EFFECTS OF ATTENDING ANT SPECIES ON THE FATE OF COLONIES OF AN APHID, *MACROSIPHONIELLA YOMOGICOLA* (MATSUMURA) (HOMOPTERA: APHIDIDAE), IN AN ANT-APHID SYMBIOSIS[1]

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ABSTRACT: An aphid, *Macrosiphoniella yomogicola*, establishes an obligate symbiosis with ants and cannot survive without attending ants. Several species of ant attend this aphid, but differences in the effects of attending ant species on the fate of aphid colonies are unknown. Here, we report that parasitism rates by parasitic wasps and the survival of aphid colonies differ depending on the species of attending ants in this symbiotic system in autumn 2017. The proportions of mummies (parasitized aphid individuals) were significantly higher in colonies attended by ants (*Tetramorium tsushimae* and *Pheidole fervida*) other than *Lasius japonicus*, which is the most abundant attender. Only the aphid colonies attended by *L. japonicus* survived to the end of the observational period. Our results indicate that the species of attending ant has a crucial effect on the fate of symbiotic aphid colonies.

KEY WORDS: Ant-aphid symbiosis, *Macrosiphoniella yomogicola*, *Lasius japonicus*, parasitism rate, mummy, effects of attending ant species

INTRODUCTION

The ant-aphid symbiosis is one of the representatives of mutually beneficial relationships in nature. Ants receive honeydew from aphids, and aphids are protected from predation by ants (Buckley, 1987). Both the contributors gain fitness benefits from this symbiotic system (Way, 1963). In some cases, aphids adjust the contents of the honeydew to follow ant preferences to obtain their attendance (Stadler and Dixon, 1998) by sacrificing their own reproduction (Yao et al., 2000; Yao and Akimoto, 2002) because this cost is necessary for the survival of an aphid colony (Banks, 1962; Fischer et al., 2001; Watanabe et al., 2016).

An aphid, *Macrosiphoniella yomogicola* (Matsumura), establishes an obligate symbiosis with ants. The aphids cannot survive without the attending ants because predators prey upon aphids exhaustively when ants have been removed (Watanabe et al., 2016). Thus, protection by ants is necessary for the reproductive success of the aphids. There are several species of ants that attend this aphid in our study area. *Lasius* (s. str.) *japonicus* Santchi is the most abundant attender for this aphid, but *Lasius (Dendrolasius) fuji* Radchenko, *Pheidole fervida* Smith, *Tetramorium tsushimae* (Emery), *Formica japonica* Motscholsky, and *Myrmica kotokui* Forel are found as rare attenders of this aphid in this area.

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[1] Received on October 25, 2017. Accepted on April 8, 2018.

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If there are differences in the ability for attending aphid colonies among these species, the fitness of an aphid individual is affected by the attending ant species. A previous study demonstrated that *L. japonicus* repels most of the natural enemies of this aphid (Watanabe et al., 2016). However, it is unknown whether there are any differences among attending ant species in terms of their ability to maintain aphid colonies in such ant-aphid symbiotic systems. In addition, we have sometimes observed that some aphids become "mummies" from which parasitic wasps appear. Parasitic wasps are assumed to be the most difficult enemies for the attending ants to prevent from parasitizing the cared-for aphids because they can fly and lay eggs on the aphids within a very short time. Thus, differences in the attending ability of ants would be reflected more strongly in such enemies. The aim of this study was to compare the mummification rate in *M. yomogicola* colonies that are attended by different ant species. In addition, we investigated the final fates of the aphid colonies that are attended by the different ant species.

MATERIALS AND METHODS

We selected a total of 9 *M. yomogicola* colonies parasitizing shoots of a host plant (a mugwort; *Artemisia montana*) community on the property of Hokkaido University in Sapporo, Hokkaido, Japan. Among these colonies, three were attended by *L. japonicus*, three were attended by *P. fervida*, and the rest were attended by *T. tsushimae*. In this area, we could find only 6 shoots (3 shoots each) with aphids that were attended by species other than *L. japonicus*. From 27 August to 14 September 2017, we counted the numbers of live aphids and mummies in each colony. The counts were conducted 4 times (27 August and 4, 8, 14 September) during the observational period.

For each colony on each surveyed day, we calculated the proportion of mummies in the colonies attended by different ants, and these values were compared among the ant species. Furthermore, changes in the parasitism rates were compared among the attending ants for each surveyed day. Changes in the pattern in aphid numbers were examined by comparing the slopes of lines from linear regressions of aphid numbers over the days of the study for each ant species.

Statistics: The proportion data were compared using Fisher's exact probability test. Differences in the regression slopes were examined using ANCOVA. When the analysis of multiple comparisons was conducted, the significance levels were corrected using the Bonferroni correction for multiple comparisons (Sokal and Rohlf, 1994). All statistical tests were conducted using R ver. 3.2.1 (R Core Team, 2016).

RESULTS

Fig. 1 shows the change in average aphid number during the observational period by attending ant species. Only colonies with *L. japonicus* (Lj) survived to the end of the observational period. All the colonies attended by *T. tsushimae* (Tt) and *P. fervida* (Pf) had become extinct by 14 September. Figs. 2a-c represent the

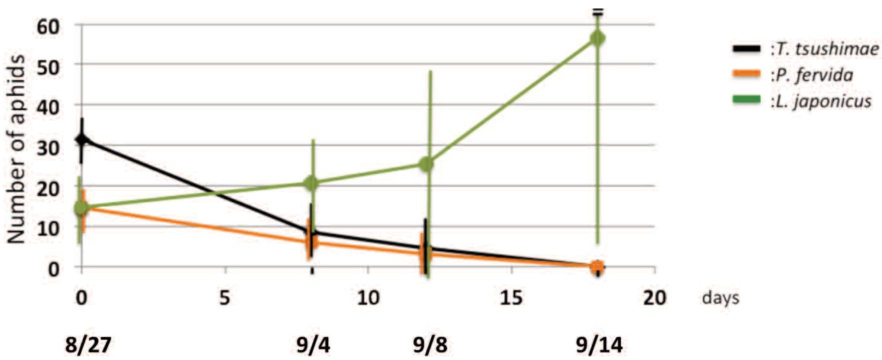

Fig. 1. Changes in the average number of aphids in the 3 colonies attended by each of the 3 ant species. For the 4 observed days, mean ± S.D. are shown. Black diamonds, orange squares and green circles show the data for *T. tsushimae, P. fervida* and *L. japonicus*, respectively. (Actual Printing done in Black and White only)

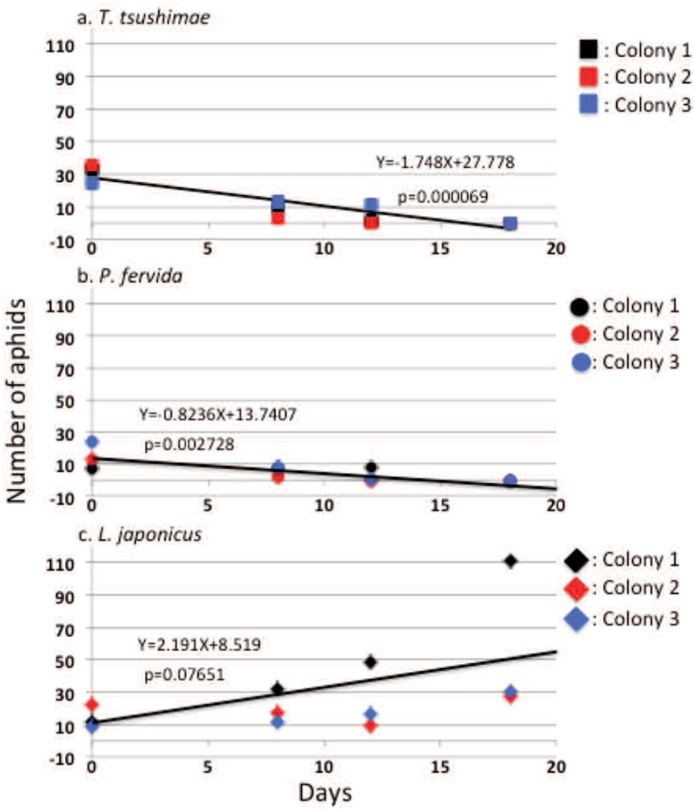

Fig. 2. Changes in the number of aphids in the 3 colonies attended by each of the ant species. (a) Change in aphids attended by *T. tsushimae*. (b) Change in ants attended by *P. fervida*. (c) Change in ants attended by *L. japonicus*. All the colonies attended by *T. tsushimae* and *P. fervida* became extinct before the end of the observational period, whereas all the colonies attended by *L. japonicus* survived until the end of the observational period.

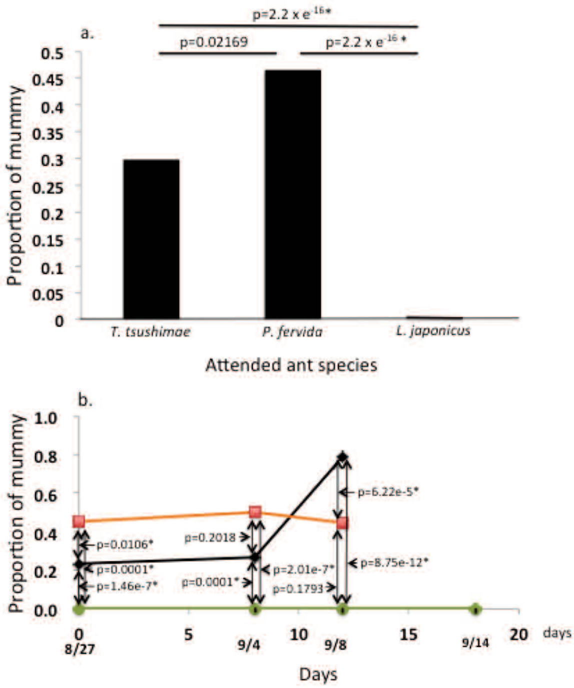

Fig. 3. Pairwise comparisons of the regression slopes for colonies attended by the different ant species. The differences were tested by ANCOVA.

* represents a significant difference between the two slopes after the Bonferroni correction for multiple comparisons.

linear regressions of aphid numbers on total days by each attending ant species. For Tt and Pf, the slopes are significantly negative, and for Lj, the slope is marginally significantly positive (Fig. 2a, Tt; Y=-1.748X+27.778, F=42.25, df=1,10, p=0.000069; 2b, Pf; Y=-0.824X+13.741, F=15.61, df=1,10, p=0.002728; 2c, Lj; Y=2.191X+8.519, F=3.90, df=1,10, p=0.07651). Fig. 3 shows significant differences among the slopes. All the pairs of the slopes were significantly different (Tt vs. Pf; F=7.3865, df=1, 20, p=0.0133: Tt vs Lj; F=11.9098, df=1, 20, p=0.0025: Lj vs. Pf; F=7.1320, df=1, 20, p=0.0147; all the p values were significant after the Bonferroni correction for multiple comparisons). Therefore, the number of aphids changed differently according to the attending ant species.

For the parasitism rates by parasitoid wasps, there were no mummies in the aphid colonies attended by Lj, whereas mummies were found in the aphid colonies attended by the other 2 ant species. Fig. 4a shows the proportion of mummies observed during the whole period by attending ant species. There was no difference in the parasitism rate between the Tt and Pf colonies. However, both types of colonies contained a highly significant proportion of mummies compared to

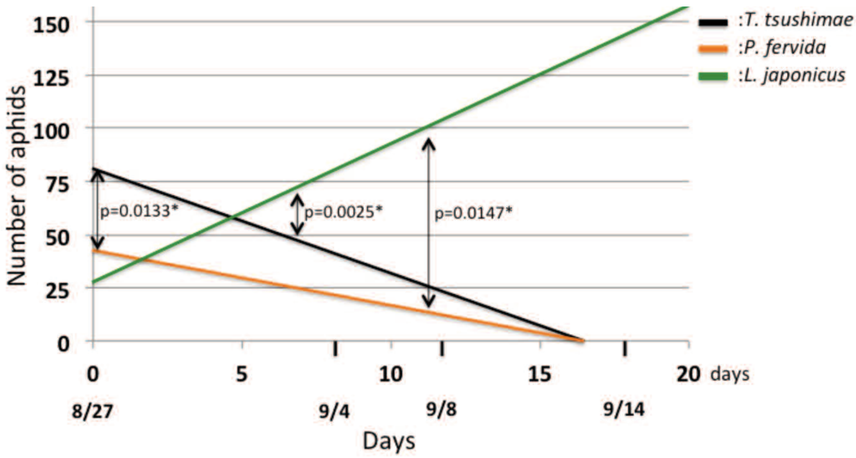

Fig. 4. Differences in mummification rates among aphid colonies attended by different ant species. (a) Total proportions over the observational period. (b) Comparisons for each observation day.
* represents a significant difference between the two ratios after the Bonferroni correction for multiple comparisons.

the colonies attended by Lj. This trend was the same when the data were analyzed separately by the observed days (Fig. 4b; p values were calculated using Fisher's exact probability test). These results indicate that *L. japonicus* is a better partner than the other 2 ant species for *M. yomogicola* because the aphid colonies attended by *L. japonicus* survived longer and were more protected from parasitism than those attended by the other two ant species.

DISCUSSION

Our results demonstrate that there are differences in the ability of ant species to care for aphid colonies. *L. japonicus* perfectly protected their aphids from parasitism by parasitic wasps (the mummification rate was 0 for all the aphid colonies on all the observation days; Fig. 4), and the aphid colonies attended by this ant survived longer than those attended by the other two ant species (Fig. 1). The aphid colonies attended by *T. tsushimae* and *P. fervida* suffered from parasitism by parasitic wasps and were extinct before the end of the observational period. As *M. yomogicola* produce sexuparae in mid-October in this area, it would be difficult for an aphid colony to reproduce when it is attended by ants other than *L. japonicus*.

Why does this difference arise? One possibility is that the ant species belonging to the genus *Lasius* depend mostly on the honeydew from aphids and scale insects for food (R Core Team, 2016; Itioka and Inoue, 1999; Pekas et al., 2011).

This fact suggests that they are specialists in terms of rearing the host symbionts, while *T. tsushimae* and *P. fervida* are omnivorous species that gather various foods, such as dead insects, plant seeds and honeydew (Endo and Itino, 2012; Hölldobler and Wilson, 1990). The latter two species do not crucially depend on the honeydew of aphids, and they would thus invest less in the maintenance of aphid colonies. This difference in the habits of the attending ant species might be the cause of the observed differences.

The reason ants attend aphids is to exploit their honeydew. Ants obtain honeydew from the aphids, and the aphids are protected from predators by ant attendance (Watanabe et al., 2016). Thus, this is a type of mutually beneficial symbiosis. However, *L. japonicus* are known to selectively prey upon aphid individuals that excrete less honeydew in a symbiosis with another aphid species (Wilson, 2003). In addition, symbiotic aphids have been known to change the content of their honeydew when attended by ants (Yao and Akimoto, 2002). Thus, attending ants would be sensitive to the quality of their host's honeydew. *L. japonicus* (previously *L. niger*) prefers particular types of sugar over other sugars (Sakata, 1995). They have especially shown a strong preference for melezitose (Detrain et al., 2010; Völkl et al., 1999). Our preliminary experiments showed that the honeydew of *M. yomogicola* consists of only melezitose and sucrose (Watanabe et al, unpubl.). As shown in this study, when they are attended by ants other than *L. japonicus*, the final fate of the aphids is likely to be extinction. Thus, there is a possibility that *M. yomogicola* excretes honeydew to which only *L. japonicus* shows a strong preference. Most wild colonies of *M. yomogicola* are attended by *L. japonicus* (Watanabe et al., 2016). In fact, we found only 6 shoots (3 shoots each) with aphids attended by different ant species (*P. fervida* and *T. tsushimae*) in the investigated host plant community. Chemical analyses of the honeydew contents and bioassays of the preferences of each ant species for each substance in the honeydew are required to examine this hypothesis.

The cause of the differences in the patterns of the number of aphids is unclear. *M. yomogicola* colonies are abundant in summer but rapidly decrease after the budding of the inflorescences of the host plants in mid-August. Perhaps the physiological condition of the host plant changes with the budding of inflorescences and becomes inadequate for the aphid's reproduction. Only a few colonies survived to mid-October and produced sexuparae, which lay overwintering eggs after copulation. The observational period is the period during which the fate of a colony will separate into either extinction or survival. All 6 colonies attended by ants other than *L. japonicus* went extinct within the observational period. However, whether this extinction was caused by the species of attending ants is unclear based on the data obtained in this study. However, it is a definite fact that all 3 colonies that had been attended by *L. japonicus* survived until the end of the observational period. Ant colonies are perennial and can maintain a source of resources into the future if the current aphid colony persists to the next year. As mentioned above, *L. japonicus* seems to be a specialist feeder on the honeydew of insects. Maintaining a source of honeydew into the future would be more important for *L. japonicus* than for the other omnivorous ants. Therefore, this species might have evolved to facilitate the persistence of the attended aphid colonies into the next year. Further studies will bring interesting insights into the evolution of symbiosis in nature.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports Science and Technology of Japan to E. H. (no. 26440228).

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