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Parasitic mites as part-time bodyguards of a host wasp

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Some bees and wasps that host mites have peculiar pocket-like structures called acarinaria. These have long been considered as morphological adaptations to securely transfer beneficial mites into nests, and thus are thought to be the product of a mutualistic relationship. However, there has been little compelling evidence to support this hypothesis. We demonstrated that the parasitic mite *Ensliniella parasitica*, which uses acarinaria, increases the reproductive success of its host wasp *Allodynerus delphinalis* by protecting it from parasitoid wasps. Every time the parasitoid *Melittobia acasta* accessed a prepupal or pupal wasp host cell, adult mites attacked it, continuously clinging to it and possibly piercing the intersegmental membrane of the parasitoid with their chelicerae. Subsequent mortality of the parasitoid depended on the number of attacking mites: an average of six mites led to a 70% chance of mortality, and 10 mites led to a 100% chance of mortality. In this way, parent mites protect the food source (juvenile wasps) for themselves and ultimately for their offspring. We propose that wasps evolved acarinaria to maintain this protective guarding behaviour.

Keywords: acarinaria; mutualism; parasitism; parasitoid; phoresy

1. INTRODUCTION

Mutualisms are ubiquitous in nature and are of fundamental importance in ecosystems. However, we face several challenges in characterizing these interactions due to their instability across environments, as well as in defining particular relationships as mutualisms (Boucher 1985; Cushman & Beattie 1991; Douglas 1994; Herre *et al.* 1999; Wäckers *et al.* 2005). For example, a broad continuum of heterospecific interactions exists among two or more organisms that provide unequal reciprocal benefits, and the relationships among all associated organisms cannot always be clearly and directly defined. For example, many ants live on plants that provide the ants shelter and nutritious nectar, while the ants attack and thus protect the plant from herbivores that would otherwise damage the plant; yet, if no herbivore enemy appears, the plant receives no benefit, while the ant continues to benefit from the food and shelter (Wäckers *et al.* 2005; Bronstein *et al.* 2006). In addition, the nature of many interactions are difficult to demonstrate because the extent of benefit to each organism can be spatiotemporally unstable (Bronstein *et al.* 2003, 2006; Sachs & Simms 2006) or one interaction may be masked by another (e.g. mycorrhizal fungi and endosymbiotic bacteria of insects; Fitter & Moyersoen 1996; Scarborough *et al.* 2005).

In interspecific relationships between mites and other organisms, among the most intriguing phenomena are the distinctive external structures found on some hosts. For example, some plants develop leaf domatia, tufts of hair or small invaginations on the undersides of leaves, which function as shelters for predatory or fungivorous arthropods, including mites, and are assumed to mediate mutualisms (Walter 1996; Agrawal & Karban 1997). Pockets on some lizards are similar to domatia in that they harbour mites, although they may have developed to concentrate blood-sucking chiggers in less sensitive locations, thus avoiding large-scale damage to the skin (Arnold 1986; Benton 1987). Other interesting but puzzling structures are the acarinaria found in some groups of Hymenoptera (figure 1; Skaife 1952; Soika 1987; OConnor & Klompen 1999; Makino & Okabe 2003). Acarinaria are considered one of the best examples of a mutualistic adaptation because they are apparently specialized to shelter mites, and exhibit a high specificity between hosts and mites. This hypothesis assumes that associated mites benefit hosts by destroying harmful pathogens or parasites (Eickwort 1994; OConnor & Klompen 1999), although no supporting evidence to date exists, and Klimov *et al.* (2007) suggested that acarinaria on apid bees developed to control harmful mites.

Several genera of eumenine wasps have well developed acarinaria on both sides of their scutellum, propodeum or the second matasomal tergite, in which they harbour specific enslinielline mites (Soika 1987; Eickwort 1994; Klompen & OConnor 1995; Makino & Okabe 2003). Among them, *Allodynerus delphinalis* (Giraud 1866) is the only species whose life history with its associated mite *Ensliniella parasitica* (Vitzthum 1925) is known. The wasp, which ranges from Europe to Japan (Yamane 1990; Klompen & OConnor 1995), is a small (adult body length 6–10 mm) solitary hunting wasp that nests in dead plant stems by excavating their pith (Enslin 1922; Benno 1945; Crèvecoeur 1945). This wasp makes one to seven brood cells (approx. 4.5 mm in diameter, 20 mm in length) in a nest, and its life cycle is similar to that of other tube-nesting eumenine wasps (see Krombein 1967). A female adult lays one egg in a brood cell, which she provisions

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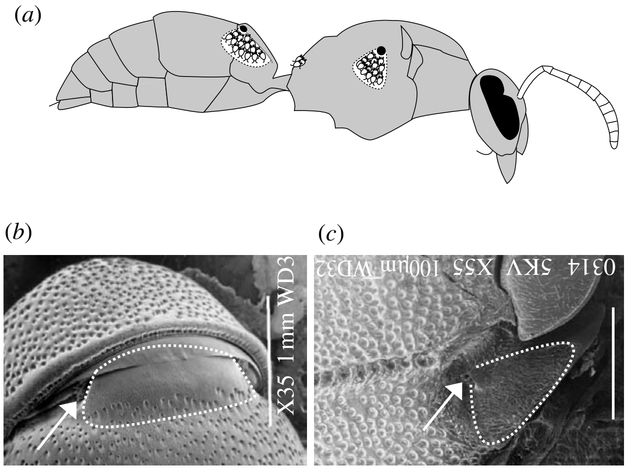

Figure 1. Acarinaria of *Allodynerus delphinalis* (female) harbouring deutonymphs of *Ensliniella parasitica* on both sides of the scutellum, propodeum and second metasomal tergite. (*a*) Diagram indicating the locations of acarinaria. Black dots indicate holes opening to the scutellar and metasomal acarinaria, the approximate shapes of which are indicated by the dotted lines; mites are also shown. (*b*,*c*) The inside of the (*b*) metasomal and (*c*) scutellar acarinaria are indicated by dotted lines, and openings are indicated by arrows on *A. delphinalis* taken with a scanning electron microscope (JEOL type JXA840A). Scale bars, (*b*) 1000 µm, (*c*) 500 µm.

with paralysed microlepidopterous prey (mostly Gelechiidae in Japan; Okabe & Makino 2008). After provisioning, the female closes the brood cell with mud mixed with her saliva. While the wasp is nesting, the *E. parasitica* deutonymph invades the wasp cell, moults to the tritonymph and adult (idiosomal length 400–700 µm; K. Okabe 2007, unpublished data) while sucking haemolymph from host–prey and then from the host itself, and lays eggs on the pupa. When the eggs hatch, the larvae and the protonymphs feed on the pupa without killing it and develop into deutonymphs by the time of host eclosion (Okabe & Makino 2008). The life cycle is basically similar to that of *Ensliniella kostylevi* (Klompen *et al.* 1987). Okabe & Makino (2008) found that approximately 90% of *A. delphinalis* cells (more than 380) collected in the field over 2 years (2006 and 2007) in Tsukuba, Japan, harboured one or more *E. parasitica*. Although no obvious negative or positive effects of mite presence were observed on host survival, dead mites were sometimes found in cells accessed by the parasitoid *Melittobia acasta* (Walker), a cosmopolitan species that attacks a wide range of Hymenoptera (Evans & West Ebehard 1970; Tepedino *et al.* 1979). A mated adult female *M. acasta* (body length 1–1.5 mm; S. Makino 2007, unpublished data) invades brood cells of hosts either before or after cell partitioning and lays eggs on the prepupal or early pupal hosts. After hatching, the parasitoid larvae feed on the host's body fluids, often resulting in death of the host (Maeta 1978; Dahms 1984; González *et al.* 2004). Because the survival of *E. parasitica* mites depends entirely on the *A. delphinalis* wasp, the parasitoid is also a natural enemy of the mite. We performed a series of field and laboratory experiments on *A. delphinalis*, *E. parasitica* and *M. acasta* to better understand the relationships among these three species.

2. MATERIAL AND METHODS

(a) *Collections and experiments*

In 2006 and 2007, we collected nests of *A. delphinalis* in an approximately 5×10 m area of grassland dominated by *Solidago altissima* L. and *Conyza sumatrensis* Walker (Compositae) in Tsukuba, Ibaraki Prefecture, Japan (36°05′58″ N, 140°04′59″ E). In the laboratory, wasp nests in dead *S. altissima* stems were opened and nest contents were recorded.

Prepupae of *A. delphinalis* used in the mite–parasitoid interaction experiments were obtained by rearing wasps in the laboratory using the methods of Okabe & Makino (2008). *Melittobia acasta* was originally collected from *A. delphinalis* nests in the field and was maintained by transferring a mated female to a prepupa of *A. delphinalis* for reproduction. After newly emerged females mated, they were placed in a small acrylic tube and maintained at 8°C for at most 5 days until the experiment. To examine the interactions among wasps, mites (*E. parasitica*) and parasitoids (*M. acasta*), 3 (female : male = 2 : 1), 7 (4 : 3 or 5 : 2) or 10 mites (6 : 4 or 5 : 5) were transferred to an acrylic tube (5 mm diameter and 50 mm length) containing a prepupal host (1–2 days after meconium excretion). When possible, mites were taken from the same host cell; otherwise, they were taken from different host cells of the same age. We used only adult mites because every mite was an adult at the time when the parasitoid attacked a host in the prepupal or early pupal stage (Okabe & Makino 2008). We transferred one inseminated female *M. acasta* into the tube and plugged both ends with cotton. We examined the tube contents daily under a stereomicroscope and terminated the experiment when all mites or the wasp had died. Each mite treatment was replicated 10 times; 10 mite-free tubes were used as controls.

(b) *Video recording of the interaction*

We used a 3CCD camera with a video recording system (colour video camera, DXC-390, Sony; connected to a camera adaptor, CMA-D2, Sony; hard disk recorder, VR-509, Victor) for videotaping mite–parasitoid interactions. To record these interactions, three tubes with three or seven mites were videotaped until either all mites or all parasitoids died. Any physical contact between mites and the parasitoid was considered mite attack, regardless of the duration of clinging. The counter-attack by the parasitoid was also recorded. Using video playback, we counted the number of mite attacks. The number of attacks per mite was calculated by dividing the total number of attacks by the number of mites. Every 12 or 24 hours (for experiments with three or seven mites, respectively), the interactions between the mites and the parasitoid were analysed for the subsequent 3 hours.

(c) *Statistics*

We analysed whether the distribution of mite numbers in a cell (figure 2) fit Poisson distribution or concentrating distribution. For concentrating distribution, we calculated variance : mean. To analyse the relationship between mortality of the parasitoid and the number of mites present, we used logistic regression analysis (binominal error distribution). To compare the number of attacks by all mites or by individual between treatments with three and seven mites, we used a generalized linear model (Poisson error distribution). All statistical analyses were conducted using the statistics package of R v. 2.7.0.

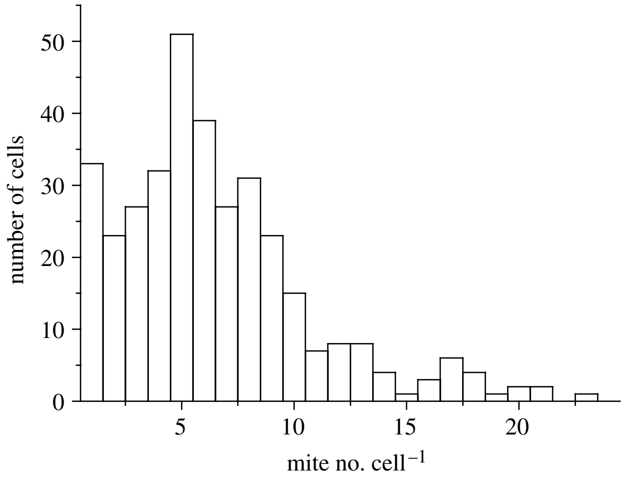

Figure 2. Frequency distribution of mites in *A. delphinalis* brood cells from nests collected in the field between 2006 and 2007. In total, 348 nests containing one to five cells were examined.

3. RESULTS

In 92.8% of host cells infested by mites, mite numbers ranged from 1 to 23 (figure 2). The mode of mites per cell was 5 and the average was 6 ± 4.3 (mean±s.d., $n=348$). The distribution of mite numbers in a cell (figure 2) was not random ($\chi^2=160.154$, $p=0.00$) but concentrated (variance : mean $=2.94$, $p=0.00$). Nests were sometimes infested with other natural enemies, including the parasitoid wasp (*M. acasta*), a kleptoparasitic fly and unknown pathogens. The latter two enemies occurred infrequently, with annual infestation rates of less than 5% (figure 3). Although very low in the first year, the infestation rate of *M. acasta* greatly increased in the second year (figure 3), in parallel with increases in the population density of *A. delphinalis*. In the field, 70% of cells invaded by *M. acasta* had a single parasitoid (with an average of 1.52 ± 1.0, $n=33$). When the parasitoid and adult mites co-occurred in a host cell, either all mites or all parasitoids died. In cells without parasitoids, the mites completed their normal life cycles on the host.

For a more detailed analysis of interactions between the parasitoid and the mites, we observed their behaviour in the laboratory. At the beginning of the experiment (for the first 1–3 days), the introduced parasitoid occasionally walked on the surface of the tube or the host, but spent most of the time hiding near the cotton plug. By contrast, the mites usually crawled on the surface of the host or the cell wall (see electronic supplementary material 1). Although the mites did not interact much with each other, even during occasional encounters, when mites encountered the parasitoid, both females and males clung to it, whether it was on or off the host, and the parasitoid attempted to escape from the clinging mites (electronic supplementary materials 1 and 2). In some cases, attacked parasitoids eventually died. Based on observations of mites clinging to an injured parasitoid, the mites may pierce the intersegmental membrane of the parasitoid with their chelicerae. However, in other cases, the parasitoid counter-attacked mites by biting them repeatedly on their dorsum (electronic supplementary material 3). Although the trigger of this aggression in *M. acasta* was unclear, physical contact with the host (*A. delphinalis*) appeared to promote the behaviour during the first 12–72 hours.

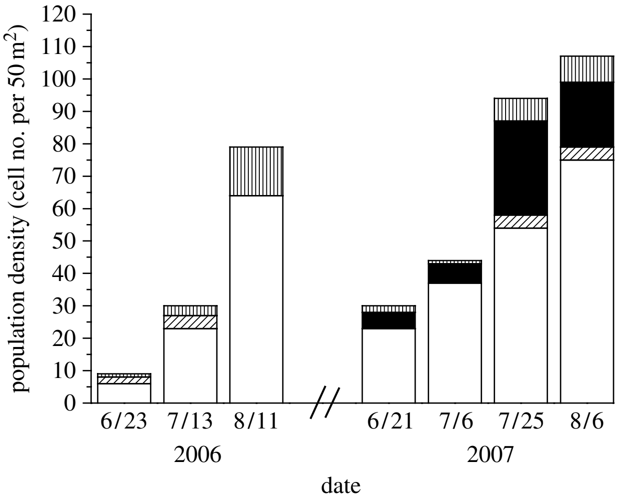

Figure 3. Number of juvenile wasps surviving to adulthood (white areas), killed by a kleptoparasitic fly (diagonally striped areas), killed by the parasitoid *M. acasta* (black areas) or died for unknown reasons (vertically striped areas) in 2006 and 2007 at the study site (5×10 m quadrat within an approx. 50×1500 m strip of *S. altissima*).

The probability of mites killing the parasitoid depended on the number of mites present (logistic regression analysis, $\chi^2_3=42.448, p<0.00001$; figure 4*a*). Under mite-free conditions, the parasitoid laid several eggs on the host, similar to previous reports for many *Melittobia* species (Maeta 1978; Dahms 1984). While the number of attacks on a single parasitoid was significantly higher with seven than with three mites ($z=3.473$, $p=0.000515$), the number of attacks per mite was not significantly different between seven and three attacking mites ($z=0.333$, $p=0.7388$; figure 4*b*,*c*). When the parasitoid died, almost all mites survived to produce offspring on the pupal host. However, when the parasitoid successfully laid eggs on the host, all mites and the host died.

4. DISCUSSION

Our results provide the first evidence that the relationship between the endosymbiotic mite *E. parasitica* and its host wasp *A. delphinalis* is a mutualism that is usually masked by parasitism. The mutualism is mediated by protection–reward: the mite protects the host wasp and feeds on the host's haemolymph as a reward that does not kill the host. Solitary wasps generally employ various strategies for their offspring in terms of nest structure (Tepedino *et al.* 1979) or behaviour (O'Neill 2001) against natural enemies. However, the use of symbionts as 'bodyguards' against natural enemies has never been documented in either solitary or social Hymenoptera (Schmid-Hempel 1998; O'Neill 2001). Predatory organisms may become reliable defenders of their hosts, as in many ant–plant mutualisms (Bronstein *et al.* 2006); however, *E. parasitica* and related species are not known to prey upon other species, although some might be accidental predators (OConnor 1982). Our results provide the first evidence of any astigmatid mite having antagonistic, often lethal, confrontations with a parasitoid. However, the mite does not seem to have a physical structure particularly adapted to killing parasitoids, and we suspect that by clinging to the enemy with their chelicerae, in the same way they pierce the skin of the wasp host to feed on it, they haphazardly

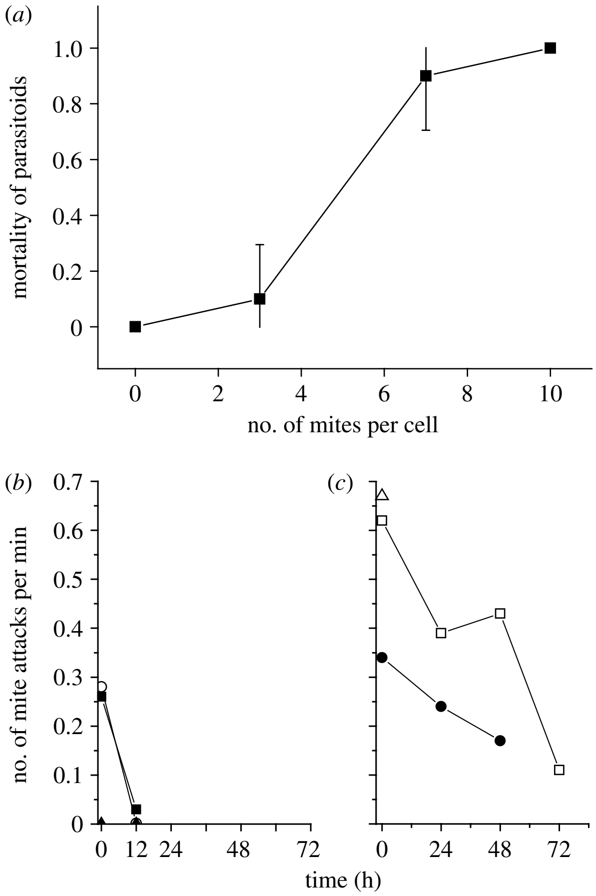

Figure 4. Mortality of the parasitoid, presumably due to mite attacks. (*a*) Logistic regression showed that significant differences in mortality existed depending on mite abundance ($\chi^2_3=42.448$, $p<0.00001$). Error bars indicate 95% CIs. (*b*,*c*) Number of mite attacks (physical contact) against the parasitoid per minute. (*b*) Three or (*c*) seven adult mites were introduced together with a parasitoid into an acrylic tube containing a prepupal host. Results from six different tubes are indicated by open squares, an open triangle, open circles, filled squares, filled triangles and filled circles. In the three-mite experiments, all mites died within 24 hours. In the seven-mite experiments, all mites died within 72 hours in two tubes, whereas the parasitoid died within 24 hours in one tube (triangles).

injure the parasitoid. Mite density did not facilitate attacks by individual mites, but parasitoid mortality increased with the number of mites, presumably because the total number of injurious attacks increased (figure 4, electronic supplementary material 2).

However, parasitoids sometimes counter-attacked, which almost always resulted in the death of all mites (electronic supplementary material 3). An average number of mites in a host cell can kill a parasitoid with over 70% probability (figure 4*a*). Under natural conditions inside a host cocoon, the likelihood that mites and parasitoids would encounter each other is greater, suggesting that even fewer numbers of mites might be sufficient to kill a parasitoid. These data, together with the fact that 67% of host cells contained more than five mites and 84% contained more than three mites (figure 2), suggest that the mite is a reliable contributor to its host survival. Mite numbers in a cell were not distributed randomly but were concentrated with unknown mechanisms. For the host wasp, keeping sufficient numbers of mites are crucial to protect offspring. Among wasps associated with symbiotic mites, species without acarinaria harbour fewer mites on their bodies (approx. 100 *Kurosaia jiju* on *Anterhynchium flavomarginatum micado*; Okabe & Makino 2003) and in their brood cells (1.92±2.4 in *K. jiju*; Okabe & Makino 2003) compared to species with acarinaria (approx. 300 mites in acarinaria and 3–10 *Vespacarus* mites per cell with *Stenodynerus* hosts; this study; Krombein 1967). Therefore, acarinaria may ensure beneficial numbers of mites in each cell.

For development and maintenance of the acarinaria, the selection pressure by the parasitoid must have been high. When wasps and bees nest in high densities at the same location for several years, their natural enemies (e.g. coleopterans, dipterans and hymenopterans) easily locate and infect nests (O'Neill 2001). Our results also showed the same tendency in the field with limited nesting resources (figure 3). Because the wasp occurs in patchy habitats in grasslands or bushes (Okabe & Makino 2008), its continuous use of the same habitat increases the numbers of natural enemies. *Melittobia* parasitoids are one of the most threatening natural enemies because they parasitize a wide range of insects including bees and wasps (Maeta 1978; Dahms 1984); they can invade eumenine cells even after they are sealed with mud (Maeta 1978), and a single individual can kill a juvenile wasp (Maeta 1978; Dahms 1984; González *et al.* 2004; this study). Many cells are probably abandoned by mother wasps due to infestations (Okabe & Makino 2008) and empty cells are sometimes built near the nest entrance (K. Okabe & S. Makino 2007, unpublished data), a common protective strategy used by solitary bees and wasps (e.g. Münster-Swendsen & Calabuig 2000). However, empty cells are not very effective against *Melittobia* parasitoids (Maeta 1978). Therefore, their mite bodyguard is essential for their survival against this parasitoid.

Interspecific interactions can drive the evolution of morphology and life history. For example, pollination mutualisms lead to specific morphologies in each pair (Boucher 1985), and a parasite can modify the behaviour of its intermediate host so that it is more easily preyed upon by the definitive host for subsequent parasite dispersal (e.g. nematode parasitism of ants; Yanoviak *et al.* 2008). Because the mite does not reduce the reproductive success of its host wasp (Okabe & Makino 2008), it can be considered a commensal partner in the absence of the parasitoid. Therefore, we hypothesize that *A. delphinalis* evolved acarinaria to maintain *E. parasitica* on their bodies for the eventual guarding of their offspring in the wasp nest. The mite–wasp system studied here is unique because adult costs (acarinaria development by the adult wasp or guarding by the adult mite) directly benefit juvenile partners.

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