

Functional and Nonfunctional Female Receptivity Signals in the Parasitoid Wasp *Spalangia endius* (Hymenoptera: Pteromalidae)

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ABSTRACT In many taxa, females signal during courtship when they are receptive. However, just because a female signals does not mean that the male responds to the signal. This study examines female signaling of receptivity (readiness to copulate) and male response in the parasitoid wasp *Spalangia endius* Walker. Females folded their antennae against their heads when they were receptive, and antennal folding has been shown to be effective in eliciting male copulation attempts in a confamilial. However, male *S. endius* did not respond to antennal folding; males did not contact the female's antennae during courtship, and how quickly a male attempted copulation was independent of whether or not the female had antennae. Males courted from on top of the female's abdomen and appeared to detect receptivity directly from the female's abdomen rising as her genital orifice opened. On females whose abdomens did not rise, initiation of male copulation attempts were delayed but not eliminated. Based on its current lack of function as a receptivity signal and on comparisons to published reports of mating behavior in confamilials, we hypothesize that female antennal folding at receptivity is a vestigial trait in *S. endius*.

KEY WORDS courtship, parasitoid wasp, receptivity, signaling, vestigial

Females can signal receptivity in a variety of ways including pheromones (Grant et al. 1987, Carlson et al. 2007), acoustic signals (Sueur 2003), and positioning of various body parts (Spieth 1974). What signal a female uses may change as she proceeds through the mating sequence. For example, she may use a different signal when she is receptive to receiving courtship than when she is receptive to being mounted. In species where courtship precedes copulation, female signaling can tell the male when to switch from courtship to copulation. However, the location of the male during courtship may constrain the evolution of the signal to copulate and vice versa.

Here, we examine female signaling of receptivity to copulate and male response to it in the parasitoid wasp *Spalangia endius* Walker (Pteromalidae). In some parasitoid wasps, the female must expose her genital aperture to copulate, and the abdomen rises substantially as she opens. In such species, if the male courts from far forward on the female, it may be difficult for him to detect the movement of the female's abdomen directly because he is usually smaller than the female (van den Assem 1974, 1976). Thus, in these species, females may evolve alternative receptivity signals, such as a folding of the female's antennae against her head. That males use female antennal movements as a copulation signal has been shown in *Nasonia vitripennis* (Walker), a confamilial of *S. endius* (van den

Assem and Jachmann 1982). The male detects the female's antennal position with his mouth palpi (van den Assem and Werren 1994). Even in species where males do not respond to the female's behavior at receptivity, it is still correct to call the behavior a signal because this simply means that it provides information (Bradbury and Vehrencamp 1998).

Spalangia endius is an ≈2- to 3-mm-long parasitoid of the pupal stage of certain fly species (Rueda and Axtell 1985). These hosts are found in carrion, manure, and rotting vegetation. Male *S. endius* begin emerging from their hosts 1 or 2 d before females (King 2000) and stay and wait for the females to emerge (B.H.K., unpublished data). Males do not exhibit any overt aggression among themselves, but they will mount courting pairs and attempt to copulate (King 2000).

Here we examine whether female *S. endius* fold their antennae against their heads at receptivity, whether the male is in a position to detect any female antennal movements by tactile means, and whether the male attempts copulation in response to the position of the female's antennae or in response to her raising her abdomen.

Materials and Methods

General Methods. The *S. endius* were from a Florida strain (King et al. 2005), which was maintained using a natural host, *Musca domestica* L. pupae, which were reared following the methods of King (1988). Vouch-

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ers are at the Illinois Natural History Survey Center for Biodiversity, catalog numbers "Insect Collection 6035 through 6054." Parasitized fly pupae were individually isolated in glass vials before the wasps' emergence to obtain virgin wasps. Wasps were virgin and <1 d from eclosion at testing, except where noted. Each wasp was used only once.

Environmental conditions, e.g., temperature, were controlled within experiments by temporal blocking; each replicate consisted of one trial of each treatment performed at nearly the same time and in random order. In such a set up, when there are significant correlations between treatments across replicates, paired *t*-tests are more powerful than independent *t*-tests and so were used; otherwise, independent *t*-tests are more powerful and were used (Zar 1999). In no case was the *t*-test assumption of normality violated strongly ($P < 0.001$; SPSS 2000). Temporally blocking treatments was not feasible in the videotapes in the abdomen rising experiment; however, temperature was constant, and there was no significant effect of humidity ($P > 0.30$). Data are presented as mean \pm SE (range). Two-tailed *P* values are presented.

Position of Courtship and Antennae. Virgin females are almost always receptive to copulation, and mated females almost never are (King et al. 2005). Thus, to begin to determine how a male detects when a female is receptive and whether it might be related to his courtship position, a female and a male ($n = 20$ pairs) were videotaped from before they mated, i.e., when they were virgin, until they completed mating and had one subsequent reencounter. The position of each of the male's legs and how quickly the female opened her genital orifice were not clear for all 20 pairs; therefore, for those observations, samples sizes are less (see Results). Videotaping was through a dissecting microscope at $\times 30$ magnification, with each pair of wasps in a glass vial (12 mm in diameter by 75 mm in height). Wasps were <3 d from eclosion. Temperature was 22–23°C, and relative humidity was 22–24%. We were particularly interested in whether the female moved her antennae when she became receptive and in whether the courtship position of the male put him in contact with her antennae or with the movement of her abdomen that was associated with her opening her genital orifice.

Antennaeless Female Experiments. Data from the previous experiment allowed us to address whether males could detect by tactile means any antennal signaling by the female. To test whether males depend on a female antennal signal using any sensory mode, e.g., vision, we observed the response of males to antennaeless females. In these and other experiments in which a body part was removed, the female was immobilized on a cryolizer, and ablation was with a sharpened insect pin. Females recovered within a few minutes of surgery, as evidenced by their walking; at which time a male was introduced.

In the first antennaeless experiment, each antennaeless female ($n = 15$) was observed with a male, and we recorded whether he attempted copulation, and if so, when he began to attempt copulation relative to

when the female exposed her genital orifice. Beginning to attempt copulation was defined as his beginning to curl his abdomen under the female's abdomen. The abdomen curling appeared to be for the purpose of copulation because his aedeagus eventually came out as the curling became more exaggerated.

In the second antennaeless experiment, quickness to attempt copulation was compared between males exposed to antennaeless females versus males exposed to one of two different controls: intact females and females with their middle legs removed to control for surgery effects ($n = 17$ per treatment). Quickness to attempt copulation was defined as the duration from the start of courtship, i.e., when his body began vibrating, until the male began to attempt copulation.

Abdomen Rising Experiment. To test whether males might be responding to the female's abdomen rising in deciding when to begin to attempt copulation, individual males were exposed to either a virgin female or a mated female. Virgin females usually open their genital aperture when mounted, causing their abdomen to rise, whereas mated females do not (King et al. 2005). Thus, if males use the abdomen rising as a cue, they should attempt copulation sooner on virgin females than on mated females. Both virgin and mated females had their hind legs removed before testing. This was to prevent mated females from immediately trying to brush the male off with their hind legs; such brush off behavior would prevent copulation attempts because the male would just dismount. After a virgin male was introduced, the male-female pair was observed directly ($n = 11$ usable pairs of each treatment) or by videotaping them ($n = 60$). Only 28 of the 60 videotaped pairs were useable, either because the necessary wasp behaviors were not clearly visible or because the male retreated from the female without mounting. Quickness to attempt copulation was measured with a stopwatch. The stopwatch measured to the nearest 0.01 s, but our reaction time on it was 0.19 ± 0.004 s ($n = 20$). In the directly observed pairs, temperature was $21.82 \pm 0.67^\circ\text{C}$ (range: 19–25°C), and relative humidity was $26.82 \pm 3.14\%$ (range: 16–54%). In the useable videotaped pairs, temperature was $19 \pm 0^\circ\text{C}$, and relative humidity was $56.46 \pm 1.24\%$ (range: 44–64%).

The duration from the start of courtship until the male began to attempt copulation was analyzed by a two-way analysis of variance (ANOVA) with observation method (video versus direct observation) and receptivity (receptive versus not) as fixed effects. One pair in which the female walked excessively (more than a few steps) during courtship was excluded because we were concerned that the male's need to hang on interfered with his ability to begin to attempt copulation. Including that pair would have increased the magnitude of the difference between treatments but made the previously significant difference marginally nonsignificant because it would have necessitated a switch to less powerful nonparametric statistics.

Dead Female Experiment. To address whether males were simply beginning to attempt copulation at a set time after courtship began or were responding to

the female's behavior, e.g., the folding of her antennae or her abdomen rising, a male was exposed to either a live female or a dead female ($n = 15$ of each treatment). Quickness to attempt copulation was recorded and compared between treatments. Testing was in a small plastic petri dish (3.4 cm diameter by 1.1 cm high) that was half full of damp sand and with a glass cover ($n = 8$ of each treatment) or in a test tube containing a small drop of water that was used to hold the dead female's anterior body in place ($n = 7$ of each treatment). There was no statistical difference in male response between the two different types of containers ($P > 0.20$), so they were combined for analyses. In all replicates, dead females were dorsal side up. The dead females had been killed by freezing.

Results

Position of Courtship and Antennae. The male mounted the posterior end of the female's dorsal side and positioned himself so that his head was located over her thorax, with his forelegs on the female's thorax ($n = 15$) or abdomen ($n = 2$); his hind legs on the underside of her abdomen; and his middle legs tapping her lateral abdomen ($n = 16$) or thorax ($n = 1$). During courtship, the male would have been able to contact the base of the female's antennae if he extended his antennae, but instead his antennae were bent down at the elbow at an acute angle and to either side of the female's thorax.

A female usually responded to this courtship behavior by remaining stationary and then opening her genital orifice $\approx 5.8 \pm 0.64$ s (range: 4–14 s; $n = 15$) after the male had mounted. The male's position on top of the length of the female's abdomen should have enabled him to feel her abdomen rise as she opened. As the female opened, she usually pulled her antennae toward her head loosely ($n = 18$ of 20), forming an $\sim 45^\circ$ angle at the flagellum-scape elbow. The 2 of 18 females that did not fold their antennae as they became receptive included one female whose antennae were already folded because she had drawn her legs and antennae in toward her body at initial male contact and one female whose antennae remained extended as she became receptive.

Males quickly attempted to copulate even on the two females that had not folded their antennae, and some males began to attempt copulation before the female had folded in her antennae. Because males were so far back on females, the males only had to back up a head length or less to copulate. Females extended their antennae during postcopulatory courtship, at the same time that they began to walk and to try to dislodge the males with their hind legs ($n = 18$ of 19 with previously folded antennae). There was no obvious change in male behavior immediately after the female extended her antennae.

Antennaeless Female Experiments. In the first antennaeless female experiment, males fanned, mounted, courted, and copulated in 14 of 15 pairs. (In the other pair, the male fanned but then walked away.) The males began to attempt copulation as the

female's abdomen rose and opened ($n = 14$ of 14). How far the abdomen had opened before the male began was variable.

In the second antennaeless female experiment, there was no significant difference in the male's quickness to attempt copulation between the two controls (intact females versus females with missing middle legs: $t = 1.35$, $df = 32$, $P = 0.19$), so they were combined into a single control of females with intact antennae before comparison to the antennaeless treatment. Males were just as quick to attempt copulation on females with missing antennae as on females with antennae intact (3.13 ± 0.32 s [range: 1.41–6.23 s], $n = 17$ versus 3.18 ± 0.19 s [range: 1.08–6.05 s], $n = 34$; $t = 0.15$, $df = 49$, $P = 0.89$).

Abdomen Rising Experiment. There was no significant effect of observation method ($F = 0.16$; $df = 1,1$; $P = 0.69$) or the interaction between receptivity and observation method ($F = 0.82$; $df = 1,46$; $P = 0.37$) on quickness to attempt copulation. There was a significant effect of female receptivity ($F = 5.34$; $df = 1,1$; $P = 0.03$). Specifically, males were quicker on receptive females, who opened their genital aperture when the male mounted, than on unreceptive females, who did not (directly observed: 3.67 ± 0.42 s, $n = 11$ versus 5.12 ± 0.76 s, $n = 11$; videotaped: 3.90 ± 0.31 s, $n = 15$ versus 4.53 ± 0.29 s, $n = 13$). Looking just at all virgin females, the pattern was the same; males were quicker to attempt copulation on receptive than on unreceptive females (3.80 ± 0.25 s, $n = 26$ versus 5.32 ± 0.38 s, $n = 3$; $t = 2.02$, $df = 27$, $P = 0.05$). Among unreceptive females, how quickly the male attempted copulation did not differ significantly for virgin versus mated females ($t = 0.52$, $df = 22$, $P = 0.61$).

Dead Female Experiment. Males were quicker to attempt copulation on live females than on dead females (3.69 ± 0.24 s [range: 1.66–5.34 s] versus 6.30 ± 0.37 s [range: 4.59–9.37 s]; $t = 5.94$, $df = 28$, $P < 0.001$).

Discussion

Almost all *S. endius* females provided an antennal signal at receptivity. However, the results of this study suggest that males are not responding to the folding of the female's antennae to know when to begin to attempt copulation. Males were too far back to detect the female's antennal movements by physical contact, and how soon males began to attempt copulation was independent of whether or not the female had antennae. An alternative explanation for the persistence of the folding in *S. endius* is that it has been maintained because of some sort of signaling function associated with the later restraightening of the female's antennae rather than the folding per SE. However, this seems unlikely given that male behavior did not change on females straightening their antennae. That the folding is used by the female to signal other females or unmounted males also seems unlikely because it is hard to imagine what advantage signaling her receptivity to them would provide to a female, particularly given the signal's brevity.

Although our results were inconsistent with males using the female's antennal signal to know when to attempt copulation, our results were consistent with males responding to an alternative signal, the female's abdomen rising. Males were slower to attempt copulation when the female's abdomen did not rise, as on unreceptive females and on dead females. Although males appeared to respond to the female's abdomen rising, it was not a necessary condition for males to attempt copulation. Even in the absence of any female behavior, males automatically began to attempt copulation a short time after beginning courtship. Female behavior just sped up the attempt.

We hypothesize that the loose antennal folding seen in *S. endius* is a vestigial trait, a remnant of a functional trait in an ancestor. *Spalangia* are in the subfamily Spalangiinae. A more fully developed version of the trait, i.e., tighter, antennal folding, is seen in a related subfamily, Pteromalinae. It is seen in 13 of the 14 Pteromalinae species for which there are data (Barrass 1960, van den Assem 1974, 1976, van den Assem and Werren 1994, Ruther et al. 2000). For one of the Pteromalinae with tight folding, there is experimental evidence that the signal is functional, i.e., used by males (van den Assem and Jachmann 1982), and for another, there is evidence that it is not used (van den Assem 1976). The evolutionary history of the antennal signal may become clearer as data on additional species are collected and mapped onto phylogenies of the family (Dzhanokmen 2000, Török and Abraham 2001, Desjardins et al. 2007).

Receptivity signals seem to be quite variable not only among insects generally (see Introduction), but also within parasitoid wasps. Some species signal receptivity with their antennae but in ways other than the folding seen among the Pteromalidae (van den Assem 1969, 1970, Vinson 1978, Nordlander and Grijpma 1991, Field and Keller 1993, Rungrojwanich and Walter 2000, Ovruski and Aluja 2002, Cheng et al. 2004, Pannebakker et al. 2005), but not all parasitoid wasps signal receptivity with their antennae even when their position would make it possible (Orr and Borden 1993). In the parasitoid wasp *Melittobia chalybii* Ashmead, females do not use their antennae to signal receptivity, but instead step forward as they open their genital orifices (Hobbs and Kronic 1971). The presence of receptivity signals has been documented much more frequently than the functionality of those signals. How common the presence of nonfunctional receptivity signals is among other taxa is an interesting question for future studies.

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