Infection behavior of a parasitoid fly, *Emblemasoma auditrix*, and its host cicada *Okanagana rimosa*

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Abstract

Males of the cicada *Okanagana rimosa* (Homoptera: Cicadidae) that produce calling songs are parasitised by the parasitoid fly *Emblemasoma auditrix* (Diptera: Sarcophagidae). An ethogram of the infection behavior was extracted from videotaped experiments with tethered hosts. The infection behavior can be divided into three phases, each involving different sensory cues: long-range host detection via acoustic signals, visual short-range orientation, and a contact phase with tactile/chemical cues. After phonotaxis by flight, the fly lands on or near the host cicada. It walks around the host to identify the caudal end and squeezes underneath the wings (with a 64–67% preference of the left side). Finally, *E. auditrix* cuts into the timbal and deposits a larva into the sound producing organ of *O. rimosa*. This highly specific behavior restricts the host range to cicadas, only two species of which occur simultaneously with the parasitoid. During the infection behavior, the fly does not discriminate between male and female hosts. However, females were not successfully infected during the experiments. The host *O. rimosa* shows only weak defense behavior (mainly flapping their wings) and seems to rely on parasitoid avoidance. Production of the protest song does not prevent infection.

This paper includes one video that can be accessed at http://insectscience.org/4.36

Introduction

Host finding and selection often involves different processes such as habitat localization, host localization, host discrimination, and host acceptance (Vincent 1976). Such host finding and selection strategies are not very well understood in parasitoid Diptera (Feener and Brown 1997). Therefore we analysed host infection behavior in the sarcophagid fly *Emblemasoma auditrix* that parasitizes singing males of the cicada *Okanagana rimosa* (Soper et al. 1976b).

The parasitoid possesses an ear at the prothorax (Lakes-Harlan et al. 1999; Robert et al. 1999) and uses acoustic cues for host location (Soper et al. 1976b; Lakes-Harlan et al. 2000). The acoustic cue seems to be a major and sufficient cue for host finding, because flies can be attracted to loudspeakers broadcasting the calling song or song models (Soper et al. 1976b; Lakes-Harlan et al. 2000). The signal is a long-range signal allowing homing in on hosts from distances of 2–4 m (Köhler and Lakes-Harlan 2001). Furthermore, the neuronal networks are adapted to the characteristics of most likely a single species (*O. rimosa*), as can be seen in the specific phonotactical response (Lakes-Harlan et al. 2000; Köhler and Lakes-Harlan 2001). Specialized parasitoids can adapt to host-specific signals, which may be advantageous for host finding, compared to generalist parasitoids (Godfray 1994). Using the described system, our goals were to evaluate the specificity of host infection behavior, to identify possible sensory cues involved in the process, and to analyse the defense behavior of the host. Such an infection behavior has not yet been reported for sarcophagid flies. Therefore, experiments with tethered hosts were performed accompanied by field observations.

Materials and Methods

The natural host infection behavior was observed at six different times in northern Michigan between 1996 and 2002. In June 2000, experiments were set up in Emmet County, Michigan to videotape the parasitoid-host interactions. Experiments were performed at the margin of a forest where both host and parasitoid occur. A small portable box (30 cm × 50 cm) containing the electronic equipment was placed on the ground. A piezo loudspeaker was mounted into the lid of the box for broadcasts of a phonotactically attractive signal. A song model of the host calling song (for details on the signal and its generation, see Lakes-Harlan et al. 2000) was stored on a compact disc. The song model consisted of near rectangular sound pulses with a carrier frequency of 9 kHz and a repetition rate of 83 Hz. The signal was replayed with a discman...
In June 2002 an additional experiment was performed to test the alertness and defense behavior of unrestrained hosts. Male (n = 50) or female (n = 40) cicadas were carefully approached in the habitat ensuring that they did not react to the approaching person. Generally males continued singing or females continued laying eggs. After a fly arrived at the experimental box, the sound was turned off again and the flies immediately continued with infection behavior and the sound was turned off again. After each experiment the host was checked for the presence of a larva or indications of infection (ruptures in the timbal). Parasitoids were caught only in a minority of the experiments, because they usually suddenly left the host and flew away. In order to minimize pseudoreplication, we waited for approximately five minutes before starting the next experiment, disturbed the nearby vegetation in order to scare flies away and changed the location, each about 200 m apart, after 5–7 experiments.

Notes and videos were carefully analysed and an ethogram of the infection was extracted. Hosts were preserved in 70% ethanol and later dissected under a dissection microscope (Leica M5). Special attention was given to injuries of the timbal and the presence of larvae within the abdomen. Additionally, a sample of infected male cicadas collected in June 1999 in the same area was also dissected. Timbals were photographed with a CCD camera (1048*768 pixel) attached to the dissecting microscope.

Some timbals of cicadas and the abdomen of the parasitoid were viewed with scanning electron microscopy. Tissue was dehydrated, critical point dried and gold sputtered. Preparations were viewed with a Leo 438VP microscope and digital pictures (1024*768 pixel) were obtained.

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T-test and chi-square tests were conducted using Prism software (GraphPad, www.graphpad.com).
Figure 1. Infection behavior of the parasitoid *Emblemasoma auditrix* and the host *Okanagana rimosa*. A Ethogram illustrating the steps of the three different phases: long range host finding with acoustic signal and approach by flight; short range host location in which the fly orientates visually and walks towards the host; contact phase leading to larva deposition and departure from the host. The long-range approach can also lead to direct contact. If the parasitoid is disturbed it either starts again at any position of the sequence or it leaves the host. B Duration of the short-range phase for male and female host candidates (n = 15 in both cases; no significant difference). C Duration of the contact phase (n = 24 for males and n = 33 for females; p < 0.05). D Percentage of attacks with completed behavioral elements of the contact phase (n = 23 for males, n = 29 for females). The shading indicates percentages of attacks with positioning, timbal cut and larva deposition. The element “timbal cut” is defined as abdominal movement, not as completed cut of the timbal. The latter was only seen with males where a successful larva deposition took place.
Figure 2. Four pictures from a video showing the infection behavior of *Emblemasoma auditrix*. A male cicada was attached on top of a loudspeaker. The fly approaches the host by walking (A), visually identifies the wings and the abdomen (B, C) and squeezes caudally underneath the wings (C, D). During the contact phase (D) it pushes the left wings aside, moves anteriorly with the head on top of the cicada and attempts to locate the timbal with its ovipositor. Scales: 1cm.

**Left-right asymmetry of infection**

On their first attack, most flies squeeze underneath the host wings from the left side: 65% in males (n = 26) and 68% in females (n = 22). If the attack is interrupted or incomplete, the flies can also move to the right side. The bias to the left side is not induced by the artificial situation of the infection experiments because an analysis of a sample of infected cicadas from the same area confirmed the result. Cicadas infected with larvae of *E. auditrix* always had injuries in a timbal (n = 36) and in 64% the ruptures were in the left timbal. This asymmetry is statistically significant (d.f. 4.463, p = 0.0376).

**Defense of the host**

In field observations and during the experiments, little defense by the cicada was seen. Two defense behaviors are possible: to scare off the parasitoid and/or to escape. Scaring includes holding the fly with the wings, flipping the wings, and production of protest song. The effectiveness of each of the behaviors has not been determined due to the use of restrained hosts. During “holding,” the cicada presses the wings strongly against the fly and suppresses movements of the parasitoid. The holding is difficult to quantify, but the behavior seems to occur more often in females than in males. At least in some cases, the parasitoid leaves the host after the release without completing the infection. In about 13% of the infection experiments, both sexes of *O. rimosa* flipped their wings after contact of the fly (Fig. 3A). However, despite the movements the flies often manage to stay on the host. Occasional field observations in which flying cicadas were attacked show that even during flight the flies managed to stay on the host. When cicadas are handled or scared they often produce a protest song. Such songs were produced by *O. rimosa* in 32% of the experiments. This sound has a mean duration of 13 seconds (SEM 2.6; n = 8) and ceases thereafter although the fly is still in contact with the host.

By contrast, males of the related species *Okanagana canadensis* show more defensive behavior. In 85% of the attacks, male *O. canadensis* flip their wings (Fig. 3A) and they always produce a protest song as long as the fly contacts the host. However, even on this species flies manage to stay on the host, and no obvious influence of the protest song on the fly’s behavior has been detected.

Figure 3. Defense behavior of the host candidates during the infection experiments (A) and experiments in which the animals were approached with a stick (B). A Comparison of two elements of defense behavior (display of wing flips and production of protest song; Dist. noise) between males of the sister species *Okanagana canadensis* (O.c.; n = 13) and *Okanagana rimosa* (O.r.; n = 24). B Defense behavior elements of males (n = 50) and females (n = 40) of *O. rimosa*. 
Escape seems to be the only effective behavior for parasitoid avoidance, and the difficulty to approach *O. canadensis* in the field is an indication for the alertness of the species.

In order to test the escape reaction and defense behavior elements in freely moving *O. rimosa*, a separate experiment was performed in the field. The animals were approached carefully with a thin stick from behind, attempting to lift the left wings and to touch the region of the timbal. Sixty-two percent of the females reacted with wing flips and 35% escaped by flight (Fig. 3B; n = 40). Males displayed wing flips in 28% of the experiments and only 20% escaped by flight (n = 50). In these experiments, 30% of the males produced a short protest song, a similar percentage to the experiments with restrained cicadas.

**Morphology of the timbal and the ovipositor**

Light microscopy and scanning electron microscopy were performed to analyse morphological structures involved in larval deposition. Female *E. auditrix* possesses a sclerotized genital plate at the ventral abdomen, which is used to cut into the timbal (Figs. 4A, B). The plate is formed at the sternite of the fifth abdominal segment and has a curved shape without sharp teeth or other obvious structures (Fig. 4B). The ovipositor, dorsal of the genital plate, is covered with many hairs.

Male cicadas have bilateral symmetrical timbals at the first abdominal segment that are used for sound production. The timbal of *O. rimosa* is a thin membranous structure supported by 7–8 dorso-ventrally running sclerotized ribs (Fig. 4C). Cicadas infected with a parasitoid larva always have injuries in a timbal. The injury occurs anterior-ventrally at one timbal (Figs. 4D, Figs. 5A–D). The larval deposition involves breaking of 1–3 rips (Figs. 5A–D) and rupturing of the membrane (Fig. 4D). An injury of the tympanum has been never been found even not in the females (which lack a timbal).

**Discussion**

We have studied the infection behavior of females of the sarcophagid parasitoid *E. auditrix*. The fly phonotactically locates its hosts, which are the singing males of the cicada *O. rimosa*. The infection behavior was only rarely observed in the field. Therefore we set up experiments with restrained hosts to analyse the behavior in detail. This experimental approach involves some possible differences compared to the freely moving hosts in the field: males
did not produce the calling song by themselves, animals could not escape and the effectiveness of defense might be different from unrestrained animals. However, the occasional field observations confirm that the restraining does not change the behavior in general.

**Specificity of infection behavior**

*Emblemasoma auditrix* is adapted to specific parameters of the calling song of the only known host species (*Lakes-Harlan et al. 2000; Köhler and Lakes-Harlan 2001*). This adaptation correlates with the highly specific infection behavior: the fly pushes underneath the wings from behind and injures the timbal of the male cicada for larva deposition. The parasitoid shows the same behavior when interacting with female cicadas. In the field a very low percentage of females were infected (*Soper et al. 1976b*). It is unknown how the larvae enter the female body, because females lack a timbal. Why are females not discriminated against, relative to males, for more efficient foraging? It is likely that the primary cue, the auditory signal from the male, makes it unnecessary to select for discrimination between males and females. Perhaps in crowded populations of cicadas the flies might be attracted by a calling male, but instead of finding the male, encounter a nearby female. In these populations new males can be located rather fast and a waste of energy is probably not a limiting factor. An infection via the timbal has further consequences: infected males cease to produce calling song, which might allow a much higher survival rate of larvae.

This specific infection mechanism reduces the potential host range to male cicadas and up to now *E. auditrix* has only been found in *O. rimos.* Other acoustically hunting parasitoids (tachinids) deposit their larvae either on the host, on the substrate nearby the host or even expel larva into the direction of the host (*Wineriter and Walker 1990; Walker 1993; Fowler and Martini 1993; Adamo et al. 1995b; Allen et al. 1999*). Deposition on the substrate reduces the larval survival (*Allen et al. 1999*) and, in all cases, the larva enters the host’s body by itself. Whereas the tachinids have several hundred larvae (*Allen et al. 1999*), *E. auditrix* has a mean number of 35 larvae at the beginning of the season (*deVries and Lakes-Harlan, unpubl. results*). Thus, the low number correlates well to the specific infection behavior, which might allow a much higher survival rate of larvae.

**Possible sensory cues in host infection**

The described sequence of infection behavior might involve different sensory signals for host infection: in the long range the acoustic signal is most important, in the short range visual signals might be important, and in the contact phase mechanical and chemical signals may be involved. The sequence of different stimuli might elicit a chain of infection behavior, which is also seen in other parasitoids (e.g. *Karamauna and Copland 2000*). However, the sequence does not represent a strict hierarchical order, for example, a visual identification of the wings and a subsequent caudal approach is not necessary, although often seen. The primary cue for host finding is certainly the auditory signal. A silent cicada in a quiet environment was never observed to be approached by a fly in the field. The secondary cue is probably a visual signal. Visual objects influence the phonotaxis response of *E. auditrix*, although the auditory signal is dominant (*Schniederkötter and Lakes-Harlan 2001*). The short-range approach with identification of the caudal end of the host, also involves visual input. This scanning of the host resembles to some extent the visual scanning behavior of honeybees (*Lehrer et al. 1985*). Generally, the visual system of Diptera is highly evolved and contributes to many different behaviors, including host location (*Morehead and Feener 2000; Stireman III 2002; Yamawaki et al. 2002*). In the tachinid *Homotrixia allenii*, visual cues enable females to find the direction of the host, but sound alone can trigger larviposition (*Allen et al. 1999*). It is not yet clear whether mechanical and chemical stimuli play a role in assessing host suitability and larva deposition. Chemical stimuli could be important for discrimination of hosts with existing infections of a fungus (*Soper et al. 1976a*) or other parasitoids. The tachinid *O. ochracea* deposits larvae on dead *Gryllus sp.*, on plastic coated crickets and cricket models, suggesting that chemical cues are less important (*Cade 1975; Adamo et al. 1995a*). The importance of the different sensory cues for host infection and possible host discrimination has now to be tested experimentally.

Whether sensory cues are responsible for the asymmetry in the infection side is unknown. No anatomical correlate has been found on either the cicada or on the parasitoid. The biology of the host does not give any hints for a necessity of a lateral asymmetry. Both timbals are equally involved in sound production (*Stöllting et al. 2004*) and also the behavior of the cicada does not suggest any lateral bias. Thus, so far the asymmetry in infection behavior remains unexplained.

**Defense of the host**

During the experiments and in observed parasitoid attacks in the field, *O. rimos* usually showed only weak defense behavior. For defense, male cicadas could produce a protest song, flip their wings, hold the parasitoid with wings or escape. An internal defense (e.g., encapsulation of the parasitoid larva) has never been observed. Interestingly, the production of the protest song does not seem to influence the parasitoid. Male *O. rimos* produce it only for a short time, but also the long, ongoing noise of *O. canadensis* does not repel flies. For the attacked individual, rigorous movements, especially flipping of wings, are more effective. This behavior does not provide complete protection against parasitization because parasitoids manage to stay on the host. Nevertheless, such movements are a common strategy of defense (*Godfray 1994*) and might enhance the chances to escape parasitization at relatively low cost. Therefore, it is surprising that the frequency of individuals displaying such behavior is rather low.

Only early escape by flight seems to be an effective defense. However, the field data suggest that many cicadas do not escape. By contrast, the partly sympatric species *O. canadensis* is much more alert. It produces long protest songs, rigorously flips its wings, and readily escapes. No parasites have been found in this species; however, it is not known whether this defense is the only decisive factor. A low attractiveness of the acoustic signal might also be important (*Lakes-Harlan et al. 2000*). In *O. rimos*, the rate of successful infections in the field is unknown. However, the limited possibilities to avoid infection suggest that the host relies more on avoidance of discovery by the parasites in the first place, again a common strategy in many insects (*Godfray 1994*). An absolute
avoidance cannot be archived because the signal used by the parasitoid is also used for sexual behavior and reproduction of the host. Thus, the selective pressure by the parasitoid might act directly on the sound production of the cicada, which is subject to further studies.

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References

Adamo SA, Robert D, Hoy RR. 1995a. Effects of a tachinid parasitoid, Ormia ochracea, on behavior and reproduction of its male and female field cricket hosts (Gryllus spp). Journal of Insect Physiology 41: 269–277.

Adamo SA, Robert D, Perez J, Hoy RR. 1995b. The response of an insect parasitoid, Ormia ochracea (Tachinidae), to the uncertainty of larval success during infestation. Behavior, Ecology and Sociobiology 36: 111–118.

Allen GR, Kamien D, Berry O, Byrne P, Hunt J. 1999. Larviposition, host cues, and planidial behavior in the sound-locating parasitoid fly Homotrixa alleni (Diptera: Tachinidae). Journal of Insect Behavior 12: 67–79.

Cade W. 1975. Acoustically orienting parasitoids: fly phonotaxis to cricket song. Science 190: 1312–1313.

Feener DH, Brown BV. 1997. Diptera as parasitoids. Annual Review of Entomology 42: 73–97.

Fowler H, Martini A. 1993. Larval dispersion of Ormia depleta (Towns.) (Dipt., Tachinidae). Journal of Applied Entomology 115: 107–110.

Godfray HCJ. 1994. Parasitoids. Princeton. Princeton University Press.

Karamaouna F, Copland MJW. 2000. Oviposition behavior, influences of experience on the host size selection, and niche overlap of the solitary Leptomastix epona and the gregarious Pseudaphycus flavidulus, two endoparasitoids of the mealybug Pseudococcus viburni. Entomologia Experimentalis et Applicata 97: 301–308.

Köhler U, Lakes-Harlan R. 2001. Auditory behavior of a parasitoid fly (Emblemasoma auditrix, Sarcophagidae, Diptera). Journal of Comparative Physiology A 187: 581–587.

Lakes-Harlan R, Stölting H, Moore TE. 2000. Phonotactic behavior of a parasitoid (Emblemasoma auditrix, Sarcophagidae, Diptera) in response to the calling song of the host (Okanagana rimosa, Cicada, Homoptera). Zoology 103: 31–39.

Lakes-Harlan R, Stölting H, Stumpner A. 1999. Convergent evolution of insect hearing organs from a preadaptive structure. Proceedings of the Royal Society London B, 266, 1161–1167.

Lehrer M, Wehner R, Srinivasan M. 1985. Visual scanning behavior in honeybees. Journal of Comparative Physiology A 157: 405–415.

Morehead S, Feener D. 2000. Visual and chemical cues used in host location and acceptance by a dipteran parasitoid. Journal of Insect Behavior 13: 613–625.

Robert D, Miles RN, Hoy RR. 1999. Tympanal hearing in the sarcophagid parasitoid fly Emblemasoma sp.: the biomechanics of directional hearing. Journal of Experimental Biology 202: 1865–1876.

Schniederkötter K, Lakes-Harlan R. 2001. Auditory and visual cues during host finding of the parasitoid fly Emblemasoma auditrix (Sarcophagidae). In: Elsner N, Kreutzberg GW, editors. Göttingen Neurobiology Report. 371. Stuttgart. Thieme Verlag.

Soper RS, Delyser AJ, Smith LFR. 1976a. The genus Massospora entomopathogenic for cicadas. II. Biology of Massospora levispora and its host Okanagana rimosa, with notes on Massospora cicadina on the periodical cicadas. Annals of the Entomological Society of America 69: 89–95.

Soper RS, Shewell GE, Tyrrell D. 1976b. Colcondamyia auditrix nov. sp. (Diptera: Sarcophagidae), a parasite which is attracted by the mating song of its host, Okanagana rimosa (Homoptera: Cicadidae). Canadian Entomologist 108: 61–68.

Stireman III JO. 2002. Host location and selection cues in a generalist tachinid parasitoid. Entomologia Experimentalis et Applicata 103: 23–34.

Stölting H, Moore TE, Lakes-Harlan R. 2004. Acoustic communication in Okanagana rimosa (Say) (Homoptera: Cicadidae). Zoology in press.

Vinson SB. 1976. Host selection by insect parasitoids. Annual Review of Entomology 21: 109–133.

Walker TJ. 1993. Phonotaxis in female Ormia ochracea (Diptera: Tachinidae), a parasitoid of field crickets. Journal of Insect Behavior 6: 389–410.

Wineriter SA, Walker TJ. 1990. Rearing phonotactic parasitoid flies (Diptera: Tachinidae, Ormiini, Ormia spp.). Entomophaga 35: 621–632.

Yamawaki Y, Kainoh Y, Honda H. 2002. Visual control of host pursuit on the parasitoid fly Exorista japonica. Journal of Experimental Biology 205: 485–492.