



Larvae of the exotic predatory ladybird *Platynaspidium maculosus* (Coleoptera: Coccinellidae) on citrus trees: prey aphid species and behavioral interactions with aphid-attending ants in Japan

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Abstract

An aphidophagous ladybird, *Platynaspidium maculosus* (Weise) (Coleoptera: Coccinellidae), is originally distributed in China, Taiwan, and Vietnam. The ladybird has recently intruded into the southern and central parts of Japan. The present study found that the larvae of this ladybird preyed on three aphid species, *Aphis spiraecola*, *Aphis gossypii*, and *Toxoptera citricidus* (all Hemiptera: Aphididae), feeding on young shoots of various *Citrus* species in August to early October in Shizuoka Prefecture, central Japan. Laboratory rearing of the sampled larvae confirmed that the larvae completed their development (adult emergence) by consuming each of the three aphid species. The ladybird larvae were observed foraging in aphid colonies attended by one of the four ants, *Lasius japonicus*, *Pristomyrmex punctatus*, *Formica japonica*, and *Camponotus japonicus* (all Hymenoptera: Formicidae). Field observations revealed that the foraging/feeding larvae were almost completely ignored by honeydew-collecting ants even when they physically contacted each other. Thus, in Japan, the larvae of the exotic ladybird exploit colonies of the three aphid species attended by one of the four ant species on many *Citrus* species. On the basis of the results, I discuss the possibility of the ladybird's reproduction on citrus trees in Japan, probable adaptations of the ladybird larvae to aphid-attending ants, and potential impacts of the ladybird on native insect enemies attacking ant-attended aphids on citrus.

Keywords *Platynaspidium maculosus* · Exotic ladybird · Prey aphids · Ants · Citrus

Introduction

Alien organisms have the potential to considerably diminish or even destroy populations of indigenous species occupying common ecological niches in the areas that they have invaded (e.g., Elton 1958; Mack et al. 2000; Williamson 1996). Establishment of exotic aphidophagous ladybirds and the following substantial reduction in the abundance of native ladybird species have been demonstrated in North America and Europe (e.g., Brown et al. 2011; Elliott et al. 1996; Evans 2004). Interspecific relationships

between exotic and native aphidophagous insects, including ladybirds, lacewings, syrphids, and parasitoid wasps, are expected to be affected by their ecological characteristics, such as prey species, foraging habitats, seasonal occurrence, and tolerance to ants attending aphids.

An aphidophagous ladybird, *Platynaspidium maculosus* (Weise, 1910) (Coleoptera: Coccinellidae) is originally distributed in China, Taiwan, and Vietnam (Sasaji and Taniguchi 2003; Tao and Chiu 1971). The ladybird has recently intruded into the southern parts of Japan: Okinawa Island in 1989 (Matsubara et al. 1998) and then Kyushu Island in 2006 (Imasaka 2006). Thereafter, the ladybird was detected in Shizuoka City, located in the central portion of Honshu Island (the main island of Japan), in 2010 (Kaneko 2013). In addition, the capture of *P. maculosus* adults in 3 consecutive years, 2010–2012, in multiple citrus groves in Shizuoka City suggests that the ladybird has already become established in the area (Kaneko 2013). The exotic ladybird *P. maculosus* might exert negative impacts on the abundance of native aphidophagous insects in the areas that it has

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invaded. However, little has been elucidated about the ecological attributes of *P. maculosus* in Japan, including prey aphid species, host plant species, and interrelationships with aphid-attending ants.

Monitoring using yellow sticky traps set inside citrus tree canopies in my previous surveys in 2010–2012 showed that *P. maculosus* adults occur continuously from late April through October in Shizuoka City, being more abundant in late spring (late April–mid June), mid-summer (early July–early August), and early autumn (early September–early October) (Kaneko 2013). During these periods, aphid densities on citrus trees are high because of abundant young citrus shoots available for the aphids (Korenaga et al. 1992). These facts imply that the exotic ladybird *P. maculosus* preys on aphids on citrus, thereby reproducing in citrus groves in Japan. However, the occurrence of the larvae of *P. maculosus* on citrus trees has not been confirmed in Japan, except a preliminary observation by Kaneko (2013). In addition, the prey aphid species of *P. maculosus* on citrus and the citrus species it utilizes have not been identified. Furthermore, many aphid species on citrus are frequently attended by honeydew-collecting ants in Japan (Kaneko 2003a, 2003b), but interactions between *P. maculosus* and aphid-attending ants have not been revealed.

In the present study, I surveyed the larvae of the ladybird *P. maculosus* in two citrus groves in Shizuoka Prefecture and recorded the prey aphid species that the larvae were feeding on, the citrus species being infested with the prey aphids, and the ant species attending the aphids. In addition, to examine developmental completion of *P. maculosus* larvae through preying on the aphid species, the larvae found in the citrus groves were sampled and then reared in the laboratory by providing them with the aphid species that they had been consuming on citrus trees. Behavioral interactions between *P. maculosus* larvae foraging in aphid colonies and honeydew-collecting ants in their encounters were also observed to assess the ability of *P. maculosus* to exploit ant-attended aphids. On the basis of the results, I discuss the possibility for the exotic ladybird *P. maculosus* to reproduce on citrus trees in Japan, probable adaptations of the ladybird larvae to various ants attending aphids, and potential impacts of the ladybird's establishment on populations of native aphidophagous insects, especially those mainly attacking ant-attended aphids on citrus.

Materials and methods

Ladybird

The body of the *P. maculosus* adult is ca. 2–3 mm in length. The pronotum is black and the elytra are light red–orange, with two pairs of large black spots surrounding a smaller

black spot (see the photo in Kaneko 2013). The larva has a peculiar feature: a flat, oval-shaped body, with the dorsum weakly convex and the entire outer margin very thin (Sasaji 1992, and see the photo in Kaneko 2013). The larva of *P. maculosus* resembles that of a native aphidophagous ladybird, *Phymatosternus lewisii* (Crotch); however, the two species can be distinguished by the presence of a pair of black spots on the pronotum of the *P. maculosus* larva (Sasaji 1998).

Occurrence of *P. maculosus* larvae on citrus trees and laboratory rearing

Larvae of *P. maculosus* were surveyed in a citrus grove cultivating Satsuma mandarin, *Citrus unshiu* Marcow. (Rutaceae), at Shizuoka Prefectural Fruit Tree Research Center (hereafter referred to as SPFTRC), located in Komagoe, Shizuoka City, in August to September in 2011–2012, and in a citrus grove containing various species and varieties, such as orange, *C. sinensis* (L.) Osbeck, and 'Iyokan', *C. iyo* Hort. ex Tan. (both Rutaceae), at Shizuoka Prefectural Izu Agriculture Research Center (hereafter referred to as SPIARC), located in Inatori, Higashi-Izu Town, in August to early October in 2013–2014.

When a second- or third-instar larva of *P. maculosus* (body length > 2 mm) was found, the aphid species that the larva was feeding on, the plant (citrus) species infested with the aphid colony, and the ant species attending the aphid colony were noted and then the larva was sampled. Each collected larva was reared in a plastic Petri dish (30 mm diameter, 10 mm depth) at an ambient temperature of 20–25 °C in the laboratory, with being provided daily with more than 20 nymphs of the aphid species that the ladybird larva had been preying on when it had been found. The aphids were collected from citrus trees in the groves where each aphid species was abundant enough to be used for the prey. Two to three weeks after the beginning of the rearing, adult emergence was determined for each larva. The proportion of the total (pooled) number of *P. maculosus* adults that emerged to the total (pooled) number of collected larvae (hereafter referred to as the adult emergence ratio) was calculated for each category (the study site, the plant species, the aphid species, and the ant species). Voucher specimens of the adults have been deposited at SPIARC.

Comparisons of the number of *P. maculosus* larvae per aphid colony were made between study sites, plant species, aphid species, and ant species using Mann–Whitney *U* test or Scheffé's multiple comparison test with Kruskal–Wallis test.

Behavioral interactions between *P. maculosus* larvae and ants

I observed behavioral interactions between honeydew-collecting workers of each ant, *Lasius japonicus* Santschi and *Pristomyrmex punctatus* Smith (both Hymenoptera: Formicidae), and foraging or feeding larvae (third-instar) of *P. maculosus* when they encountered each other in colonies of the spirea aphid *Aphis spiraeicola* Patch (Hemiptera: Aphididae) attended by either ant species on *C. unshiu* trees at SPFTRC in September 2011–2012. In addition, I noted behaviors of aphid-attending workers of each ant, *Formica japonica* Motschoulsky and *Camponotus japonicus* Mayr (both Hymenoptera: Formicidae), and *P. maculosus* larvae (third-instar) when encountering each other in colonies of the brown citrus aphid *Toxoptera citricidus* (Kirkaldy) (Hemiptera: Aphididae) attended by either ant species on citrus trees at SPIARC in August and September 2014.

In encounters between an ant worker and a *P. maculosus* larva, their behaviors were recorded as follows. The ant displayed either of the following two different behaviors to the larva: *ignoring*—the ant physically contacted the larva with the antennae or legs but otherwise did not respond to it, and *antennal tapping*—the ant made physical contact with the larva and tapped it with the antennae for a short period of time (5 to 10 s). The larva responded to the ant with either of the two distinct behaviors: *ignoring*—the larva continued its activities (feeding on aphids or walking) after the contact with the ant, and *cowering*—the larva pressed its body tightly against the plant surface when touched by the ant and then remained motionless for a short period of time (5 to 10 s).

A total of seven *P. maculosus* larvae were observed for each ant species, *L. japonicus* and *P. punctatus*, and five and four larvae for *F. japonica* and *C. japonicus*, respectively. Behavioral interactions were noted in ten encounters in a row for each *P. maculosus* larva; a total of 20–40 encounters per larva, with intervals of approximately 10 min between each 10–encounter set. In total 220, 200, 130, and 100 encounters were recorded for *L. japonicus*, *P. punctatus*, *F. japonica*, and *C. japonicus*, respectively.

Results

Occurrence of *P. maculosus* larvae on citrus trees and laboratory rearing

A total of 29 *P. maculosus* larvae (second- or third-instar) were collected in a citrus grove at SPFTRC in Komagoe, Shizuoka City, in 2011–2012, and a total of 91 larvae (second- or third-instar) were sampled in a citrus grove at SPIARC in Inatori, Higashi-Izu Town, in 2013–2014 (Table 1). The larvae recorded in 2011–2014 were found

on a total of seven species of citrus (*C. unshiu*, *C. sinensis*, *C. natsudaidai* Hayata, *C. hanaju* Siebold ex Shirai, *C. iyo*, *C. maxima* Merr., and *C. canaliculata* Y. Tanaka) with colonies of the three aphid species (the cotton aphid *Aphis gossypii* Glover, *A. spiraeicola*, and *T. citricidus*), which were being attended by one of the four ant species (*L. japonicus*, *P. punctatus*, *F. japonica*, and *C. japonicus*).

The number of *P. maculosus* larvae per aphid colony showed no significant difference between the two study sites (Table 2; $p = 0.44$, Mann–Whitney *U* test). The adult emergence ratio was almost the same between the two sites.

There were no clear differences among the seven plant species in the larval numbers per aphid colony (no significant difference among *C. sinensis*, *C. unshiu*, and *C. iyo*; $p = 0.89$, Kruskal–Wallis test) and in the adult emergence ratio (Table 3).

The larval numbers per aphid colony did not differ significantly between *T. citricidus* and *A. spiraeicola* colonies (Table 4; $p = 0.89$, Mann–Whitney *U* test). The adult emergence ratio was high in colonies of both aphid species.

All the *P. maculosus* larvae were noted in aphid colonies attended by one of the four ant species (Table 5). The larval numbers per aphid colony was significantly smaller when being attended by *L. japonicus* than when attended by *P. punctatus* ($p < 0.05$, Scheffe's multiple comparison test with Kruskal–Wallis test). The adult emergence ratio was similar with respect to the ant species that had been attending the aphid colonies from which the larva had been collected.

Behavioral interactions between *P. maculosus* larvae and ants

In 218 out of 220 encounters (99.1%) between a honeydew-collecting *L. japonicus* worker and a foraging/feeding *P. maculosus* larva (third-instar) in aphid colonies, the ant ignored the larva without exhibiting any aggressive behavior and the larva also ignored the ant, continuing the activity. In just two out of the 220 encounters (0.9%), a *L. japonicus* worker tapped a *P. maculosus* larva with the antennae for 5–10 s, and the larva cowered and remained motionless during the period, and then the ant walked away without showing any aggression. In all 200 encounters, a worker of *P. punctatus* ignored a *P. maculosus* larva and the larva also ignored the ant. A worker of *F. japonica* or *C. japonicus* ignored a *P. maculosus* larva that also ignored the ant in all 130 or 100 encounters, respectively. Thus, none of the four ant species treated *P. maculosus* larvae aggressively.

Table 1 Records of the larvae of the ladybird *Platynaspidius maculosus* collected in citrus groves and results of laboratory rearing of the larvae

Year	Month	Day	Study site	Plant species	Aphid species	Ant species	No. of aphid colonies	No. of larvae collected	No. of adults emerging
2011	Sep	13	Komagoe, Shizuoka	<i>Citrus unshiu</i>	<i>Aphis spiraeicola</i>	<i>Lasius japonicus</i>	4	15	13
2012	Aug	9	Komagoe, Shizuoka	<i>Citrus unshiu</i>	<i>Aphis gossypii</i>	<i>Lasius japonicus</i>	1	1	1
2012	Sep	11	Komagoe, Shizuoka	<i>Citrus unshiu</i>	<i>Aphis spiraeicola</i>	<i>Pristomyrmex punctatus</i>	3	13	12
2013	Oct	4	Inatori, Higashi-Izu	<i>Citrus sinensis</i>	<i>Toxoptera citricidus</i>	<i>Lasius japonicus</i>	2	5	5
2014	Aug	4	Inatori, Higashi-Izu	<i>Citrus natsudaikai</i>	<i>Toxoptera citricidus</i>	<i>Lasius japonicus</i>	2	4	3
2014	Aug	4	Inatori, Higashi-Izu	<i>Citrus sinensis</i>	<i>Toxoptera citricidus</i>	<i>Lasius japonicus</i>	2	5	5
2014	Aug	6	Inatori, Higashi-Izu	<i>Citrus hanaju</i>	<i>Toxoptera citricidus</i>	<i>Lasius japonicus</i>	1	1	1
2014	Aug	6	Inatori, Higashi-Izu	<i>Citrus sinensis</i>	<i>Toxoptera citricidus</i>	<i>Pristomyrmex punctatus</i>	1	4	3
2014	Aug	7	Inatori, Higashi-Izu	<i>Citrus sinensis</i>	<i>Aphis spiraeicola</i>	<i>Lasius japonicus</i>	1	1	1
2014	Aug	8	Inatori, Higashi-Izu	<i>Citrus iyo</i>	<i>Toxoptera citricidus</i>	<i>Formica japonica</i>	3	11	10
2014	Aug	11	Inatori, Higashi-Izu	<i>Citrus sinensis</i>	<i>Toxoptera citricidus</i>	<i>Formica japonica</i>	2	12	9
2014	Aug	11	Inatori, Higashi-Izu	<i>Citrus sinensis</i>	<i>Toxoptera citricidus</i>	<i>Pristomyrmex punctatus</i>	3	24	21
2014	Aug	12	Inatori, Higashi-Izu	<i>Citrus maxima</i>	<i>Toxoptera citricidus</i>	<i>Formica japonica</i>	1	1	1
2014	Aug	15	Inatori, Higashi-Izu	<i>Citrus iyo</i>	<i>Toxoptera citricidus</i>	<i>Pristomyrmex punctatus</i>	1	3	3
2014	Aug	15	Inatori, Higashi-Izu	<i>Citrus sinensis</i>	<i>Aphis spiraeicola</i>	<i>Pristomyrmex punctatus</i>	2	6	6
2014	Sep	12	Inatori, Higashi-Izu	<i>Citrus canaliculata</i>	<i>Toxoptera citricidus</i>	<i>Camponotus japonicus</i>	1	4	4
2014	Sep	12	Inatori, Higashi-Izu	<i>Citrus sinensis</i>	<i>Toxoptera citricidus</i>	<i>Camponotus japonicus</i>	1	2	2
2014	Sep	12	Inatori, Higashi-Izu	<i>Citrus unshiu</i>	<i>Aphis spiraeicola</i>	<i>Camponotus japonicus</i>	1	3	3
2014	Sep	19	Inatori, Higashi-Izu	<i>Citrus unshiu</i>	<i>Aphis spiraeicola</i>	<i>Formica japonica</i>	1	3	3
2014	Sep	19	Inatori, Higashi-Izu	<i>Citrus unshiu</i>	<i>Aphis spiraeicola</i>	<i>Lasius japonicus</i>	1	2	2
Total							34	120	108

Table 2 Comparison of the number of *Platynaspidius maculosus* larvae per aphid colony and the adult emergence ratio of the larvae reared in the laboratory between different study sites

Study site	No. of aphid colonies	No. of larvae collected	No. of larvae/aphid colony ^a	No. of adults emerging	Adult emergence ratio (%)
Inatori, Higashi-Izu	26	91	3.5 ± 2.1 a	82	90.1
Komagoe, Shizuoka	8	29	3.6 ± 1.4 a	26	89.7

^aMeans (± SD) followed by the same letter are not significantly different ($p > 0.05$, Mann–Whitney U test)

Table 3 Comparison of the number of *Platynaspidius maculosus* larvae per aphid colony and the adult emergence ratio of the larvae reared in the laboratory among different plant species

Plant species	No. of aphid colonies	No. of larvae collected	No. of larvae/aphid colony ^a	No. of adults emerging	Adult emergence ratio (%)
<i>Citrus sinensis</i>	14	59	4.2 ± 2.5 a	52	88.1
<i>Citrus unshiu</i>	11	37	3.4 ± 1.3 a	34	91.9
<i>Citrus iyo</i>	4	14	3.5 ± 0.6 a	13	92.9
<i>Citrus natsudaikai</i>	2	4	2.0 ± 0	3	75.0
<i>Citrus canaliculata</i>	1	4	4	4	100
<i>Citrus hanaju</i>	1	1	1	1	100
<i>Citrus maxima</i>	1	1	1	1	100

^aMeans (± SD) followed by the same letter are not significantly different ($p > 0.05$, Scheffe's multiple comparison test with Kruskal–Wallis test)

Table 4 Comparison of the number of *Platynaspidius maculosus* larvae per aphid colony and the adult emergence ratio of the larvae reared in the laboratory among different aphid species

Aphid species	No. of aphid colonies	No. of larvae collected	No. of larvae/aphid colony ^a	No. of adults emerging	Adult emergence ratio (%)
<i>Toxoptera citricidus</i>	20	76	3.8 ± 2.3 a	67	88.2
<i>Aphis spiraeicola</i>	13	43	3.3 ± 1.2 a	40	93.0
<i>Aphis gossypii</i>	1	1	1	1	100

^aMeans (± SD) followed by the same letter are not significantly different ($p > 0.05$, Mann–Whitney U test)

Table 5 Comparison of the number of *Platynaspidius maculosus* larvae per aphid colony and the adult emergence ratio of the larvae reared in the laboratory among different ant species attending aphid colonies

Ant species	No. of aphid colonies	No. of larvae collected	No. of larvae/aphid colony ^a	No. of adults emerging	Adult emergence ratio (%)
<i>Lasius japonicus</i>	14	34	2.4 ± 1.2 a	31	91.2
<i>Pristomyrmex punctatus</i>	10	50	5.0 ± 2.3 b	45	90.0
<i>Formica japonica</i>	7	27	3.9 ± 1.8 ab	23	85.2
<i>Camponotus japonicus</i>	3	9	3.0 ± 1.0 ab	9	100

^aMeans (± SD) followed by the same letter are not significantly different ($p > 0.05$, Scheffe's multiple comparison test with Kruskal–Wallis test)

Discussion

Many studies have shown so far the occurrence of the adults of the exotic ladybird *P. maculosus* in Japan (Imasaka 2006; Ishikawa 2011; Kaneko 2013; Kido 2010; Kusakabe 2013; Matsubara et al. 1998; Sasaji and Taniguchi 2003). However, the larvae of *P. maculosus* have not been recorded in this country, except a preliminary observation by Kaneko (2013). The present survey detected a large number of *P. maculosus* larvae foraging in two citrus groves in central Japan (Table 1). Thus, in practice, this is the first study that unequivocally confirmed the occurrence of *P. maculosus* larvae in Japan.

Platynaspidius maculosus has been described as an aphidophagous coccinellid in Taiwan (Tao and Chiu 1971), but no information is available on its prey aphid species. The present study identified the prey aphid species

of *P. maculosus* larvae in Japan, which were *A. gossypii*, *A. spiraeicola*, and *T. citricidus*, on the basis of field observations in citrus groves and laboratory rearing (Table 1). The ladybird larvae were found almost exclusively in colonies of *A. spiraeicola* and *T. citricidus* in August to early October. These two aphids are predominant among aphids on citrus trees in summer to early autumn, when *A. gossypii* is less abundant, in Japan (Korenaga et al. 1992). The same was true of the two citrus groves examined in this study (S. Kaneko, personal observation). These facts suggest that these two aphids are the main prey of *P. maculosus* larvae on citrus in summer to early autumn. On the other hand, in late April to early June, *A. gossypii* and *A. spiraeicola* occur more abundantly on citrus (Korenaga et al. 1992), so *P. maculosus* larvae might exploit *A. gossypii* and *A. spiraeicola* on citrus in late spring to early summer. The species composition of native aphidophagous insects on citrus trees differs among the three aphid species and

the seasons (Kaneko 2003a, 2003b; S. Kaneko, personal observation). Therefore, the main prey aphid species of *P. maculosus* larvae on citrus and its seasonal differences need to be documented to assess the potential impact of *P. maculosus* on each native aphid enemy. On the other hand, the proportion of *P. maculosus* larvae that finally became adults did not differ largely between when being fed with *A. spiraecola* and with *T. citricidus* (Table 4). This result implies that the larvae complete their development at an almost equal possibility when consuming each of the two aphids. The nutritional values of the three aphid species as prey for *P. maculosus* larvae need to be compared through laboratory rearing.

Foraging *P. maculosus* larvae were recorded on a total of seven species of *Citrus* (Table 1). The three aphids noted as prey of *P. maculosus* larvae feed on various *Citrus* species and commonly occur on citrus trees from late April through October in Japan, although the seasonal prevalence in the occurrence differs among the three aphid species, as described above (Korenaga et al. 1992). These facts suggest that plants belonging to *Citrus*, on which the prey aphids of the ladybird are abundant for a relatively long period of time, are one of foraging habitats of the ladybird larvae in Japan. Furthermore, the adults of *P. maculosus* were caught on sticky traps set inside *C. unshiu* tree canopies almost continuously from late April through October in Japan (Kaneko 2013). The present study showed that *P. maculosus* larvae that were attacking aphids on citrus in August to early October could successfully become adults (Table 1). These results suggest that *P. maculosus* reproduces on citrus trees by preying on aphids, at least in mid-summer to early autumn in Japan. Also in late spring to early summer, *P. maculosus* might reproduce by exploiting *A. gossypii* and *A. spiraecola*, as stated above. Thus, the ladybird might utilize citrus trees for foraging and reproduction in late spring to early autumn in Japan. On the other hand, *A. spiraecola* and *A. gossypii* infest a wide range of plant species, including many agricultural crops (Moritsu 1983). It is therefore necessary to survey the occurrence of *P. maculosus* larvae on various plants other than citrus, as well as its prey aphid species, in Japan in order to determine its foraging/reproduction habitats in Japan that it has recently invaded.

The present study found that *P. maculosus* larvae foraged in aphid colonies that were attended by one of the four ants, *L. japonicus*, *P. punctatus*, *F. japonica*, and *C. japonicus* (Table 1). In addition, the field observations revealed that the larvae were almost completely ignored by honeydew-collecting workers of each ant species even when being touched by the ants' antennae or legs. These results suggest that the ladybird larvae adopt some strategies to avoid being recognized as an aphid enemy by aphid-attending ants, irrespective of the ant species. To verify this suggestion, field observations on behavioral interactions between the ladybird

larvae and aphid-attending ants in the ladybird's original areas are needed. The larvae of two aphidophagous coccinellids, *Platynaspis luteorubra* Goeze and *Phymatosternus lewisii* are characterized by a flat, 'coccid-like' body shape and very short legs completely hidden under the thorax, just like *P. maculosus* larvae (Kaneko 2007a; Völkl 1995). Völkl (1995) documented that *P. luteorubra* larvae were totally disregarded by aphid-attending workers of the ant *Lasius niger* L. even when they physically contacted each other, suggesting that chemical camouflage of the larvae might contribute to the avoidance of ant aggression. The larvae of *P. lewisii* foraging in ant-attended aphid colonies are also never attacked by *P. punctatus* workers (Kaneko 2007a). The larvae of the three ladybirds are expected to have several strategies for preventing ant detection in common, including chemical (mimicry or camouflage), morphological (flat body and short legs), and behavioral (inconspicuous movements) adaptations to ants. Chemical substances on the larval body surface, such as epicuticular hydrocarbon, might act an important role in behavioral interactions with ants, as demonstrated for some aphid parasitoids (Akino and Yamaoka 1998; Liepert and Dettner 1993; Völkl et al. 1996). Comparison of the larval hydrocarbon compositions across the three ladybirds might lead to the identification of key substances that function for deceiving aggressive ants.

Significantly fewer *P. maculosus* larvae were observed in aphid colonies attended by *L. japonicus* than in those attended by *P. punctatus* (Table 5). A similar pattern has been reported for the larvae of the ladybird *Scymnus posticalis* Sicard, which are also ignored by the two ants in colonies of the three aphids (Kaneko 2003a, 2003b, 2007b). These studies suggested that the difference in *S. posticalis* larval numbers between the aphid colonies attended by each of the two ants would be caused by the difference between the two ants in aggressiveness against *S. posticalis* adult females laying eggs in/nearby aphid colonies and in the consequent exclusion effects on the adult females. Also in the case of *P. maculosus*, the two ants might exhibit different intensities of defensive responses against ovipositing adult females of the ladybird. To verify this hypothesis, behavioral interactions between aphid-attending ant workers and *P. maculosus* adult females in aphid colonies need to be compared between the two ants.

The present study implies that *P. maculosus* larvae primarily prey on aphids being attended by ants, just as the larvae of the ladybirds, *P. luteorubra* and *P. lewisii*, do (Kaneko 2007a; Völkl 1995). Völkl (1995) suggested that the more frequent occurrence of *P. luteorubra* larvae in ant-attended aphid colonies than in unattended colonies could be attributed chiefly to the protection of the larvae by ants against their parasitoids and intraguild predators and to the resulting preference of the ladybird for ant-attended aphids. On citrus trees in Japan, ant-attended colonies of the three

prey aphids of *P. maculosus* (*A. spiraecola*, *A. gossypii*, and *T. citricidus*) are frequently exploited by the ladybird *P. lewisii* and other native enemies that are capable of avoiding ant attacks, including the ladybird *S. posticalis* and the parasitoid wasp *Lysiphlebus japonicus* Ashmead (Kaneko 2002, 2003b). Therefore, the colonization of citrus groves in Japan by *P. maculosus* might cause severe competition for ant-attended aphids between the exotic ladybird and these native, ant-adapted enemies, thereby significantly depressing the abundance of the natives. In fact, *P. maculosus* larvae and these enemies occurred in the same aphid colonies in the examined citrus groves (S. Kaneko, personal observation). Direct and indirect interactions between *P. maculosus* and the indigenous enemies in ant-attended aphid colonies on citrus trees need to be elucidated by performing detailed field observations and laboratory experiments.

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