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Behavioural observations of the bodyguard mite *Ensliniella parasitica**

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Abstract

In a mutualistic relation between a potter wasp, *Allodynerus delphinalis* (Giraud) (Hymenoptera: Vespidae), and its specific parasitic mite, *Ensliniella parasitica* Vitzthum (Winterschmidtiidae), behaviour of the mite guarding the wasp and attacking their common natural enemy, *Melittobia acasta* (Walker) (Hymenoptera: Eulophidae), was examined. While mite attacks to *M. acasta* occurred by accidental physical contact, the counterattack by the parasitoid occurred 24 h after both were released onto their mutual host. The two organisms fought until one of them died in our experimental arena, which the parasitoid could not escape from to avoid combat. It was not possible to determine what the behaviour of the parasitoid would be had it been able to escape. Mite phoretic behaviour was also examined to understand the mechanism by which both the host wasp and the mite could reap reciprocal benefits from the presence of acarinaria on the wasp. The results suggested that the newly emerged host wasp might have an attractant to collect the necessary number of mites in an acarinarium, which would later function as guards of its offspring, given that around 46% of mite deutonymphs were able to migrate into one of the acarinaria within only 10 min after they were put together in an experimental arena. To more fully understand the strategy of each organism involved in this mutualism, further observation on their behaviour is needed.

Key words: *Allodynerus delphinalis*, *Ensliniella parasitica*, host searching behavior, mutualism, parasitism.

Introduction

Life histories of mites and insects represent a broad spectrum of symbiotic interactions, from parasitism to mutualism and phoresy. Although the ecological status of many ectoparasitic and phoretic mites is relatively well known (e.g., parasitengone larvae are parasites and many free-living mesostigmatids and astigmatids are phoretic) (Krantz & Walter, 2009), other interactions are obscure and their ecological nature rarely proven. Some examples of other interspecific relations involve tarsonemid mites carrying ascospores of *Ophiostoma* fungi (Ascomycetes), which tend to outcompete mutualistic fungi carried by bark beetles in whose galleries they live (Lombardero *et al*., 2003), and *Poecilochirus necrophori* Vitzthum (Parasitidae), which has a mutualistic relationship with *Necrophorus* burying beetles by preying on natural enemies of juvenile beetles (Wilson, 1983). In both cases, phoresy of mites on the symbiotic insects seems to play a key role in their relationships.

Acarinaria are extraordinary structures of insects that appear to be used solely for phoretically carrying mites into their nests (e.g., Eickwort, 1994). Although insects may have different types of depressions on their exoskeleton where mites can attach, only structures that specifically facilitate mite transport are called "acarinaria" (OConnor & Klompen, 1999). These structures are present in at least three different lineages of bees and vespid wasps (OConnor & Klompen, 1999), suggesting that they have evolved independently several times within the aculeate Hymenoptera. Although lacking in evidence, mutualism between acarinarium-bearing bees and wasps and the mite users has long been suggested. Eickwort (1994) suggested that *Dinogamasus* (Laelapidae) mites carried in the acarinaria of carpenter bees might remove potentially harmful microbes from the body of the carriers, by feeding

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on scrap cuticles of juvenile bees. Acarinaria are common in eumenine wasps of the family Vespidae and are located on the thorax behind the scutellum, at the sides of the scutellum, on the propodium and/or on the second metasomal tergite of different species (Soika, 1987; OConnor & Klompen, 1999). Although interactions between wasps and mites other than phoresy are poorly described, it is known that of *Ensliniella parasitica* Vitzthum (Winterschmidtiidae) is a parasite of *Allodynerus delphinalis* (Giraud) (Hymenoptera: Vespidae) juveniles (Okabe & Makino, 2008a). Synchronism of life cycles of the mite and its host has been investigated. Deutonymphal mites migrate into a cell of *A. delphinalis,* probably during the oviposition phase of the latter, and immediately moult into tritonymphs which feed on the haemolymph of paralyzed moth larvae stored by *A. delphinalis* in the cell as a food resource. Other stages of the mite feed on *A. delphinalis* haemolymph. Adult mites lay eggs on *A. delphinalis* pupae; all mite offspring reach the deutonymphal stage between 48 h before and 24 h after the emergence of the adult host from the pupa (Okabe & Makino, 2008a). However, the phoretic behaviour of the former on the latter has not been well described.

Because the mites and their hosts reap reciprocal benefits from their interaction (the wasp transports the mites, in the acarinaria, to a new nest, where the mite has food but cause little damage to the wasp and protects the host juvenile from the attack of a more destructive parasite, as subsequently explained), there should be an effective mechanism by which the deutonymph properly enter acarinaria for phoresy before the recently moulted adult host departs from the nest. Sexual dimorphism exists in the metasomal acarinarium of *A. delphinalis*: the male has a simple indentation on the second tergite that can harbour around 10 deutonymphal mites, whereas the female has a large and deeper cavity mostly covered with exoskeleton of the second tergite that can hold nearly 200 mites (Makino & Okabe, 2003). This suggests that the female plays a more important role in delivering mites to nests. How mites discern where to go on the host is a remaining question in this interaction.

It has recently been reported that *E. parasitica* protects its food source (i.e., wasp juvenile) when the parasitoid *Melittobia acasta* (Walker) (Hymenoptera: Eulophidae) attacked *A. delphinalis* in its nest (Okabe & Makino, 2008b). Adult mites kept in a small vial frequently attacked a female parasitoid found in the same vial, but sometimes all of the mites were killed by the parasitoid (Okabe & Makino, 2008b). Eventually, either the mite killed the parasitoid or the parasitoid killed all of the mites in the same cell. Although a videotaped mite attack has been analyzed, the parasitoid counterattack has not yet been carefully observed. It would be interesting to know what strategy this generalist parasitoid uses when meeting the mites that may attack it in the nest of their common host. In a previous study, although no difference was observed in host fecundity between mite-laden and mite-free wasps under rearing conditions without parasites, mites and juvenile hosts frequently died because the hosts were parasitized by flies or by unknown pathogens (Okabe & Makino, 2008a, b). In addition, when one of the most powerful natural enemies, *M. acasta*, invaded and searched a juvenile host, frequent attacks by both female and male mites were observed (Okabe & Makino, 2008b).

In this study, we review host-guarding behaviour by the parasitic mite against a parasitoid of its host to understand the guarding mutualism from a behavioural viewpoint. We also observed the mite phoretic behaviour, to determine whether the wasp had a special mechanism for gathering the mites into its acarinaria.

Materials and Methods

Mite attack and parasitoid counterattack

Specimens of *A. delphinalis*, *E. parasitica* and *M. acasta* were collected from nests of the first species. Wasps with mites were reared in cages maintained in a glasshouse following the rearing method established by Okabe & Makino (2008b) until they were used in the experiments. The parasitoid colony was maintained in a vial (10-mm diameter and 50-mm length) with a juvenile of the *A. delphinalis*. Emerged adult parasitoids were maintained in a refrigerator (ca. 5°C) for a few hours to a few days until used in the experiments.

A prepupal *A. delphinalis*, three or seven adult mites and an adult female parasitoid were transferred into a vial (5-mm diameter and 50-mm length) with an internal volume equivalent to that of a cell of the wasp (Okabe & Makino, 2008b). The behaviour of the mites and the parasitoids was videotaped with a 3CCD camera attached to a video recording system (colour video camera, DXC-390, Sony; connected to a camera adaptor, CMA-D2, Sony; hard disk recorder, VR-509, Victor) (Okabe & Makino, 2008b). We recorded mite and parasitoid behaviour for 3 h in up to three consecutive days, to determine the time the parasitoid remained on the wasp, physical contact between the mite and the parasitoid, and mite attack against the parasitoid. Three replications were made for each setup (three vs. seven mites). The numbers of mites to be used in the experiment were determined based on the different effects according to mite number in a cell observed by Okabe & Makino (2008b)

Mite migration into acarinaria

We used wax to attach a newly emerged female wasp to the bottom of a Petri dish by her ventral mesosoma. A mite deutonymph was released onto the anterior end of the dorsal mesosoma of the wasp with a thin brush. Mite behaviour was videotaped for 10 min after mite release. We examined the time it took for the mite to get into an acarinarium, and mite behaviour that resulted in reaching or not the acarinaria. A total of 9–11 different mites were individually released to each female wasp. Over all, 52 mites and five female wasps were used.

Results

Mite attack and parasitoid counterattack

Adult mites moved vigorously around the surface of the host prepupa. Almost all of the adults except the small males survived until adult host emergence, in a continuous apparent lookout behaviour. Mites also actively moved around on the surface of the pupal host. In both prepupa and pupa, the movement of the mite would stop only when it was feeding on the host´s hemolymph. Parasitoid wasps initially tended to stay at the edges of the vial, with occasional contacts with the host pupa. Once on the host, the parasitoid slowly moved around in a seemingly exploratory manner, stopping occasionally, probably to bite the surface of the host. The duration of halting on the host became gradually longer along the duration of the experiment.

The average number of attacks by all mites on each parasitoid was much higher when seven mites were present in each cell, but the average number of attacks per mite was about the same at both mite densities. Mites were active and attacked the parasitoid more often immediately after the latter was released into the vial than 12 or 24 h later (Figs. 1, 2). However, it was not clear whether the length of time the parasitoid stayed on the host correlated with the number of mite attacks (Figs. 1, 2). Mite attack did not seem directed from a distance, but rather resulted when a mite encountered the parasitoid during the mite's walking over the host's body. When two mites met, they investigated each other and then quickly moved away. Mite attack on the parasitoid involved clinging to it; the first attack was usually a short bite on a parasitoid appendage. Once the parasitoid became weaker and moved slowly, multiple individual mites clung to it, not only on appendages but also on the body. Most likely, the mites bit the membranous parts of the parasitoid body.

Within 24 h, the parasitoid began counterattacking by biting the idiosoma of the mites, when both

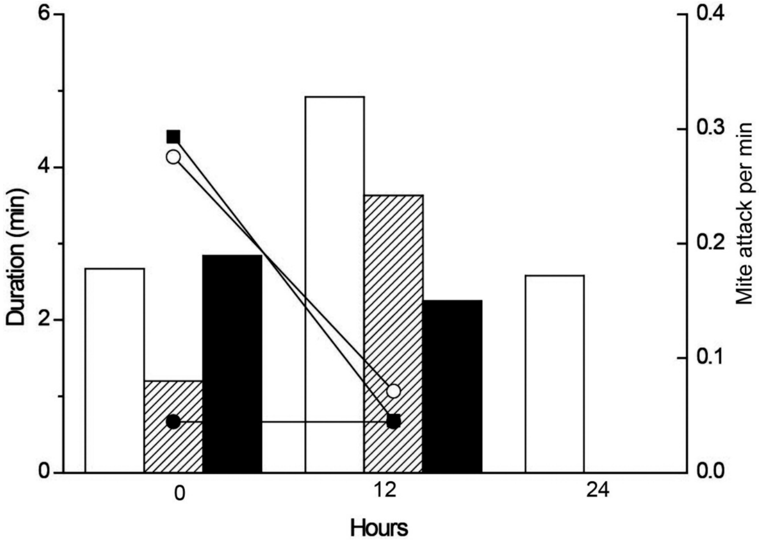

FIGURE 1. Number of attacks (physical contacts between mites and parasitoid) by three mites at each hour (each line with circles or squares representing a vial) and average duration of parasitoid residence on a host per hour (each bar representing a vial). In the vial where mites were indicated by white circles, the parasitoid was indicated by white bars; mites indicated by black circles were associated with the parasitoid indicated by black bars; the rest (hashed bars and black squares) were associated together within the same vial. All mites were killed by the parasitoid within 24 h, when the experiment was discontinued.

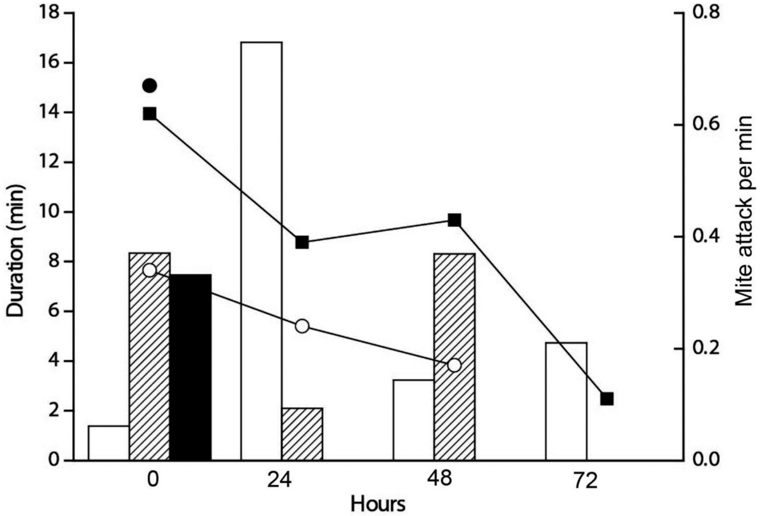

FIGURE 2. Number of attacks (physical contacts between mites and parasitoid) by seven mites at each hour (each line representing a vial) and average duration of parasitoid residence on a host per hour (each bar representing a vial, with a host and a parasitoid). All legends are the same as in Fig. 1. In the vial where mites were indicated by black circles, the parasitoid was indicated by black bars; this parasitoid died within 24 h. In the vial where mites were indicated by white circles, the parasitoid was indicated by slashed bars; this parasitoid became weak at around 48 h and died within 72 h. In the rest of the vials, the parasitoid died within 48 h, but most mites survived.

encountered each other on the host wasp or on the wall of the vial. The mites escaped when the parasitoid tried to bite them, but they were eventually grasped by the parasitoid's mandibles. There was no chasing behaviour during attack and counterattack. Surviving parasitoides began laying eggs within about 72 h. By the time the parasitoid oviposited, all mites were already dead. Mite attack lasted at most three days.

Mite migration into acarinaria

Forty-six percent (24 individuals) of the mites migrated into one of the host acarinaria within 10 min after being released onto the host. In three cases out of 52 replications, mites sequentially entered the same acarinarium while one mite did not enter the acarinarium where the former mite had entered. Most mites entered either mesosomal acarinaria (see Fig. 1 in Makino & Okabe, 2008b); only three entered the metasomal acarinaria. The average duration to get to an acarinarium was 180.74±133.9 seconds (n = 19 mites). It took four seconds for the fastest mite to get to an acarinarium. When a mite reached a mesosomal acarinarium within a few minutes, it either took the shortest course or went along the margin of the scutum.

All mites that entered the mesosomal acarinarium walked into it backwards, with the gnathosoma entering last. Most mites did not exit the acarinarium they had entered but three stayed around the openings of one of the mesosomal acarinaria, moving in and out repeatedly. When a mite was present in an acarinarium and another mite moved close to it, an interaction occurred between the two deutonymphs: either the latter one pushed the other into the acarinarium and also entered, remained at the opening of the acarinarium, or left the acarinarium. However, whether this constituted antagonism between the deutonymphs was unclear.

Discussion

Although it could not be determined what specifically triggered counterattack by the parasitoid wasp, we suggest that it started after the parasitoid recognized the prepupa as a favorable food source. As defensive action when *E. parasitica* were present in the host cell, the parasitoid would have two choices: to attack the mites, to protect the food source (immature *A. delphinalis*) and/ or itself, or to escape, to avoid a potentially lethal mite attack to it and to look for a new cell without mites. Although the parasitoid could not leave the vial in our experimental system, a parasitoid could conceivably escape when mite attack was very serious (i.e., too many mites, perhaps at the early invasive stage). A complementary experimental setup, giving the parasitoid the chance to escape, could indicate the preferred strategy to be adopted. We suspect that because the parasitoid is a generalist that can find alternative host easily, it might often escape rather than confront the attacking mites. Escaping by the parasitoid should be considered a victory of the mite.

Tachinid and phorid flies are also serious natural enemies for both the wasp and the mite, although no attacks by mites on these insects have been observed (Okabe & Makino, 2008a, b). However, we suspect that the mite seldom wins such contests, given that we have never collected dead immature flies (Okabe & Makino, 2008a). Therefore, careful observation on mite behaviour against all natural enemies of the host species is also important to understand the optimal strategy of the mite.

Both social and solitary bees and wasps have numerous parasites and predators of their offspring (Schmid-Hempel, 1998; O'Neil, 2001). While social insects take care of their offspring until juveniles become mature, solitary bees and wasps develop strategies to provide protection for their juveniles when the mothers abandon their offspring after completing the nests. Some eumenine wasps set up an empty cell between those occupied by eggs to discourage natural enemies (Tepedino *et al.*, 1979), while other eumenine wasps avoid repeatedly nesting in the same site, probably to avoid serial mortality of their broods by a local buildup of parasitic species (Itino, 1997). We suspect that several solitary wasps and bees adopt a defensive strategy based on their acarinaria. In this sense, it seems important for newly eclosed adult hosts to pack into their acarinaria as many mites as they can before departing from their nests. For that purpose, they should have proper means to gather or direct mites into the acarinaria. Based on the videotaped mite behaviour and the fact that not many mites are left in a cell after the host left the nest (Okabe & Makino, 2008a), we suspect that the host uses chemical(s) to attract the mite into its acarinaria and/or that the mites take a particular route that might be morphologically adaptive for their locomotion to enter one of the acarinaria. In the future, gland openings of the acarinaria should be examined carefully to understand mite behaviour.

To assure benefits to both partners of a mutualistic relation, one of the organisms often provides a reward to its mutualistic counterpart, which might also serve as an attractant. As examples, a flower provides nectar to attract pollinators, while aphids provide honeydew to attract ants that guard them (Boucher, 1985). If there were no acarinaria on the host wasp, mites could still attach to the surface of their host, perhaps preferring particular locations of the surface (Houck & OConnor, 1991). However, the general surface of an insect's body may not be well suited for that purpose, and some mites may fall off the host. The existence of acarinaria allows safer transportation of mites. Therefore, acarinaria certainly represents a mechanism to benefit both the mites, facilitating their safe transportation from one to another habitat, and their hosts, having the mites as bodyguards. For the wasps, the size of the acarinarium might also be useful to limit mite numbers for invasion into a juvenile cell (Okabe & Makino, 2010). Further examination is needed to clarify such mechanisms.

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References

Boucher, D.H. (1985) *The biology of mutualism: ecology and evolution*. Oxford University Press, Oxford.

Eickwort, G.C. (1994) Evolution and life-history patterns of mites associated with bees. In: Houck, M.A. (eds) *Mites: ecological and evolutionary analysis of life-history patterns*. Chapman and Hall, New York, pp. 218–251.

Houck, M.A., OConnor, B.M. (1991) Ecology and evolutionary significance of phoresy in the Astigmata. *Annual Review of Entomology*, 36, 611–636.

Itino, T. (1997) Comparative behavioral ecology and population dynamics of eumenid wasps. *Memoirs of Faculty of Agriculture, Kagawa University*, 62, 1–206 [in Japanese with English summary].

Krantz, G.W. & Walter, D.E. (2009) *A manual of acarology, 3rd edition*. Texas Tech University Press, Lubbock.

Lombardero, M.J., Ayres, M.P., Hofstetter, R.W., Moser, J.C. & Lepzig, K.D. (2003) Strong indirect interactions of *Tarsonemus* mites (Acarina: Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). *Oikos*, 102, 243–252.

Makino, S. & Okabe, K. (2003) Structure of acarinaria in the wasp *Allodynerus delphinalis* (Hymenoptera: Eumenidae) and distribution of deutonymphs of the associated mite *Ensliniella parasitica* (Acari: Winterschmidtiidae) on the host. *International Journal of Acarology*, 29, 251–258.

OConnor, B.M. & Klompen, J.S.H. (1999) Phylogenetic perspectives on mite–insect associations: the evolution of acarinaria. In: Needham, G.R., Mitchell, R., Horn, D.J. & Welbourn, W.C. (eds) *Acarology IX, vol. 2, Symposia*. Ohio Biology Survey, Columbus, pp. 63–71.

Okabe, K. & Makino, S. (2008a) Life cycle and sexual mode adaptations of the parasitic mite *Ensliniella parasitica* (Acari: Winterschmidtiidae) to its eumenine wasp host, *Allodynerus delphinalis* (Hymenoptera: Vespidae). *Canadian Journal of Zoology*, 86, 470–478.

Okabe, K. & Makino, S. (2008b) Parasitic mites as part-time bodyguards of a host wasp. *Proceedings of Royal Society B*, 275, 2293–2297.

Okabe, K. & Makino, S. (2010) Conditional mutualism between *Allodynerus delphinalis* (Hymenoptera: Vespidae) and *Ensliniella parasitica* (Astigmata: Winterschmidtiidae) may determine maximum parasitic mite infestation. *Environmental Entomology*, 39, 424–429.

O'Neil, K.M. (2001) *Solitary wasps: behavior and natural history*. Cornell University Press, Cornell.

Schmid-Hempel, P. (1998) *Parasites in social insects*. Princeton University Press, Princeton.

Soika, G.A. (1987) Sulla presenza di acarinari nei Eumenidi solitari e descrizione dell'*Acarepipona insolita* n. gen. n. sp., con un acarinario di nuovo tipo. *Bollettino del Museu Civico di Storie Naturale di Venezia*, 34 (for 1985), 189–196.

Tepedino, V.J., McDonald, L.L. & Rothwell, R.R. (1979) Defence against parasitization in mud-nesting Hymenoptera: can empty cells increase nest reproductive output? *Behavioral Ecology and Sociobiol*, 6, 99–104.

Wilson, D.S. (1983) The effect of population structure on the evolution of mutualism: a field test involving burying beetles and their phoretic mites. *The American Naturalist*, 121, 851–870.