported on prey composition of treefrogs with species level identification of the ants in an urban area in western Japan, and found a possible prey preference to some ant species (TANIGUCHI & al. 2005a). In the present paper, we examine whether the treefrogs feed on Argentine ants by investigating the stomach and gut contents of the frog in an area invaded by Argentine ants.

Materials and methods

We investigated the diet of *Hyla japonica* in Hayatani-jinjya (shrine), Hatsukaichi City, Hiroshima Prefecture, western Japan. This shrine is surrounded by forests and paddy fields. In this shrine, Argentine ants had invaded but there were still many native ant species in 2001 (F. Ito, unpubl.). We do not know when Argentine ants invaded into this area. Treefrogs were collected in mid July 2001. These frogs were preserved in 80% ethanol, and brought back to the laboratory. Snout-vent length (SVL) of each frog was measured to the nearest 0.1 mm. They were then dissected to check their stomach and gut contents. Prey animals were identified to the order, but ants were identified to the genus or species level. Maximum length (L) and width (W) of each prey item were measured, and volumes of prey insects were calculated by using the formula for an ellipsoid as in other research of feeding habit of frogs and lizards (DUNHAM 1983): $V = 4 / 3 * \pi * (L / 2) * (W / 2)^2$. To examine prey composition, we used only data from stomach contents, because gut contents were usually completely de-

Tab. 1: Ant species found in the study site and the number of samples (No.) they were found in.

Ant species	No.
Linepithema humile (MAYR, 1868)	10
Paratrechina sakurae (ITO, 1914)	5
Formica japonica MOTSCHOULSKY, 1866	3
Camponotus japonicus MAYR, 1866	2
Camponotus vitiosus F. SMITH, 1874	2
Tetramorium tsushimae EMERY, 1925	2
Pristomyrmex punctatus (F. SMITH, 1860)	2
Monomorium chinense SANTSCHI, 1925	1
Monomorium intrudens F. SMITH, 1874	1

stroyed except for ants, of which the heads remained intact, allowing identification based on morphological characteristics. For analysis of predation on ants, we therefore used both stomach and gut contents.

The ant species composition in this site was assessed in September 2001 and 2002, as a part of an annual survey of the effects of Argentine ant invasion on native ants (see MIYAKE & al. 2002). Using an aspirator, we collected as many species of foraging ants as possible on the ground, grasses, and trees, during a five-minute interval. In one interval, we walked along the forest and the shrubbery approximately 30 to 50 m, and looked for ants. Sampling was repeated six times in each investigation (in total twelve sampling units).

Ants collected in the field and in the stomachs and guts of the frogs were identified according to taxonomic keys provided by the JAPANESE ANT DATABASE GROUP (2007). To show the body size of ants collected in the field and in the stomach and gut of frogs, we used the data of body length given by the JAPANESE ANT DATABASE GROUP (2007). Voucher specimens of both ants and frogs will be deposited in the Kagawa University Museum.

Results

We collected 10 species of ants in 12 time samples of a 5-minute collection (Tab. 1). The average number of ant species collected during a 5-minute collection was 2.3 ± 1.2 SD (range 1 - 4, N = 12). Argentine ants were the most common, found in 10 of 12 sampling units. All individuals (N = 42) of *Hyla japonica* collected in this survey were small, less than 2.0 cm SVL, and may have only recently undergone metamorphosis. All but three frogs contained 1 - 21 prey items in their stomach (average 5.8 ± 5.0 SD). Arachnida, Gastropoda, and 7 orders of Insecta were found (Tab. 2). Insects were the most common prey items, constituting more than 99% in number and 98% in volume. Ants were found in the stomachs of 34 frogs. In numerical composition, ants were the most abundant taxon among prey animals (82.4% of all prey items), followed by aphids

Tab. 2: Stomach contents of *Hyla japonica* collected in area invaded by Argentine ants. 244 prey items (183.8 mm^3) were found in 42 frogs. F, frequency of occurrence; N, numeric proportion; V, volumetric proportion.

	Frequency (%)		
	F	**N**	**V**
INSECTA			
Hymenoptera			
Formicidae	80.9	82.4	49.9
Non-Formicidae	14.3	2.8	13.2
Hemiptera			
Aphididae	33.3	6.7	2.5
Heteroptera	2.4	0.4	6.4
Homoptera	2.4	0.4	0.4
Diptera	19.0	3.9	1.4
Lepidoptera	9.5	1.7	12.0
Coleoptera	7.1	1.8	10.0
Dermaptera	2.4	0.4	0.3
Diplura	2.4	0.4	0.01
Neuroptera	2.4	0.4	1.63
ARACHNIDA			
Araneae	2.4	0.4	0.5
GASTROPODA	2.4	0.4	1.1

(6.7%) and Diptera (3.9%). In volumetric proportion, ant constituted 49.9% of the prey.

Ants were found in the stomach and / or gut in all 42 frogs (average number of ants per frog: 15.4 ± 12.3 SD). In all, 12 ant species were found inside frogs (Tab. 3): the Argentine ant, *Linepithema humile*, was the most abundant, 333 workers (51.6% of all ants) were found in 36 frogs (85.7% of frogs examined), followed by *Paratrechina flavipes* (F. SMITH, 1874) (14.4% of ants, 28.6% of frogs), *P. sakurae* (ITO, 1914) (12.1% of ants, 45% of frogs), *Pheidole fervida* F. SMITH, 1874 (8.8% of ants, 11.9% of frogs), and *Solenopsis japonica* W.M. WHEELER, 1928 (7.4% of ants, 40.5% of frogs). Of the 12 ant species found inside frogs, only 5 species were also collected in our 5-minute surveys, which makes for a total of 17 ant species sampled in the course of this study. The reasons of the difference of species composition between the field and the frog stomach are unknown; however, one of the possible reasons could be that frogs foraged not only in open land but also in the forest while we collected ants only in open

Tab. 3: Species composition of ants found in stomachs and guts in young *Hyla japonica* collected in area invaded by the Argentine ant. 645 ant workers from 42 frogs were examined. F, frequency of occurrence; N, numeric proportion.

Ant species	F	N
Linepithema humile (MAYR, 1868)	85.7	51.6
Paratrechina flavipes (F. SMITH, 1874)	28.6	14.4
Paratrechina sakurae (ITO, 1914)	45.2	12.1
Pheidole fervida F. SMITH, 1874	11.9	8.8
Solenopsis japonica WHEELER, 1928	40.5	7.4
Pyramica membranifera (EMERY, 1869)	19.0	2.6
Lasius japonicus SANTSCHI, 1941	9.5	1.4
Pristomyrmex punctatus F. SMITH, 1860	9.5	0.9
Temnothorax congruus F. SMITH, 1874	2.4	0.2
Strumigenys sp.	2.4	0.2
Crematogaster sp.	2.4	0.2
Monomorium intrudens F. SMITH, 1874	2.4	0.2

land and at forest edges. Furthermore, the body size of the ants seems to be one of the possible reasons (Fig. 1): large sized ants (*Formica japonica* MOTSCHOULSKY, 1866, *Camponotus japonicus* MAYR, 1866, and *Camponotus vitiosus* F. SMITH, 1874) were not eaten by the frogs.

The average number of Argentine ant workers in a frog was 9.3 ± 7.0 SD, with a maximum of 27 workers (Fig. 2). Some frogs also took more than 10 individuals of *P. flavipes, P. sakurae, Solenopsis japonica*, or *Pheidole fervida*. These results indicate that Argentine ants, as well as these native ants, are palatable to the treefrogs.

Discussion

We demonstrated that the majority of prey items in young treefrogs are ants, which is in line with our former report on an urban area of Takamatsu-shi, Shikoku Island, western Japan (TANIGUCHI & al. 2005a). Both volumetric and numeric proportions of ants in the young treefrogs are almost equal to ant specialist frogs like *Dendrobates* spp. and *Microhyla ornata* (DUMERIL & BIBON, 1841) (CALDWELL 1996, HIRAI & MATSUI 2000c). The survey of the ant fauna in our study site was not adequate to evaluate the relative abundance of each ant species. Therefore it is difficult to conclude about the ant species preference of *Hyla japonica*. In our laboratory experiments, if a treefrog took very unpalatable ants like *Lasius* (*Dendrolasius*) spp. and *Polyrhachis lamellidens* F. SMITH, 1874, which have very strong toxic substances and strong large spines, respectively, the treefrog usually stopped feeding on the unpalatable species after a few experiences (TANIGUCHI & al. 2005b; K. Taniguchi & F. Ito, unpubl.). In the case of *Polyrhachis lamellidens*, a large spine in the petiole is evidently an effective defence apparatus (K. Taniguchi & F. Ito, unpubl.). Dendrolasin and / or other chemicals produced

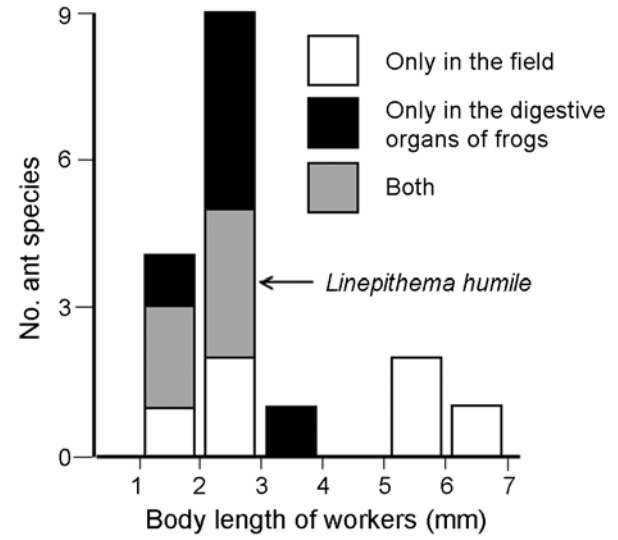

Fig. 1: Body length of ants collected in the field and in the stomachs and guts of Japanese treefrog, *Hyla japonica*, specimens.

in the mandibular glands of *Lasius* (*Dendrolasius*) spp. seem to be an effective defence against predators, although no direct experimental evidence has been provided. In the field collected treefrogs, the average number of Argentine ants was nine workers. The abundance of Argentine ants in the frogs' digestive system apparently indicates that the treefrogs continuously take the ants. Thus, Argentine ants are likely palatable for the treefrogs. In addition to Argentine ants, two species *of Paratrechina*, *Pheidole fervida* and *Solenopsis japonica* may also be palatable.

The species composition of ants in the diet of treefrogs in the area invaded by Argentine ants clearly reflected the effects of invasive ants on the Japanese native ants. In Kagawa Prefecture, where Argentine ants have not invaded, *Tetramorium tsushimae* EMERY, 1925, *Crematogaster osakensis* FOREL, 1900, and *Paratrechina flavipes* were common prey items for young treefrogs (TANIGUCHI & al. 2005a; K. Taniguchi & F. Ito, unpubl.). MIYAKE & al. (2002) reported that *T. tsushimae* suffered negative effects from the Argentine ant invasion. As a result, Argentine ants replaced these native ants in the prey spectrum of treefrogs in the invaded area. In contrast, *P. sakurae*, which can coexist with Argentine ants (MIYAKE & al. 2002), was a common prey in the invaded area while this ant species was not frequently found among the prey found in uninvaded areas (TANIGUCHI & al. 2005a; K. Taniguchi & F. Ito, unpubl.).

SUAREZ & al. (2000) reported that to coastal horned lizards Argentine ants may constitute suboptimal prey because of their small size, aggressive behavior, and defensive substances, which resulted in a remarkable depopulation of these lizards in the area invaded by Argentine ants. In the case of *Hyla japonica*, the effects of Argentine ants may not be that serious if compared to the case of the horned lizards, because Japanese treefrogs are generalist predators, attacking a range of animals with variable body size (HIRAI & MATSUI 2000a, and the present paper), and they do feed on multiple Argentine ant workers as shown in this study. Recently, GLENN & HOLWAY (2008) showed that pit-building ant lions *Myrmeleon* spp. fed on Argen-

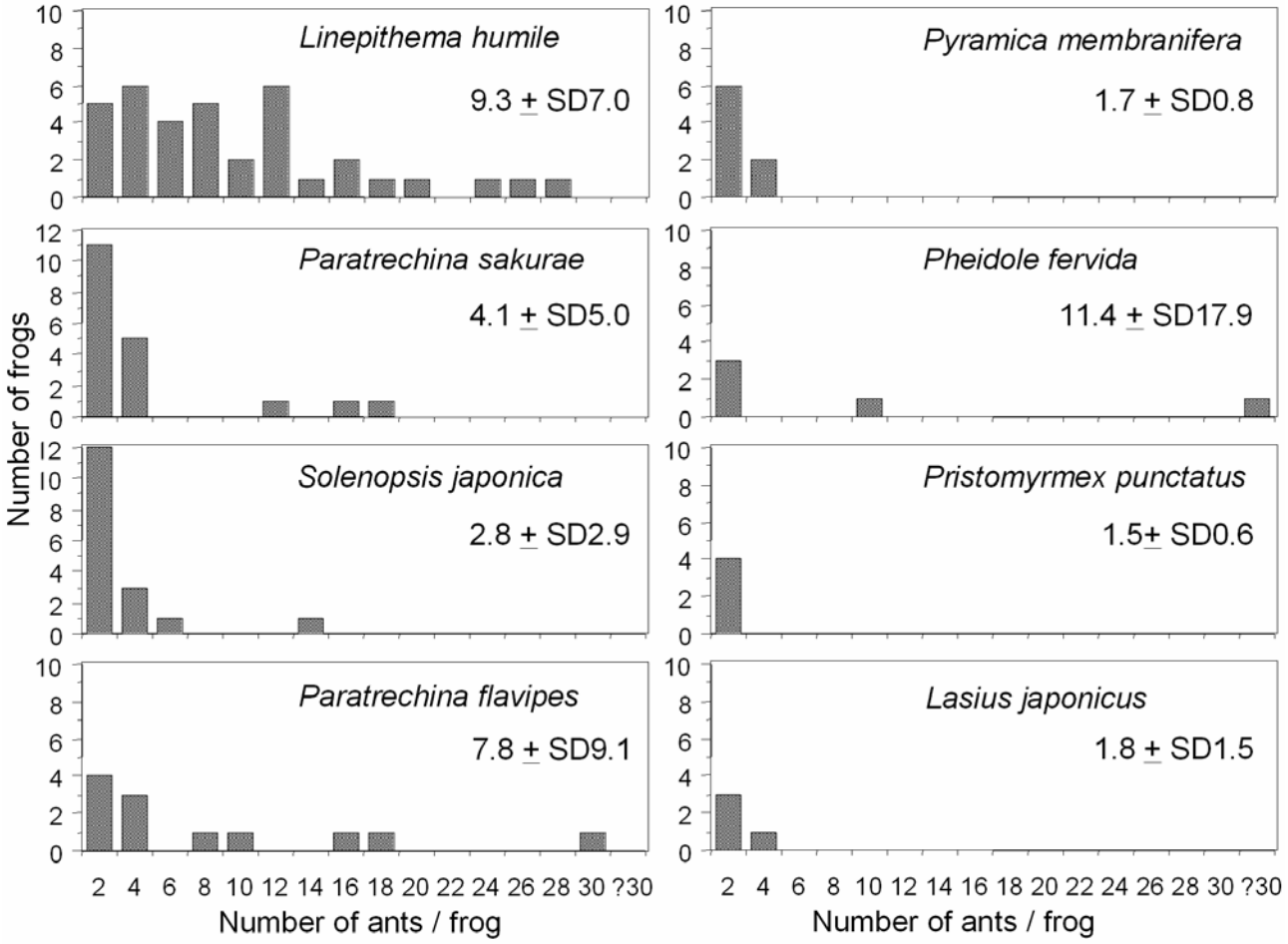

Fig. 2: Frequency distribution of ant numbers found in stomachs and guts of the Japanese treefrog, *Hyla japonica*. Average number of ants and SD per one frog are shown for those eight ant species that were found in more than one frog.

tine ants grew more quickly than those fed on native ants. TOUYAMA & al. (2008) reported that the density of the native myrmecophagic jumping spider *Siler cupreus* SIMON, 1889 increased in Argentine ant invaded areas. It seems that palatability of Argentine ants varies among predators. Whether the treefrogs indeed quantitatively prefer Argentine ants over other native ants still awaits closer scrutiny. To evaluate the effects of Argentine ants, also the nutritional value such as effects of the ants on the growth of frogs, and palatability compared to the various native ants species by species, as assessed in horned lizards by SUAREZ & al. (2000, 2002), must be examined.

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Zusammenfassung

Das Beutespektrum des Japanischen Laubfrosches, *Hyla japonica* GÜNTHER, 1859, wurde in einem Gebiet untersucht, in das die Argentinische Ameise, *Linepithema humile* (MAYR, 1868), vorgedrungen ist. Ameisen waren die am häufigsten von jungen Laubfröschen erbeuteten Tiere, mit einem Anteil von 82,4 % an der Zahl der insgesamt erbeuteten Tierindividuen und von 49,9 % am Beutegesamtvolumen in den Froschmägen. Innerhalb der Ameisen war die Argentinische Ameise am häufigsten: 333 Arbeiterinnen (52,2 % aller Ameisenindividuen) wurden in 36 Fröschen (85,7 % der untersuchten Froschindividuen) gefunden. Wir schlussfolgern, dass die Argentinische Ameise für den Laubfrosch genießbar ist.

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