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An analysis of mutualistic interactions between exotic ants and honeydew producers in the Yanbaru district of Okinawa Island, Japan

Abstract Invasive exotic ants often have a mutualistic relationship with other insects excreting honeydew, and this is considered to play a key role in their invasion success. We investigated the multispecies association patterns between ants and hemipteran insects in the Yanbaru forests, Okinawa, Japan, an Asian biodiversity hotspot. We especially focused on roadside environments, which are the frontlines of invasion for exotic ants. We found that only a small number of herbaceous and pioneer plants were predominant on the roadsides. Four honeydew producers, *Melanaphis formosana*, *Dysmicoccus* sp. A, *Heteropsylla cubana*, and *Sogata hakonensis*, living on these roadside plants accounted for 94.9% of the total honeydew-producer aggregations observed. Only a few exotic ants, such as *Technomyrmex brunneus* and *Anoplolepis gracilipes*, were observed with these honeydew-producer aggregations, and densities of these ants and honeydew producers were often positively correlated. An ant exclusion experiment showed that exotic ant occurrence improved the survival of some of the hemipteran colonies. Interestingly, the abundance of native ants was not correlated with the abundance of honeydew producers, and the local density of *Pheidole noda* was negatively correlated with that of *M. formosana*. These findings, i.e., only a few ants, all exotic, tended to hemipteran honeydew producers despite the existence of many native ants, and the abundances of those exotic ants and those hemipteran insects had positive correlations, provide some insights into the mechanism of biological invasion and provide information for the management of exotic ants.

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Introduction

Biological invasion by exotic ant species is an increasing threat to biodiversity worldwide (Holway et al. 2002; McGlynn 1999; Passera 1994). Some invasive exotic ants are known to displace native ants and other arthropods through competition and/or predation (Hoffmann et al. 1999; Holway 1998; Holway et al. 2002; O'Dowd et al. 2003). However, invasive exotic ants often have a mutualistic relationship, called trophobiosis, with other insects excreting honeydew. Honeydew contains sugary substances and amino acids, which provide nutrients to ant workers. As a reward for this carbohydrate-rich food, the exotic ants strongly protect the honeydew-producing insects from their natural enemies (Helms and Vinson 2002; Holway et al. 2002; Ness and Bronstein 2004), and this occasionally results in massive outbreaks of the honeydew producers. The consequences of ant invasion can therefore be extensive by indirectly damaging vegetation and altering local ecosystems (Hill et al. 2003; O'Dowd et al. 2003; Dunham and Mikheyev 2009) and can have important implications for agriculture (e.g., González-Hernández et al. 1999; Phillips and Sherk 1991; Reimer et al. 1990).

Trophobiosis between exotic ants and honeydew producers has been shown to have positive effects on honeydew-producer populations (e.g., Abbott and Green 2007; Bach 1991; González-Hernández et al. 1999; Grover et al. 2008; Helms and Vinson 2002; Hill et al. 2003; Kaplan and Eubanks 2002, 2005; O'Dowd et al. 2003). However, most of these studies so far have focused on mutualism between single species or at most trophobiosis between an ant species and a few particular honeydew producers. In reality, however, many areas

have been invaded by multiple exotic ant species (e.g., Boer and Vierbergen 2008; Clark 1982; King and Tschinkel 2007; McClelland and Jones 2009; Pfeiffer et al. 2008; Sarty et al. 2007; Wetterer et al. 2006), and these exotic ants usually coexist with multiple species of honeydew-producing insects. Yet, few studies have tried to comprehensively describe the pattern of such multi-species mutualism in actual communities.

Okinawa Island is the main island of the Ryukyu Islands, which are located in the subtropical region of southwestern Japan. The northern part of this island, called Yanbaru, supports relatively well-conserved primary and semi-primary forests that contain many unique, endemic, and rare species and is considered a biodiversity hotspot in the world (IUCN 2000; Olson and Dinerstein 1998). A number of invasive ant species, including the yellow crazy ant, *Anoplolepis gracilipes*, and the big-headed ant, *Pheidole megacephala*, are found there, and 11 of approximately 70 ant species reported from the area so far are considered to be nonnative (Eguchi 2001; Yamauchi and Ogata 1995). In Yanbaru, exotic ants tend to be dominant in open habitats and are rare in primary forests (Yamauchi and Ogata 1995). However, even in primary and semi-primary forests, nonnative ants are often found along roadsides with native ants (Katayama and Tsuji 2010; Suwabe et al. 2009), suggesting that the forest roadside environment is a potential invasion frontline for exotic ants.

In Yanbaru, researchers have studied the relationship between exotic and native ants (Suwabe et al. 2009; Yamauchi and Ogata 1995) and between ants and a myrmecophilous plant (Katayama and Tsuji 2010), but no one has focused on trophobiosis between exotic ants and honeydew-producing insects. Indirect negative effects on local biodiversity of honeydew producers protected by *A. gracilipes*, one of the two worldwide invasive ants found in Yanbaru, are well documented on some tropical oceanic islands (Abbott and Green 2007; Hill et al. 2003; O'Dowd et al. 2003). However, to our knowledge, no comparable study concerning trophobiosis with *A. gracilipes* has been carried out in an East Asian continental island, such as Okinawa Island.

In this study, we focused on roadside environments of the forests of Yanbaru where exotic and native ants coexist (Katayama and Tsuji 2010; Suwabe et al. 2009). We carried out a comprehensive survey on the quantitative pattern of associations, namely what species of honeydew producers are attended by what species of ants at what frequencies. We then analyzed the positive effects of exotic ants on the population increase of honeydew producers. This consisted of two parts: (a) detection of any statistically significant positive correlation between the local abundance of each ant species and that of each honeydew-producer species in the field, using multivariate generalized linear models (GLMs) to screen for ant species that might have a positive influence on honeydew-producer abundance, and (b) ant exclusion experiments to test directly the positive effects of some ants on the abundance of honeydew producers.

We finally discuss that future conservation biological studies should assess exotic ants' indirect impacts on plant communities and biodiversity through honeydew producers in Yanbaru.

Materials and methods

Preliminary census on vegetation as host communities for honeydew producers

We surveyed plant species composition and identified the dominant plants of roadside environments of the forests of Yanbaru. We randomly chose 97 points (except points in coastal regions where forests are scarce) from 203 permanent study points that had been established for another long-term study. These 203 permanent points were located on the ground surface of roadsides along paved roads at 1-km intervals so as to roughly cover the entire Yanbaru region (26°37′–52′N, 128°06′–19′E). For each of the 97 study points, we set two quadrats (20 m × 0.5 m for each side of the road). Plant species within the quadrats and each species' abundance, which was assessed in terms of ground cover (0–100% with a 5% interval), were determined by viewing from directly above. These censuses were carried out from April 10 to April 24, 2010.

From the 97 study points, we randomly surveyed 36 points from October 25 to October 31, 2008 to determine which plant species were used by honeydew producers. Vegetation in each quadrat was observed for 15 min (30 min for each point) by standing adjacent to the quadrat so as not to disturb the vegetation and recording plant species hosting honeydew producers. The number of aggregations (colonies) of honeydew producers on each plant species was also recorded. In this census, we did not distinguish between honeydew-producer species.

Field census

From the above preliminary censuses we found that two herbaceous plants, Japanese silver grass (*Miscanthus sinensis*) and Spanish needle (*Bidens pilosa*), were dominant, together covering 36% of the roadside ground surface (Table 1). We also noticed that 84% of the honeydew-producer aggregations were found on these two species of plants (Table 2). For this reason, in the main census described below, we used only sites where either of these two plants existed. We also excluded sites that were not surrounded by primary or semi-primary forests, such as sites surrounded by orchards, because exotic ants are known to be dominant in these artificial environments in Yanbaru (Yamauchi and Ogata 1995). In addition, we excluded sites that were weeded by local road managers during the study period.

Table 1 Plant species and their relative abundances in 97 roadside plots in the Yanbaru forests

Plant species	Relative abundance (%)
Miscanthus sinensis	20.62
Bidens pilosa[a]	16.29
Pteridopsida spp.[b]	8.56
Dicranopteris linearis	3.92
Erianthus formosanus[a]	2.53
Rubus spp.	2.32
Leucaena leucocephala[a]	1.70
Mallotus japonicus	1.34
Boehmeria nivea	0.82
Oreocnide pedunculata	0.77
Others	15.36

Only the top ten most abundant plant taxa are shown. Relative abundance was determined based on the percentage of ground cover (mean of 97 plots). Bare area accounted for 25.77%. For details of the sampling method, see text
[a]Regarded as an exotic species by Ikehara (1979)
[b]Pteridopsida species other than *D. linearis* and *Cyathea lepifera*

Table 2 The number of honeydew-producer aggregations found on roadside vegetation in 36 plots established in the Yanbaru forests

Host plant species	Total number of honeydew-producer aggregations	% of total
Miscanthus sinensis	86	72.3
Bidens pilosa	14	11.8
Mallotus japonicus	10	8.4
Ficus erecta	2	1.7
Leucaena leucocephala	2	1.7
Maesa tenera	2	1.7
Schima wallichii	1	0.8
Pleioblastus linearis	1	0.8
Alnus formosana	1	0.8

Numbers of honeydew-producer aggregations were recorded over a 15-min sampling period for each quadrat (30 min for each site). For details of the sampling method, see text

From eight paved roads built in the forests (Benoki Forest Road, Hiji Forest Road, Iji Forest Road, Janagusuku Forest Road, Ôkuni Forest Road, Okuyona Forest Road, Uka Forest Road, and Yona Forest Road), we established 40 study points, with each point located at 500-m intervals (or longer if the regular point did not satisfy the above three environmental requirements) (Fig. 1). At each point, we set a pair of linear quadrats (20 × 0.5 m), one on the ground at each side of the road, as in the preliminary census for honeydew producers. To estimate ant abundance from workers seen on the ground, we also set ten subquadrats (0.5 × 0.5 m, each located at 2-m intervals) on the road surface at the edge of each linear quadrat in order to avoid disturbing vegetation and honeydew producers in the quadrats when counting ants. Details of a study point are schematically shown in Fig. 2.

From October 27 to November 20, 2009, we observed the vegetation once in each linear quadrat for 30 min
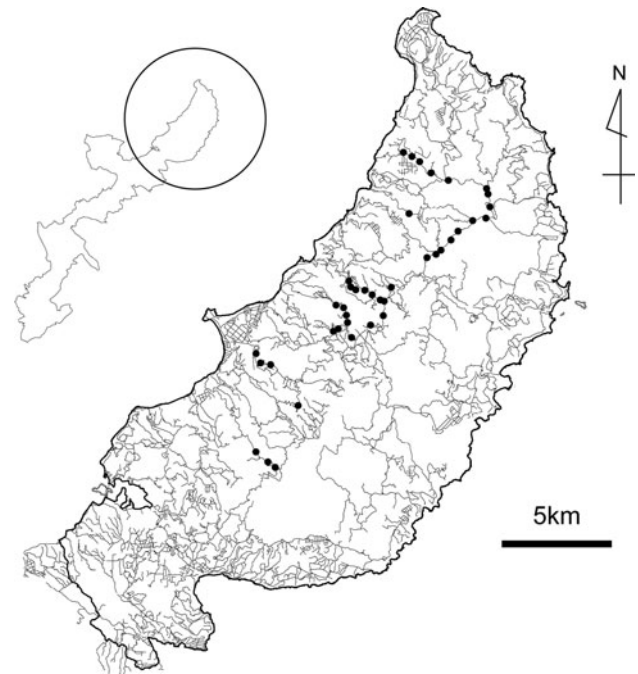
Fig. 1 Locations of study plots for the field census. Each study plot is indicated as a *closed circle*. The selection criteria for the study points are described in the text
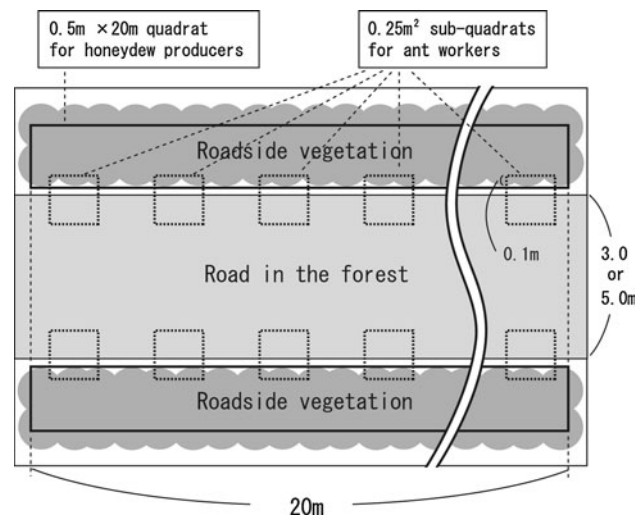
Fig. 2 Schematic of a study plot. For details, see the text

(60 min for each study point). We recorded the species (identified later in the laboratory) of honeydew producers found on the aerial parts of the vegetation, their host plant species, the number of aggregations, and their attending ant species (an "attending ant" was defined as an ant that was completely motionless for 1 s or longer within a 10-mm distance from a honeydew producer). Each subquadrat was also observed for 30 s, and all ant workers that were found were collected by an aspirator and later identified in the laboratory. We also recorded

the abundance of each honeydew producer's host plant as described previously for the preliminary census of vegetation.

The study periods for the field censuses and the subsequent ant exclusion experiment were chosen because exotic ants are most active from autumn to early spring in Yanbaru (Suwabe et al. 2009).

Ant exclusion experiments

To experimentally examine the effect of ants on the population density of honeydew producers, we focused on three ant species, *A. gracilipes*, *Ph. megacephala*, and *Technomyrmex brunneus*, because their abundance had a positive correlation with the abundance of some honeydew producers (see "Results"). For each ant species, we selected two to four study plots where the focal ant species was most dominant and almost exclusively occupied honeydew producers there. We also focused on two dominant honeydew producers, *Melanaphis formosana* (aphid) and *Heteropsylla cubana* (psyllid).

Ant exclusion experiments for M. formosana

We selected a total of 48 ramets of the Japanese silver grass infested by the aphid *M. formosana* that was exclusively occupied by *A. gracilipes* from four study plots, a total of 41 ramets of the grass infested by the aphid that was exclusively occupied by *Ph. megacephala* from two study plots, and a total of 39 ramets of the grass infested by the aphid that was exclusively occupied by *Tec. brunneus* from four study plots. In each selected ramet, we haphazardly chose two paired shoots infested by the aphid. Before ant exclusion, all but one aggregation of aphids (size of each aggregation was approximately 5–15 individuals) were removed by the soft blush. After removal, we smeared a strong sticky substance (Fuji-Tangle®) on the base of one of the two paired shoots to exclude ants but to allow access to winged predators. We left the other shoot intact. We arbitrarily clipped neighboring shoots of the focal two shoots to prevent ant workers from accessing the treated shoot via untreated shoots. These treatments were carried out from December 1–2, 2009, and we then observed and recorded the survival of aphid aggregations on these shoots every 2–8 days for approximately 1 month.

Ant exclusion experiments for H. cubana

We selected four or five shrubs of the white leadtree (*Leucaena leucocephala*) infested by the psyllid *H. cubana* in each of two plots in which *A. gracilipes* was the most dominant ant. Similarly, we chose two or three *L. leucocephala* shrubs infested by the psyllid in each of two plots in which *Tec. brunneus* was the most dominant ant. In each selected shrub, we haphazardly chose 2–14 shoots infested by the psyllid. We assigned half of the selected shoots to the ant exclusion treatment and the rest were controls. The ant exclusion treatment was carried out as described for *M. formosana* using Fuji-Tangle from December 3–4, 2009, and observations of survivorship of psyllid aggregations were carried out as previously mentioned. We found no *H. cubana* aggregation occupied by *Ph. megacephala* and therefore did not use *Ph. megacephala* for the exclusion experiments.

During observations of honeydew-producer aggregation survival, we noted any activity by natural enemies of the honeydew producers and recorded the species and the situations in which they were found, including predating, staying near the prey's aggregation, and being attacked by ants.

Data analysis

To screen the ant species that might positively influence the abundance of honeydew producers, field census data were analyzed by the "glm.nb" function in R 2.10.1(R Developmental Core Team 2009) to fit multivariate GLMs with a negative binomial distribution (which is ideal for count data of organisms showing a clumped distribution). Each study plot was regarded as the independent unit, the abundance of each honeydew producer was assigned as the response variable, and the abundances of the host plant and that of the nine ant species that were observed to attend the honeydew producers were assigned as explanatory variables. In these analyses, we defined the abundance of each honeydew producer as the total number of aggregations in two 20×0.5 m quadrats in each study plot (approximate size of each aggregation, *M. formosana*: ca. 5–15 individuals; *Dysmicoccus* sp. A: ca. 5–20 individuals; *S. hakonensis*: ca. 5–20 individuals; *H. cubana*: ca. 15–50 individuals), the abundance of each ant species as the total number of its workers collected in the 20 subquadrats of each study plot, and the abundance of each host plant as the percentage ground cover in the two quadrats (which was transformed to a binarized dummy variables on the basis of whether the score was larger or smaller than the average before analyzing). We assumed that effects of explanatory variables were independent statistically, and therefore their interaction terms were not included in the model.

To ensure robustness, we also ran a generalized liner mixed model (GLMM) with a Poisson distribution in which the study point identification number was assigned as a random factor using the "glmmML" function in R. These two models yielded similar results, but Akaike's Information Criteria value (Akaike 1974) of the GLMM models was much higher than the values of the GLM models and we have judged the GLM models are more suitable for our analyses. Therefore, only the results of the GLM with a negative binomial distribution are presented below.

Table 3 Honeydew producers and ants appearing at the study site

Species	Family	Host plant	Number[a]	%[b]	Number of plots	%[c]
Honeydew producers						
Melanaphis formosana	Aphididae	*Miscanthus sinensis*	905	62.6	30	75.0
Dysmicoccus sp. A	Pseudococcidae	*Miscanthus sinensis*	218	15.1	29	72.5
Heteropsylla cubana	Psyllidae	*Leucaena leucocephala*	200	13.8	10	25.0
Sogata hakonensis	Delphacidae	*Miscanthus sinensis*	49	3.4	21	52.5
Dysmicoccus sp. B	Pseudococcidae	*Miscanthus sinensis*	22	1.5	2	5.0
Icerya purchasi	Margarodidae	*Bidens pilosa*	20	1.4	4	10.0
Aphis nasturtii	Aphididae	*Bidens pilosa*	13	0.9	2	5.0
Miscanthicoccus miscanthi	Pseudococcidae	*Miscanthus sinensis*	7	0.5	4	10.0
Metanipponaphis cuspidatae	Aphididae	*Castanopsis sieboldii*	5	0.3	1	2.5
Uroleucon formosanum	Aphididae	*Lactuca indica*	4	0.3	1	2.5
Saissetia coffeae	Coccidae	*Bidens pilosa*	2	0.1	2	5.0
Ants						
Technomyrmex brunneus[d]	Formicidae	–	1,235	53.9	28	70.0
Pheidole megacephala[d]	Formicidae	–	461	20.1	4	10.0
Anoplolepis gracilipes[d]	Formicidae	–	187	8.2	4	10.0
Pheidole fervens[d]	Formicidae	–	104	4.5	11	27.5
Nylanderia spp.	Formicidae	–	80	3.5	19	47.5
Pheidole noda	Formicidae	–	58	2.5	14	35.0
Pristomyrmex punctatus	Formicidae	–	58	2.5	14	35.0
Monomorium chinense	Formicidae	–	28	1.2	15	37.5
Cardiocondyla kagutsuchi[d]	Formicidae	–	19	0.8	3	7.5
Nylanderia yambaru	Formicidae	–	18	0.8	5	12.5
Pachycondyla chinensis	Formicidae	–	16	0.7	8	20.0
Cardiocondyla wroughtonii	Formicidae	–	6	0.3	3	7.5
Cardiocondyla minutior	Formicidae	–	6	0.3	3	7.5
Tetramorium bicarinatum[d]	Formicidae	–	6	0.3	2	5.0
Ochetellus glaber	Formicidae	–	5	0.2	2	5.0
Tetramorium kraepelini	Formicidae	–	2	0.1	2	5.0
Monomorium floricola[d]	Formicidae	–	1	< 0.1	1	2.5
Camponotus bishamon	Formicidae	–	1	< 0.1	1	2.5
Odontomachus kuroiwae	Formicidae	–	1	< 0.1	1	2.5
Solenopsis tipuna	Formicidae	–	1	< 0.1	1	2.5

[a] Honeydew producers: the number of observed aggregations. Ants: the number of collected individuals on the ground
[b] Percentages of total aggregations (honeydew producers) or total collected workers (Ants)
[c] Percentage (%) of total study plots
[d] Regarded as exotic (tramp) species by Yamauchi and Ogata (1995)

To test the effect of attending ants on the persistence of honeydew-producer aggregations, the survival rates of ant-excluded and control aggregations were compared using the log-rank test of survival analyses with the "survdiff" function in R. The effect of different natural enemies of hemipterans on the proportion remaining at the end of the study was tested using Fisher's exact tests, using only groups that had these enemies.

Results

Species occurrence and frequency

We found only a small number of dominant herbaceous and pioneer plant species along the roadsides (Table 1). Of these pioneer plants, *B. pilosa*, *Erianthus formosanus*, and *L. leucocephala* are considered to be exotic plants by Ikehara (1979). These plants had frequent associations with honeydew producers (Table 2).

The frequencies of occurrence of honeydew producers and ants are shown in Table 3. We recorded 11 species of honeydew producers and 20 species of ants in the study plots. Of the honeydew producers, *Melanaphis formosana* (Aphididae), *Dysmicoccus* sp. A (undescribed species of Pseudococcidae, identified by H. Tanaka), *H. cubana* (Psyllidae), and *Sogata hakonensis* (Delphacidae) were the major dominant species and accounted for 94.9% of total honeydew-producer aggregations. All study plots had at least one of these four species. Other honeydew producers were observed in a few study plots, but their abundance was small (Table 3) and we excluded them in the subsequent analyses.

Of the ant species collected in the subquadrats, *A. gracilipes*, *Ph. megacephala*, and *Tec. brunneus* were found in large numbers and accounted for 82.1% of the total number of ant workers collected. Among these three, *Tec. brunneus* appeared in 70.0% of the study plots, whereas *Ph. megacephala* and *A. gracilipes* each appeared in ca. 10.0%.
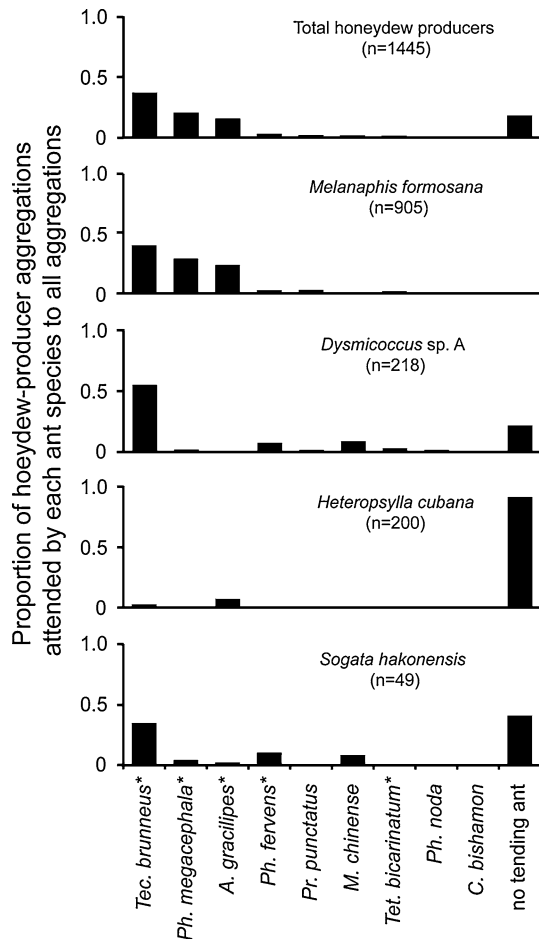
Fig. 3 Species composition of ants attending the top four most abundant honeydew-producer aggregations on roadside vegetation in the forests of Yanbaru. Exotic ant species regarded by Yamauchi and Ogata (1995) are indicated by *asterisks*

The species composition of ants attending the four most abundant honeydew producers are shown in Fig. 3. We recognized nine species of ants, *A. gracilipes*, *Camponotus bishamon*, *Monomorium chinense*, *Pheidole fervens*, *Ph. megacephala*, *Pheidole noda*, *Pristomyrmex punctatus*, *Tec. brunneus*, and *Tetramorium bicarinatum* that attended honeydew producers. Among them, eight (*A. gracilipes*, *M. chinense*, *Ph. fervens*, *Ph. megacephala*, *Ph. noda*, *Pr. punctatus*, *Tec. brunneus*, and *Tet. bicarinatum*) attended to *M. formosana*, seven (*C. bishamon*, *M. chinense*, *Ph. fervens*, *Ph. megacephala*, *Ph. noda*, *Pr. punctatus*, and *Tec. brunneus*) to *Dysmicoccus* sp. A, five (*A. gracilipes*, *M. chinense*, *Ph. fervens*, *Ph. megacephala*, and *Tec. brunneus*) to *S. hakonensis*, and two (*A. gracilipes* and *Tec. brunneus*) to *H. cubana*.

Among the nine ants, three exotic ants, *A. gracilipes*, *Ph. megacephala*, and *Tec. brunneus*, attended honeydew producers most frequently. Indeed, together they exclusively tended 89% of the total honeydew-producer aggregations. These three ants were also the three most dominant ant species on the ground surface and also on

the vegetation of the study plots. Furthermore, the abundances of each ant species on the ground and on the vegetation were positively and significantly correlated (*A. gracilipes*: $r_s = 0.995$, $p < 0.0001$; *Ph. megacephala*: $r_s = 0.997$, $p < 0.0001$; *Tec. brunneus*: $r_s = 0.922$, $p < 0.0001$). However, the species composition of attending ants differed among species of honeydew producers (Fig. 3). *Tec. brunneus* attended to *Dysmicoccus* sp. A and *S. hakonensis* at high frequencies; however, *A. gracilipes* and *Ph. megacephala* attended to these species at low frequencies, whereas all three exotic ant species attended to *M. formosana* at high frequencies. Furthermore, these three ant species rarely attended *H. cubana*.

Results of GLM analyses of the abundance of honeydew producers

Multivariate GLMs (Table 4) showed that the abundances of four exotic ants, *A. gracilipes*, *Ph. megacephala*, *Tec. brunneus*, and *Tet. bicarinatum*, were positively and significantly correlated to the abundance of *M. formosana*, while a statistically significant negative correlation was detected between the abundance of *Ph. noda* and *M. formosana*. There was also a statistically significant positive correlation between the abundance of *Tec. brunneus* and *Dysmicoccus* sp. A. Furthermore, the abundance of *Ph. fervens* had a significant positive correlation with *S. hakonensis*. In contrast, *H. cubana* had no significant correlation with any ant species. The abundance of host plants generally did not explain the abundance of honeydew producers (Table 4).

These results are consistent with the results of another GLM model in which the abundance of each ant species on "vegetation" and the abundance of the host plant were used as the explanatory variables (results not shown).

Results of ant exclusion experiments

Exclusion of all three ant species led to a statistically significant reduction in the survival rates of *M. formosana* aggregations compared to the control (log-rank test, $p < 0.0001$) (Fig. 4). In contrast, the survival rate of *H. cubana* aggregations did not change by exclusion of either *Tec. brunneus* or *A. gracilipes* (Fig. 5). Throughout the experimental period, we never observed a turnover of ant species on a Japanese silver grass shoot (control) infested by *M. formosana*.

The natural enemies of honeydew producers

Figures 6 and 7 show the percentage of honeydew-producer aggregations in which invasion by natural enemies occurred during the ant exclusion experiments. We observed attacks of hoverfly larvae ($n = 4$) and parasitoid

Table 4 Summary of GLMs (with negative binomial distributions) for abundances of honeydew producers from 40 study plots

	Estimate	SE	Z value	p value
Abundance of *Melanaphis formosana*				
Intercept	1.359	0.439	3.094	0.0020
*Technomyrmex brunneus**	0.016	0.004	3.976	0.0001
*Anoplolepis gracilipes**	0.045	0.012	3.593	0.0003
*Pheidole megacephala**	0.016	0.005	3.117	0.0018
*Tetramorium bicarinatum**	0.817	0.305	2.676	0.0075
Pheidole noda	−0.227	0.108	−2.109	0.0350
Pristomyrmex punctatus	0.075	0.052	1.433	0.1519
*Pheidole fervens**	0.011	0.037	0.305	0.7606
Monomorium chinense	−0.013	0.232	−0.058	0.9539
Host plant abundances	0.515	0.425	1.212	0.2257
Abundance of *Dysmicoccus* sp. A				
Intercept	1.081	0.404	2.677	0.0074
*Technomyrmex brunneus**	0.010	0.004	2.642	0.0083
*Pheidole fervens**	0.064	0.034	1.881	0.0600
*Pheidole megacephala**	−0.012	0.008	−1.616	0.1062
Pheidole noda	0.077	0.069	1.124	0.2609
Pristomyrmex punctatus	0.056	0.053	1.042	0.2976
Monomorium chinense	−0.146	0.214	−0.683	0.4949
Camponotus bishamon	−0.163	1.256	−0.130	0.8967
Host plant abundances	−0.046	0.419	−0.110	0.9122
Abundance of *Heteropsylla cubana*				
Intercept	−27.780	> 100	0.000	0.9998
*Anoplolepis gracilipes**	0.018	0.010	1.832	0.0669
*Technomyrmex brunneus**	−0.002	0.006	−0.350	0.7261
Host plant abundances	30.500	> 100	0.000	0.9998
Abundance of *Sogata hakonensis*				
Intercept	−0.551	0.479	−1.149	0.2507
*Pheidole fervens**	0.079	0.035	2.290	0.0220
*Technomyrmex brunneus**	0.007	0.004	1.654	0.0982
*Anoplolepis gracilipes**	−0.020	0.025	−0.794	0.4274
Monomorium chinense	0.114	0.249	0.457	0.6479
*Pheidole megacephala**	−0.003	0.008	−0.381	0.7029
Host plant abundances	0.311	0.478	0.652	0.5147

Local abundance of each ant species, estimated by the density of ants on the ground, and the host plant of each honeydew producer were included as explanatory variables in each honeydew-producer abundance model. Exotic (tramp) ant species regarded by Yamauchi and Ogata (1995) are indicated by *asterisks*. Details of explanatory variables, response variables, and methods of analyses are described in the text

wasps ($n = 37$) on *M. formosana*. Hoverfly (Syrphidae spp.) invasion of *M. formosana* aggregations was more frequently observed on ant-excluded shoots than control shoots (Fisher's exact test, $p < 0.05$ or <0.01). Parasitoid wasps (Chalcidoidea spp.) showed a similar trend, although it was statistically nonsignificant (Fig. 6). Furthermore, the proportion of aggregations invaded by any of the natural enemies was significantly higher in ant-excluded shoots than control shoots, regardless of the ant species excluded (Fig. 6).

The only natural enemy that was directly observed to attack the psyllid *H. cubana* was a hoverfly larva ($n = 1$), and the invasion ratios of hoverflies to aggregations were not significantly different between treatment and control shoots (Fig. 7).

Discussion

So far, few studies have described the pattern of multi-species mutualism between honeydew producers and ants, including exotic ants, in the field as comprehensively as our study. We revealed that most of the honeydew-producer aggregations occurring in the roadside environment of the Yanbaru forests had trophobiotic relationships with ants, and surprisingly, their honeydew seemed to be almost exclusively used by a few exotic ants (Fig. 3). These exotic ants were shown to contribute to the population increase of the honeydew producer, *M. formosana*, through their defense against natural enemies (Figs. 4, 6). The local abundance of workers of exotic ants is often positively correlated with the local abundance of honeydew producers, with some species specificity in the associations (Table 4).

Trophobiosis of ants in roadside environments of Yanbaru forests and its possible relationship with biological invasion

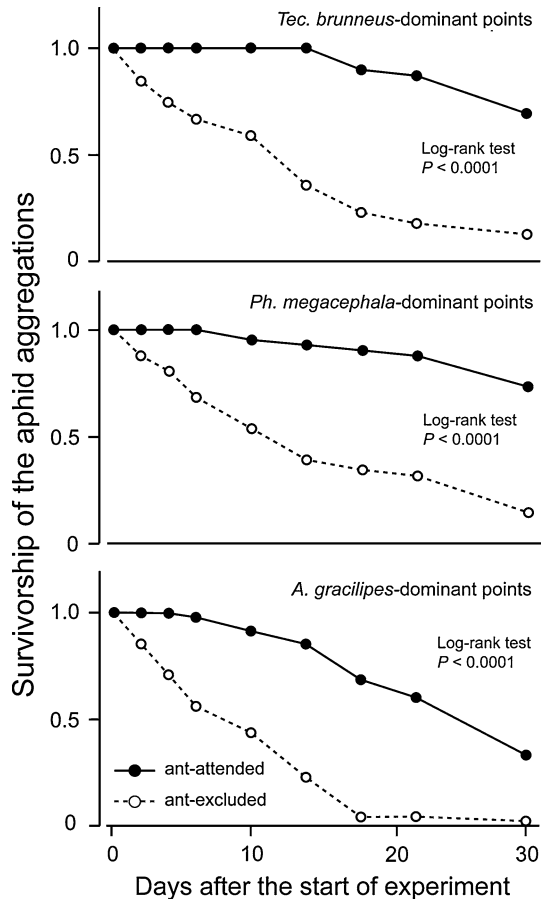Our results showed that three species of exotic ants, *A. gracilipes*, *Ph. megacephala*, and *Tec. brunneus*, were

Fig. 4 Survival rates of ant-excluded and ant-attended aggregations of *Melanaphis formosana*. Results of three ant species are separately presented. Sample size of each treatment, *Tec. brunneus*-dominant points: $n = 39$; *Ph. megacephala*-dominant points: $n = 41$; *A. gracilipes*-dominant points: $n = 48$

Fig. 5 Survival rates of ant-excluded and ant-attended aggregations of *Heteropsylla cubana*. Results of the two ant species are separately presented. Sample size of each treatment, *Tec. brunneus*-dominant points: $n = 20$; *A. gracilipes*-dominant points: $n = 30$

dominant, both in terms of abundance (Table 3) and in attendance of honeydew producers (Fig. 3), and that these three species positively affected survivorship of the honeydew producer, *M. formosana* (Figs. 4, 6). Positive effects on survivorship of the aphid was clearly demonstrated, whereas no effect was shown on survivorship of *H. cubana* (Figs. 5, 7), suggesting that *H. cubana* is not a myrmecophilous honeydew producer.

Our results suggest that in the Yanbaru region, *A. gracilipes* and *Ph. megacephala* attend nearly exclusively to *M. formosana* (Fig. 3). We know that invasions of these two exotic ants are limited to open disturbed habitats and the forest edges in Yanbaru (Suwabe 2009; Yamauchi and Ogata 1995), and we therefore consider that in Yanbaru their impact on the overall community structure of honeydew producers is small and limited. In contrast, *Tec. brunneus* is more abundant in Yanbaru (Table 3) and seems to be able to use many honeydew-producer species (Fig. 3). It can also invade the forest interior in some situations (Suwabe 2009). Furthermore, some *Technomyrmex* species, including *Tec. brunneus* (syn. *Tec. albipes*; Bolton 2007), have been considered as invasive or problematic exotic ants in some parts of the
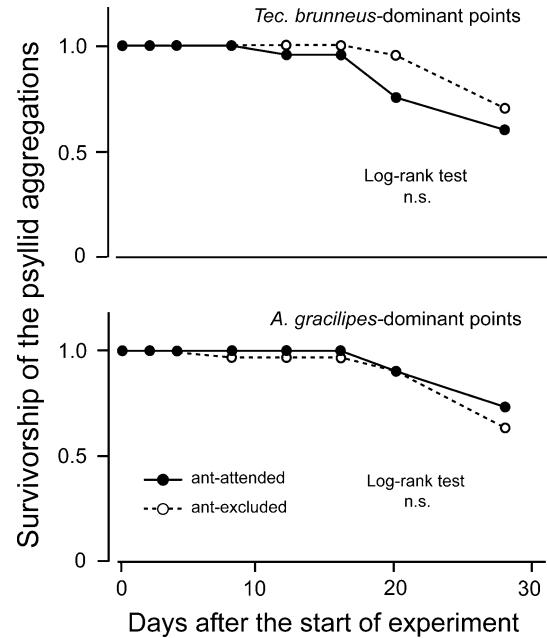
world (Hansen and Müller 2008; Holway et al. 2002; Samways et al. 1982; Sulaiman 1997). The occurrence of this species, therefore, could cause an outbreak of honeydew producers, not only on roadside vegetation but also in the forest interior. We have occasionally observed a severe occurrence of the coccid-like aphid *Metanipponaphis cuspidatae* on the chinquapin tree, *Castanopsis sieboldii*, attended by the ant (H. Tanaka pers. obs.). This tree is an important primary producer and a useful tree for the timber industry in Yanbaru.

Further empirical information is required to determine if *Tec. brunneus* causes serious outbreaks of honeydew producers in Yanbaru forests. The abundances of two other exotic ants, *Ph. fervens* and *Tet. bicarinatum*, also had positive correlations with the abundance of some honeydew producers (Table 4); however, their occurrence in honeydew-producer colonies was relatively infrequent (Fig. 3; Table 3), suggesting that their impact on environments in Yanbaru forests is small. Further studies are needed to confirm this.

In striking contrast to the exotic ants, native ants rarely attended honeydew producers (Fig. 3) and had no positive correlation with the abundance of honeydew producers (Table 4). In fact, the abundance of *Ph. noda* was significantly negatively correlated with *M. formosana* (Table 4). These results suggest that trophobiosis between native ants and honeydew producers in Yanbaru is weak and that *Ph. noda*, the most common native ant in Yanbaru (Suwabe 2009; Suwabe et al. 2009), suppresses the population growth of honeydew producers, possibly through predation.

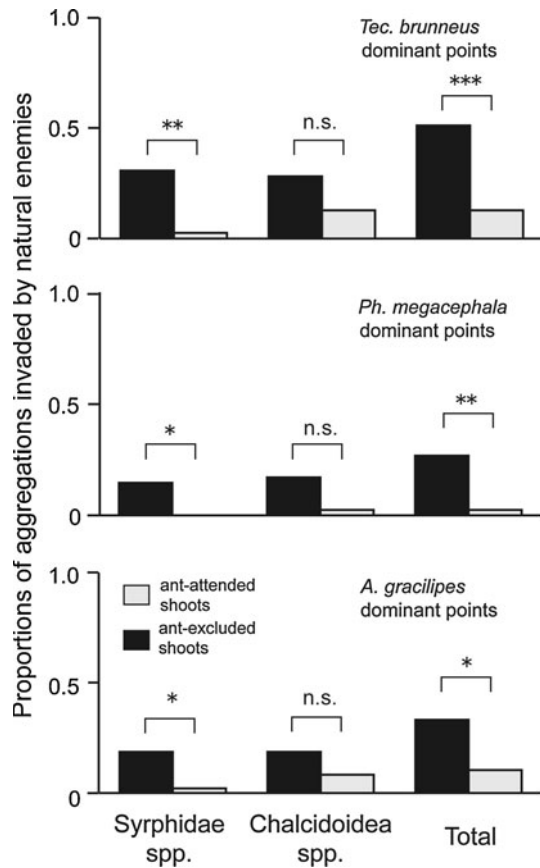Two interpretations might be possible for the differences between native and exotic ants with regard to

Fig. 6 Proportion of *Melanaphis formosana* aggregations invaded by natural enemies in the ant-excluded and ant-attended treatments. Differences in the invasion rate between ant-excluded shoots and ant-attended shoots were statistically significant at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) or nonsignificant (n.s.) (Fisher's exact test). Sample size of each treatment is the same as Fig. 4
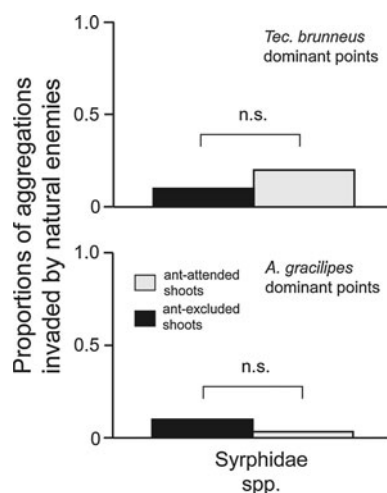

Fig. 7 Proportion of *Heteropsylla cubana* aggregations invaded by natural enemies in the ant-excluded and ant-attended treatments; n.s., nonsignificant difference. Sample size of each treatment is the same as Fig. 5

trophobiosis in Yanbaru. First, native ants do not depend on honeydew and thus had only a weak or no mutualistic relationship with honeydew producers. Second, native ants can use honeydew but were excluded by exotic ants particularly in disturbed habitats, such as roadsides, where exotic ants were most populous. The fact that there was no statistically significant negative correlation in the abundance between any pair of a native and an exotic ant (GLM with negative binomial distribution, $p > 0.05$) might suggest the first hypothesis is more plausible. However, experimental studies in the field are needed to critically test these hypotheses. If the first hypothesis is proved to be true, the question arises as to why native ants did not evolve trophobiosis with honeydew producers in the insect community of Yanbaru.

Biological invasion and road construction

The primary forests of Yanbaru were considered to be nearly free from the threat of exotic ant invasions (Yamauchi and Ogata 1995). However, as suggested by recent studies (Suwabe 2009; Suwabe et al. 2009), we also revealed that exotic ants are common in the roadside environments of Yanbaru forests. Moreover, our study showed that exotic ants are even the dominant occupants of honeydew-producer colonies there.

Exotic ants are known to dominate over indigenous ants in disturbed environments (Bolger 2007; Forys et al. 2002; Le Breton et al. 2003; Stiles and Jones 1998; Suarez et al. 1998). Nakamaru et al. (2007) theoretically demonstrated that nest founding by budding, a characteristic shared by the majority of exotic ants, is advantageous in frequently disturbed habitats. By budding, ants can occupy empty patches created by disturbances more quickly than by independent founding (see also Tsuji 2010; Tsuji and Tsuji 1996). Our results, however, provide an alternative explanation. It is known that the ecological dominance of exotic ants often depends on the presence of honeydew producers, that is, they become strongly invasive when nectar resources are available (Davidson 1998; Lach 2003). In addition, hemipteran insects, the major honeydew producers, tend to have increased numbers in disturbed or fragmented vegetation (Braschler et al. 2003; Fowler et al. 1993). Furthermore, extrafloral nectar-bearing pioneer plants, such as *Macaranga tanarius* and *Mallotus japonicus*, that are known to attract ants (Katayama and Tsuji 2010) are often well established in disturbed habitats in Yanbaru (H. Tanaka, pers. obs.). Therefore, the observed dominance of exotic ants in this study might be because roadsides in Yanbaru forests provide a suitable habitat for hemipteran honeydew producers and nectar-producing pioneer plants and thus their symbionts, exotic ants, also prosper. In other words, there may be a positive feedback between the abundance of honeydew producers and that of exotic ants. Experimental removal of honeydew producers would help determine whether

the disturbance itself or increased availability of honeydew causes the domination of exotic ants in disturbed habitats.

Why do hemipteran honeydew producers become abundant in disturbed habitats such as roads within Yanbaru forests? Soon after deforestation, the environment is often occupied by the Japanese silver grass, which is a well-known pioneer plant and very rare inside intact semi-primary forests in Yanbaru (H. Tanaka, pers. obs.). This is also the main host plant of dominant honeydew producers, especially *M. formosana* (Table 3). In our present study, the abundance of Japanese silver grass was not correlated with the abundances of any honeydew-producing insect in the roadsides (Table 4). However, when a certain requirement—it might be the existence of an ant with a high ability of symbiosis with the hemipteran insects—was fulfilled, honeydew producers would occur frequently. Such indirect effect of the plant's prevalence might partially explain the invasion of exotic ants in Yanbaru.

To prevent the invasion of exotic ants in Yanbaru, we argue that road construction, which is one of the main artificial disturbances, should be kept to a minimum. Otherwise, measures to limit the availability of sugar along roadsides, including controlling Japanese silver grass and extrafloral nectar-bearing pioneer plants, could be needed.

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