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It includes supplementary material, which is a video of *Urolepis rufipes* courtship

Courtship Behavior and Detection of Female Receptivity in the Parasitoid Wasp *Urolepis rufipes*

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Abstract Once a *Urolepis rufipes* male mounted, the female beat her antennae against his mouth and clypeus. Immediately after he swept his antennae rapidly downward and extruded his mouthparts, her abdomen rose as she opened her genital orifice. Almost simultaneously he backed up for copulation and she folded her antennae against her head. Neither her abdomen rising nor her antennal folding were essential to his backing up as determined from their timing and from experiments in which her abdomen was sealed or her antennae were removed. Females did not open their genital orifice if with a sealed-mouth male; and antennae-removed females did not open even in the few cases where untreated males extruded their mouthparts. Unlike a closely related species, females mounted by sealed-mouth males did not open in response to air from containers of mating pairs.

Keywords Courtship, mating, parasitoid wasp, pteromalid, receptivity signal

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Introduction

Traits, including behavioral ones, often reflect both ancestry and adaptation; one way to address the contribution of ancestry versus adaptation is to plot the behaviors onto independently derived phylogenies (e.g., Sillen-Tullberg 1988; Kusmierski et al. 1997). However, this depends on phylogenetic and behavioral data being available for a large number of species, and the data for

this often come from multiple published reports (e.g., Leisler et al. 2002; Mank and Avise 2006) rather than from a single report (Puniamoorthy et al. 2009). This is especially likely to be true when obtaining such data requires maintaining a colony. The present study provides a detailed description of mating behavior in the parasitoid wasp *Urolepis rufipes* (Pteromalidae) and experimentally examines some proximal mechanisms of mating behavior. Within the family Pteromalidae, *Urolepis* is closely related to *Nasonia* (Burks 1979; 2006; McAllister and Werren 1997), which is sometimes referred to as the *Drosophila* of the Hymenoptera (Pultz and Leaf 2003). There is a relatively large amount of information available on *Nasonia* species, particularly on *N. vitripennis*. This information consists of extensive evolutionary and molecular genetic data, including the first genome sequence for a parasitoid (Werren et al. 2010; Robertson et al. 2010). In addition, there have been numerous studies of mating, including the proximal mechanisms of various aspects of mating both in *Nasonia* as well as in some related species (e.g., van den Assem 1986 and references therein; van den Assem and Werren 1994; Beukeboom and van den Assem 2001; Leonard and Boake 2006; King and Dickenson 2008; Ruther et al. 2011b). However, despite its close relationship to *Nasonia*, mating in *Urolepis* has been unexamined, other than a study on the function of postcopulatory courtship (King and Kuban 2012).

One aspect of courtship behavior that seems to vary among insects generally and within pteromalids specifically is the mechanism by which males detect when a female is receptive to copulation (van den Assem 1974; King and Dickenson 2008). In many pteromalids, including *U. rufipes*, female receptivity is easy for an observer to detect because when the female opens her genital orifice, the dorsal surface of her abdomen rises significantly in the process. However, in some species, the male courts the female from so far forward on her body that it would be difficult if not impossible for him to feel the abdomen rise. This is the case in *N. vitripennis*; the male has his front tarsi on the female's head and his hind tarsi on her thorax, which does not rise (van den Assem and Jachmann 1982). He is not in contact with her abdomen. Thus an alternate way of signaling receptivity has evolved. As a female opens her genital orifice, she folds her antennae tightly against her head (the scape up against her head and the pedicel and flagellum bent down against the scape). Males have been shown to use these female antennae movements as a signal to back up and attempt copulation. The male is thought to detect the position of the female's antennae with his maxillary palpi. Because *U. rufipes* is closely related to *N. vitripennis*, their signals may be similar in form and function due to common ancestry. On the other hand, given that *U. rufipes* court from farther back on females (see Results), they may have adapted to perceive female receptivity directly, i.e. from her abdomen rising.

Female antennae may be important not only for giving males information on willingness to copulate, but also for receiving information from males. In *N. vitripennis*, males mark sex attractants from their anus on substrates, and females detect these from up to 4.5 cm away (Steiner and Ruther 2009; Ruther et al. 2009, 2010). In addition, just prior to copulation, the male extrudes his maxillary palpi, which allows the release of an aphrodisiac pheromone that induces female receptivity (van den Assem and Jachmann 1982; Ruther et al. 2010). The female likely detects this pheromone with sense organs on her antennae (Wibel et al. 1984; Slifer 1969). The present study of *U. rufipes* describes their mating behavior and uses experimental manipulations to examine the role in mating behavior of the female's antennae, the male's mouthparts, and the rising of the female's abdomen as she becomes receptive.

Methods

Biology of *U. rufipes*

U. rufipes is found developing in the pupal stage of house flies and stable flies (Muscidae) in livestock production facilities, although brine flies (Ephydriidae) are thought to be their original hosts (Smith and Rutz 1985; Gibson 2000; Gibson and Floate 2004). *U. rufipes* is a solitary species, i.e., one wasp develops per host, but is also quasigregarious, i.e., hosts can be numerous and sometimes highly clumped (e.g., Collins 1980; King 1990). Males begin emerging from their hosts as adults a day or two before females (Powell et al. 2003), and sex ratios are generally female-biased (Stenseng et al. 2003; King and Kuban 2012).

General Methods

The *U. rufipes* were a Canadian strain that originated from cattle feedlots in southern Alberta. The wasps are maintained on *Musca domestica* pupae. The *M. domestica* were reared following the methods of Nichols et al. (2010). Parasitized fly pupae were individually isolated in glass test tubes (12 x 75 mm) prior to the wasps' emergence in order to obtain virgin wasps. Wasps were 0-1 day old from emergence at testing. Each wasp was only used once, except in the second experiment of "Role of Male Mouthparts," where the same male was used in the control and the treatment. The wasps were not given honey. In experiments involving body part removal, the wasp was immobilized with cold, and the body part was then removed with a sharpened insect pin. After the wasp recovered as evidenced by normal walking, an individual of the opposite sex was introduced.

Analyses were with PASW Statistics (2009). Two-tailed *P* values are presented. Sample size discrepancies among behaviors resulted from some behaviors not being observable in every replicate. Tests of independence of categorical data were by G tests, which are also called likelihood ratio chi square tests. Comparisons of duration until mounting between treatment and control were by survival analysis, specifically Cox's regression. Survival analysis takes into account that nonmounters might or might not have mounted if the observation period had been longer (review of survival analysis in van Alphen et al. 2003).

Description of Mating Behaviors

Pairs of a male and a female were observed until ten clear observations of each behavior or its absence was achieved. Each pair of wasps was placed in a glass test tube (12 x 75 mm), which was moved as necessary to keep the wasps in view of the microscope lens. These pairs were each observed for 10 min. In this size container, copulation usually either occurs within 5 min (*n* = 28 of 35 pairs) or still has not occurred by 10 min (*n* = 7 of 35 pairs), and from mount to dismount takes approximately 1 - 3 min. Behaviors scored from these replicates were chosen based on reports of related species (e.g., van den Assem 1986; King and Dickenson 2008) and included elements of courtship, copulation, post-copulatory courtship and the first reencounter after mating. Each behavior was scored as present or not present. The video in the Online Resource was captured with Hamamatsu Orca-100 cooled CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan) attached to a macro lens and using Image Pro Plus software (Media Cybernetics, Silver Spring, MD).

Role of Female Antennae

This experiment examined the interaction between a male and a female when the female had her antennae completely removed. In addition to the antennae-removed female treatment ($N=28$), there were two controls, the intact control in which the female was not cold immobilized and had no surgery ($N=28$) and the surgery control in which the middle legs were removed ($N=28$). In both the treatment and the controls, the mating behaviors of each male-female pair were then observed for 10 min. Recorded behaviors included whether the male mounted, whether the female was receptive (opened her genital orifice) and whether the pair copulated. For each behavior, the number of pairs with versus without the behavior was first compared between the two controls. Then when controls did not differ, controls were combined for comparison to the treatment.

We chose removal of the middle pair of legs as one of the controls because observations of mating behavior indicated no major role for those legs per se, and they seemed similar in terms of severity of surgery. That the middle-legs-removal treatment was not significantly different from the intact control suggests that cold and a cut close to the body were not by themselves a major deterrent to the various aspects of mating (see Results).

Role of Female Antennae Length

This experiment examined whether the female's flagella per se are essential in male response. In the flagellum-removed treatment ($N=15$), the entirety of each flagellum was removed, leaving behind the scape and pedicel; in the tip-removed treatment ($N=15$) only the first segment of each flagellum was removed. Then courtship behaviors were recorded, excluding males that failed to mount within 5 min. Six behaviors were examined for presence or absence: mounting, male extrusion of mouthparts, female genital orifice opening, female antennae folding against her head upon opening, male aedeagus extrusions, male backing up as if to copulate (for descriptions of these behaviors, see "Description of Mating Behaviors" and "Role of Female Antennae Length" in Results.). If the male extruded his mouthparts, the number of times he did so was counted between mount and dismount. The number of times he backed up as if to copulate was also recorded.

Role of Male Mouthparts

The first male mouthpart experiment examined the interaction of females with males that were prevented from opening their mouth or extruding their mouthparts. The experiment involved a sealed-mouth male treatment ($N=10$) and a control ($N=10$). In both treatments, the male was immobilized with cold. For the sealed-mouth treatment, using a small pin, just enough super glue (ethyl cyanoacrylate) was applied to completely cover the front of the male's mouth and prevent the mouthparts from moving (van den Assem and Jachmann 1982; van den Assem 1986). In the control the male had an equivalent drop of glue placed on his thorax. After the glue was dry (indicated by its turning white), the male was placed in a test tube. Then, once the male had recovered, a female was added. Each pair was observed for 10 min or until they copulated, whichever came first. The time it took the male to mount the female and whether copulation

occurred were recorded. The sealed-mouth treatment was compared to the control in terms of relative frequency of copulation, mounting, and female receptivity.

The second male mouthpart experiment examined the number of times during courtship that males extruded their mouthparts when they were virgin versus when they had just mated. Female *U. rufipes* are less likely to become receptive to copulation by recently mated males than to virgin males (Kuban 2012). This experiment tested whether this might be a result of fewer mouthpart extrusions by mated males. A virgin male was presented with a virgin female in a test tube and observed for 10 min or until they copulated, whichever came first ($N=15$ pairs). The number of mouthpart extrusions was recorded from mount to copulation, and then from copulation to dismount. Then he was tested again (i.e., having just mated) and in the same way but with a new virgin female. Two males that failed to remate were excluded, although including them had no effect on conclusions.

The third male mouthpart experiment attempted to replicate evidence of females becoming receptive in response to male pheromone as shown for the confamilial *N. vitripennis* (van den Assem et al. 1980; van den Assem 1986). Air from a vial containing mating pairs was released in close proximity to a female's antennae while a sealed-mouth male was mounted on her. The group vial (70 mm, 20 mm) was made by combining 10-20 virgin females and 10-20 virgin males and leaving them together for 10 min, during which time matings occurred. At the same time, a sealed-mouth male was placed in a small petri dish (35 mm, 10 mm) with three virgin females in order to quickly obtain a mounted pair. As soon as a mounting occurred, 3 cc of air from the group vial was drawn up in a syringe and released directly in the mounted female's face one or more times. Six different mounted females (mounted by 5 different sealed mouth males) were tested over a course of two months. Whether or not the female opened her genital orifice was recorded.

Role of Female Abdomen Rising

This experiment examined the interaction of males with females that were prevented from opening their genital orifice. The experiment involved a sealed-abdomen treatment ($N=20$) and a control ($N=20$). For the sealed-abdomen treatment, a drop of super glue was placed on the end of the female's abdomen to prevent her from opening her genital orifice. For the control, the female had a drop of super glue placed on her dorsal thorax. Then courtship behaviors were recorded for 10 min or until they copulated, whichever came first.

Results

Description of Mating Behaviors

Prior to mounting the female, the male usually moved his antennae up and down in opposite directions of each other as he rapidly walked toward her. The male sometimes briefly fanned his wings (wings blurred from rapid movement) as he got near her or as he mounted. As the male approached, the female usually extended her antennae and turned to walk away, or, if she was already walking, she sped up, and the male chased her.

The male mounted the posterior end of the female's dorsal side until his head was above her head (Fig. 1A; see also video in Online Resource). This put the male's abdomen in contact

with the female's abdomen. His front tarsi were on her thorax, and his hind tarsi were on her abdomen. The female usually stopped walking once the male mounted her, and the male extended his antennae downward and touched the female's motionless antennae. After the male contacted her antennae, the female began to beat her antennae rapidly against the male's mouth; the two antennae did not beat in synchrony (Fig. 1B). Some males wing-flicked while on the female and prior to copulation. The male's wing movements while mounted are referred to here as wing flicks because they were often less of a blur and more isolated than the wing fanning that occurred as he was mounting. The wings generally were lifted to about a 45° angle during these flicks. The male also sometimes vaguely nodded his head sporadically during these pre-copulatory behaviors.

The male then did an antennal sweep, raising his antennae upward and then rapidly bringing them down while extruding his maxillary and labial palpi (Fig. 1C). The tip of his abdomen bent very slightly down against hers. Immediately the female raised her abdomen and opened her genital orifice and the male began to back up, both at almost the same instant. While backing up, the male extruded his aedeagus and lowered his abdomen to the side and beneath the female, allowing him to insert his aedeagus (Fig. 1D). The female folded her antennae down at their elbow, usually forming a roughly 45° angle between the flagellum and scape, at the same time as she began opening her genital orifice or slightly after. During copulation, the male's antennae were motionless and also loosely folded down. All but one of ten females remained stationary during copulation, with her antennae folded down though sometimes quivering.

After copulation, the male moved forward on top of the female so that his head was above her head and his abdomen was on hers. The post-copulatory courtship that ensued appeared to contain the same elements as the pre-copulatory courtship (the pair again touched antennae, again the female beat her antennae against the male's mouth, and again the male extended his mouthparts and antennae, and some males exhibited wing flicking.). Sometimes, the male dismounted immediately after extending his mouthparts and antennae. Other times the female continued to beat her antennae against the male's mouth, and the male extended his antennae and mouthparts a second time before dismounting. None of the females were receptive a second time during postcopulatory courtship in this experiment, although second, and even third, copulations have been observed during a single mounting event on other occasions. Females did not brush off males, i.e., brush a pair of legs across their backs as if trying to dislodge the male as has been reported in some related species (King 2010).

All males later reapproached the female, and nine of ten males proceeded to remount. As with the post-copulatory courtship, the subsequent remount courtships by mated wasps were qualitatively similar to precopulatory courtship by virgins. However, in none of the reencounters, did the female fold her antennae or open her genital orifice.

Role of Female Antennae

Mating interactions did not differ significantly between the two control groups (Table 1). In contrast, the antennae-removed treatment differed significantly from the controls in several behaviors (Table 1). Males still approached antennae-removed females, but they were slightly less likely to be mounted and much less likely to have the male extrude his mouthparts. No antennae-removed females showed receptivity (opened their genital orifice), whereas almost all control females did.

Male mouth extrusions and female receptivity (opening of the genital orifice) occur only while the male is mounted on the female (based on numerous observations of mating in this species, not just this study). However, the reduction in mouth extrusions and lack of female receptivity that were seen in the antennae-removed female treatment were not solely from males being less likely to mount. The effect was present even after restricting the analyses to just males that mounted. Relative to controls, males with antennae-removed females were still much less likely to extrude their mouthparts (3 of 23 versus 54 of 55, $G=63.06$, $df=1$, $P<0.001$) and to back up (5 of 23 versus 54 of 55, $G=52.53$, $df=1$, $P<0.001$); and receptivity and copulation were still less (0 of 23 versus 52 of 55, $G=76.01$, $df=1$, $P<0.001$).

The lack of receptivity and copulation in the antennae-removed-females treatment may explain why males in that treatment seemed very persistent. For example, more than half of the males in the treatment were still mounted on the female by the end of the 10 min observation, whereas all control males had dismounted before the end of the trial, and almost all had copulated (Table 1).

Males appeared to use the female's antennae to position themselves. Specifically, after antennating her head, with antennae-removed females but not controls, males often then moved farther forward. Among males that mounted, almost a third of the males on antennae-removed females, but none of the control males, then turned around and faced the posterior of the female (7 of 23 versus 0 of 55, $G=18.84$, $df=1$, $P<0.001$). These turning males antennated her abdomen as if searching for her antennae and then turned back to her head end, sometimes turning on her multiple times.

Role of Female Antennae Length

In both treatments the female never opened her genital orifice. However, all females were mounted and then folded their antennae. (The antennae always fold where the scape connects to the pedicel.) Once the male was mounted, he began orienting the front of his head to reach the distal-most part of what was left of the female's antennae, and she beat them as if the flagella were completely present, with them sometimes making contact with his mouth. Because her antennae were shorter, his contacting them often required his moving farther forward on the female than usual; he extruded his mouthparts shortly after making physical contact with them. However, significantly fewer males extruded their mouthparts, and they did so significantly fewer times in the flagellum-removed treatment than in the tip-removed treatment (9 of 15 versus 15 of 15 males; $G=9.83$, $df=1$, $P=0.002$; mean \pm s.e. (minimum-maximum)= 1.27 ± 0.37 (0-5) versus 2.13 ± 0.34 (1-6); Mann-Whitney $U=66.000$, $n_1=15$, $n_2=15$, $P=0.046$). In some pairs, once the male was mounted, his aedeagus protruded repeatedly and rapidly from his abdomen, with a white substance visible on the aedeagus as it happened. More males did this in the flagellum-removed treatment than in the tip-removed treatment (14 of 15 versus 9 of 15 males; $G=5.06$, $df=1$, $P=0.025$).

The treatments did not differ in number of males that backed up as if searching for the female's genital orifice to copulate or in how many times the male backed up (flagellum-removed treatment versus tip-removed treatment: 9 of 15 versus 11 of 15; $G=0.60$, $df=1$, $P=0.44$; 1.38 ± 0.45 (0-5) versus 1.36 ± 0.33 (0-4); Mann-Whitney $U=87.00$, $n_1=13$, $n_2=14$, $P=0.84$). However, within the flagellum-removed treatment, all males backed up among males that had extruded their mouthparts; whereas among males that never extruded, none backed up (9 of 9 versus 0 of 6, $G=20.190$, $df=1$, $P<0.001$). Likewise, in both treatments, males that extruded

their mouthparts more times also backed up more times (Spearman rank correlations: flagellum-removed treatment $r_s=0.98$, $N=13$, $P<0.001$; tip-removed treatment $r_s=0.57$, $N=14$, $P=0.035$).

Role of Male Mouthparts

In the first male mouthpart experiment, control males copulated more frequently than sealed-mouth males, which never copulated (Table 2). This difference was due to variation in both mounting and female receptivity. The control males mounted the females more frequently than sealed-mouth males (Table 2). If mounted, females with control males were more likely to open their genital orifice (Table 2), and females that opened always copulated. Sealed-mouth males took significantly longer to mount than control males (Cox's regression: $\beta=0.94 \pm 0.41$, Exp (β)=2.55; $\chi^2_1=5.32$, $P=0.02$; Fig. 2 shows the distribution of time-until-mounting for the treatment versus control).

In the second male mouthpart experiment, virgin males and mated males did not differ significantly in the number of mouthpart extrusions from mount to copulation or in the number between copulation and dismount (Fig. 3; sign test, $P=0.63$; sign test, $P=1.00$).

In the third experiment, with a sealed-mouth male mounted on her, no female opened her genital orifice when air that had been collected from a vial of mating pairs was released in close proximity to her antennae.

Role of Female Abdomen Rising

Males appeared to respond to sealed-abdomen females as strongly as to controls in terms of the proportion of males that mounted (Table 3) and how soon males mounted (Cox's regression: $\beta=-0.32 \pm 0.38$, Exp (β)=0.73; $\chi^2_1=0.72$, $P=0.40$; Fig. 4 shows the distribution of time-until-mounting for the treatment versus control). Likewise, males backed up for copulation with sealed-abdomen females as often as with the controls. All males copulated with the control female if she opened her genital orifice (6 of 6 pairs). Males repeatedly courted and backed up on sealed abdomen females, which were unable to open.

The inability of sealed-abdomen females to open their genital orifice did not appear to interfere with their ability to fold their antennae. The proportion of sealed females that folded their antennae was not significantly less than for control females (Table 3).

A small proportion of males backed up even when the female did not fold her antennae. However, the proportion of males that backed up was greater when the female folded her antennae than when she did not among sealed-abdomen females (8 of 8 versus 5 of 12; $G=9.60$, $df=1$, $P=0.002$) and in the control (9 of 9 versus 2 of 11; $G=17.09$, $df=1$, $P<0.001$). Note that when females folded their antennae, males always backed up, regardless of treatment. All conclusions in this experiment were the same when the analyses were restricted to pairs with mounting.

Discussion

U. rufipes males court from farther back on females than do *Nasonia* (van den Assem and Werren 1994), with the male's tarsi on the female's thorax, not her head. However, as expected given their close relatedness, some aspects of the courtship behaviors seen in *U. rufipes* have

also been reported for *Nasonia*, e.g., movement of male wings and antennal sweep, male extrusion of mouthparts, and female antennal folding upon receptivity (van den Assem 1986; van den Assem and Werren 1994). During courtship, the wings of *U. rufipes* males generally were lifted to about a 45° angle, in contrast to the subtle wing lifts seen during courtship in *N. vitripennis*, where the male's wings remain parallel to his body length (Barrass 1960; personal observation). *N. vitripennis* females are described as quivering their antennae dorsoventrally near the male's head (Barrass 1960), whereas we would describe *U. rufipes* females as rapidly beating their antennae against the male's mouth and clypeus.

The white substance visibly extruding from the abdomen of some male *U. rufipes* while mounted in the "Role of Female Antennae Length" experiment was unusual. However, *N. vitripennis* are known to release pheromone from their anal orifice when they mark substrates (e.g., Ruther et al. 2008, 2009, 2011b), and the male's rectum where this pheromone is produced is "filled with a whitish tubular accumulation (meconium)" (Abdel-latif et al. 2008). Likewise, *U. rufipes* males release a white substance when they mark substrates (Cooper 2010; Kuban 2012). *U. rufipes* males also have been observed extruding their aedeagus in and out when a couple of centimeters from a female (B.H. King personal observation); but prior to this experiment, they had not been observed doing so while they were mounted. Components of the anal sex-attractant pheromone produced by male *N. vitripennis* have been identified (Ruther et al. 2008, 2011b), but little is known about chemical communication in *U. rufipes*. Some similarities in sex pheromones seem likely given that *U. rufipes* males sometimes mount female *Nasonia*, although *Nasonia* males do not mount *U. rufipes* females (Nichols 2009). In *N. vitripennis*, male attraction to females is at least partly related to female-specific cuticular hydrocarbons (Steiner et al. 2006). Sex-specific cuticular hydrocarbons also appear to be an important source of mate recognition cues in other Pteromalinae (e.g., Sullivan 2002; Steiner et al. 2005, 2007; Ruther et al. 2011a).

Some male courtship behaviors were more sporadic or less pronounced in *U. rufipes* than in *N. vitripennis*. Wing movements were relatively sporadic in *U. rufipes*, whereas *N. vitripennis*' wing vibrations are repeated throughout courtship and at a consistent rate (Jachmann and van den Assem 1996); but *U. rufipes* wings were lifted higher. *U. rufipes* males usually extruded their mouth palpi only once prior to copulation, whereas *N. vitripennis* males extrude their palpi with the first head nod within each series of head nods during courtship (van den Assem and Visser 1976; van den Assem and Jachmann 1982). The most obvious difference between the courtships of *Nasonia* versus *U. rufipes* is that *U. rufipes* males do not usually head nod, and when they do it is subtle. The differences between the species in their wing movements and head nodding are not surprising given that neither is essential to successful mating in *N. vitripennis* (van den Assem 1986).

To what degree the differences in mating behavior between these two species reflect differences in habitat and biology is unclear. *Nasonia* is gregarious, whereas *U. rufipes* is quasigregarious. *Nasonia* are generally found associated with nests and carrion; *U. rufipes* seems to have at least initially been associated with aquatic environments and is now found along briny shores and in association with manure in livestock facilities (Gibson 2000).

Each of the male courtship behaviors described here are not unique to *U. rufipes* or to *N. vitripennis*, although they may differ in details from other species. Many of the behaviors are seen not only in confamilials, but also in parasitoid wasps from other families, e.g., wing fanning prior to mounting, the male mounting the female to court, and antennal and leg movements by males during courtship (reviewed in Hardy et al. 2005). Male head-nods and/or mouthpart

extrusions appear to be less common but are performed by many, if not all, confamilials in which the male's head is above the female's head (Barrass 1960; van den Assem and Povel 1973; van den Assem 1974; van den Assem 1976), although not in at least some confamilials in which his head is farther back (e.g., *S. endius*: King and Dickenson 2008). Leg tapping of the female by the male, as is seen in a subset of confamilials, was not observed in *U. rufipes* (e.g., Barrass 1960; van den Assem and Povel 1973; van den Assem 1974; van den Assem and Werren 1994; King 2000; Beukeboom and van den Assem 2001; King and Dickenson 2008).

In *U. rufipes*, when a female became receptive for copulation, the male was in contact with both her antennae and her abdomen and so could potentially have used cues from either. However, some males backed up in the absence of her abdomen rising or of her antennae folding or of both. Many, but not all, males backed up even on flagellum-removed females, i.e., on females that only had the scape and pedicel, although not usually on females with the entire antennae removed. Among pairs with intact antennae, males were more likely to back up if the female folded her antennae.

In *U. rufipes* the folding of the female's antennae was usually at about a 45° angle, not flat against her head as in *N. vitripennis* (Barrass 1960; van den Assem and Jachmann 1982) and most other studied members of the subfamily Pteromalinae, to which *U. rufipes* and *N. vitripennis* belong (Barrass 1960; van den Assem 1974, 1976; van den Assem and Werren 1994; Ruther et al. 2000). The partial bending of the antennae of *U. rufipes* females looked like that in the less closely related confamilial *Spalangia endius*, subfamily Spalanginae (King and Dickenson 2008).

Results of the present study are consistent with female antennae in *U. rufipes* being important even before she becomes receptive. Males almost always extruded their mouthparts if the female's antennae were intact or just had the tip removed. In contrast, almost no males extruded their mouthparts in response to antennae-removed females. Likewise, males extruded their mouthparts significantly fewer times on females with just the scape and pedicel present (flagellum-removed females) than on females with all but the first segment of their flagella present (tip-removed females). The stimulus of the female beating her antennae against his mouth and clypeus may be what elicits male mouthpart extrusion. Perhaps the female's beating is less forceful in the absence of flagella.

Despite attempting to follow the methodology used to demonstrate receptivity-inducing pheromones in *N. vitripennis* (van den Assem et al. 1980), we found no evidence of such in *U. rufipes*, although we cannot rule out the possibility. We did show that *U. rufipes* females' antennae played an important role in their becoming receptive: females missing any part of their antennae did not become receptive, whereas control females became receptive immediately after males extruded their mouthparts. The tips of the female's flagellae appear to be important in female response to male mouthpart extrusions; females did not open their genital orifice when they were antennae-removed, flagellum-removed, or even just tip-removed. The male's mouthparts also seem important to induce female receptivity in that females did not become receptive when males had their mouthparts sealed so that they were unable to open their mouths or extrude their mouthparts.

To conclude, this study provides the first detailed description of basic mating behavior in the parasitoid wasp *U. rufipes*. The behavior was similar to that of the well-studied model organism, *Nasonia*, with the most notable difference being that head nods were usually absent in *U. rufipes*. Results of the present study suggest that beating of the antennae by *U. rufipes* females is important in stimulating males to extrude their mouthparts. In turn, male mouthpart extrusion

plays a vital role in stimulating female receptivity. Although her receptivity is accompanied by her antennae folding and her abdomen rising, neither cue is essential for males to back up and attempt copulation; and if males respond to these cues, they do so almost instantaneously. Studies such as this one contribute to understanding signaling in mating. Our hope is that this study and similar ones on related species will contribute to the understanding of patterns of evolution within the family Pteromalidae. A recent analysis of strepsid flies suggests relatively rapid evolution of mating behavior, high species specificity, and frequent loss and gain of behavioral elements (Puniamoorthy et al. 2009). Whether this is true more generally in insects awaits further study.

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Table 1 Mating behaviors, roughly in sequence, for three types of females: antennae-removed treatment, intact control and surgery control^{a,b}

Behavior	Antennae-removed Treatment	Intact Control	Surgery Control	Control versus Control	Treatment versus Combined Controls ^c		
	%	%	%	G_I	P	G_I	P
Male approached female	96	100	100	0	1.00	2.22	0.14
Male mounted female	82	100	96	1.40	0.24	6.92	0.01
Male moved farther forward on female	79	0	0	0	1.00	67.51	<0.001
Male wing flicked	57	16	18	0.03	0.86	13.68	<0.001
Male turned around on female	25	0	0	0	1.00	16.70	<0.001
Male extended mouthparts and antennae	11	100	93	2.85	0.09	69.17	<0.001
Male backed up	18	100	93	2.85	0.09	58.75	<0.001
Female opened	0	96	89	1.13	0.29	82.82	<0.001
Copulation occurred	0	96	89	1.12	0.29	82.82	<0.001
Male still mounted at end of 10 min trial ^d	57	0	0	0	1.00	43.13	<0.001

^a $N=28$ for each behavior within each treatment except $N=25$ for wing flicked in the intact control

^bAnalyses were on all pairs; see text of Results for additional analyses that were restricted to just pairs in which the male mounted

^c Comparisons by G -tests

^dAs opposed to having dismounted by then

Table 2 Mounting, copulation and female receptivity for sealed-mouth males and control males

	Sealed-mouth males		Control		<i>G</i> -test	
	<i>N</i>		<i>N</i>		<i>G</i> ₁	<i>P</i>
% of all males that mounted	20	45%	20	90%	9.92	0.002
% of all pairs that copulated	20	0%	20	70%	27.36	<0.001
% of mounted females that were receptive	9	0%	18	78%	18.32	<0.001
% of mounted pairs that copulated	9	0%	18	78%	18.32	<0.001

Table 3 Mounting, antennal folding, and female receptivity for sealed-abdomen females and control females

	Sealed-abdomen females		Control		<i>G</i> -test	
	<i>N</i>		<i>N</i>		<i>G</i> ₁	<i>P</i>
% of all males that mounted	20	80%	20	65%	1.14	0.29
% of all females that folded antennae	20	40%	20	45%	0.10	0.75
% of all pairs that male backed up	20	65%	20	55%	0.42	0.52
% of mounted females that folded antennae	16	50%	13	69%	1.11	0.29
% of mounted pairs in which male backed up	16	81%	13	85%	0.06	0.81

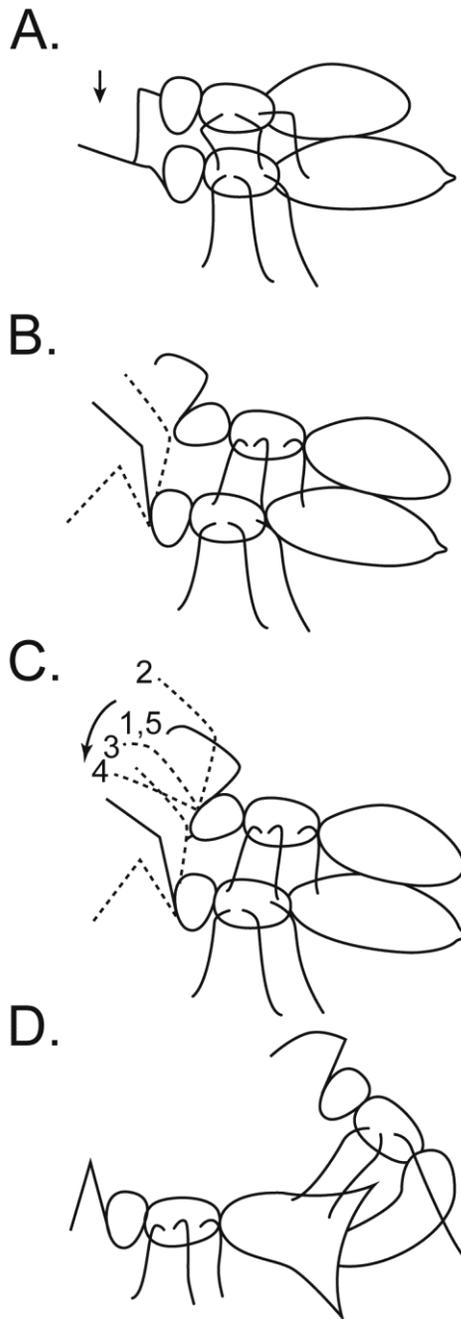


Fig. 1. A. Once mounted, the male moved his antennae down, contacting the female's antennae; B. the female beat her antennae rapidly against the male's mouth; C. the male swept his antennae back and then down; D. the female folded her antennae while opening her genital orifice; the male backed up: then they copulated. Wings are not shown: females did not move their wings during mating and males did inconsistently as described in the text. Only one of each pair of antennae and of legs is shown.

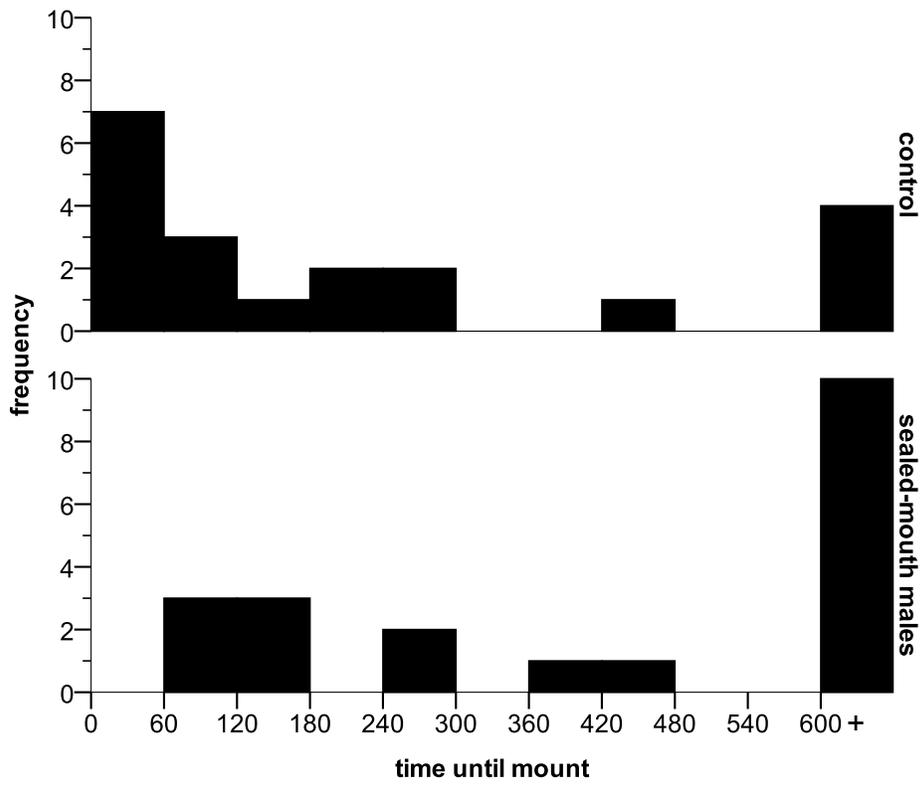


Fig. 2. Time (s) until control males and sealed-mouth males first mounted the female

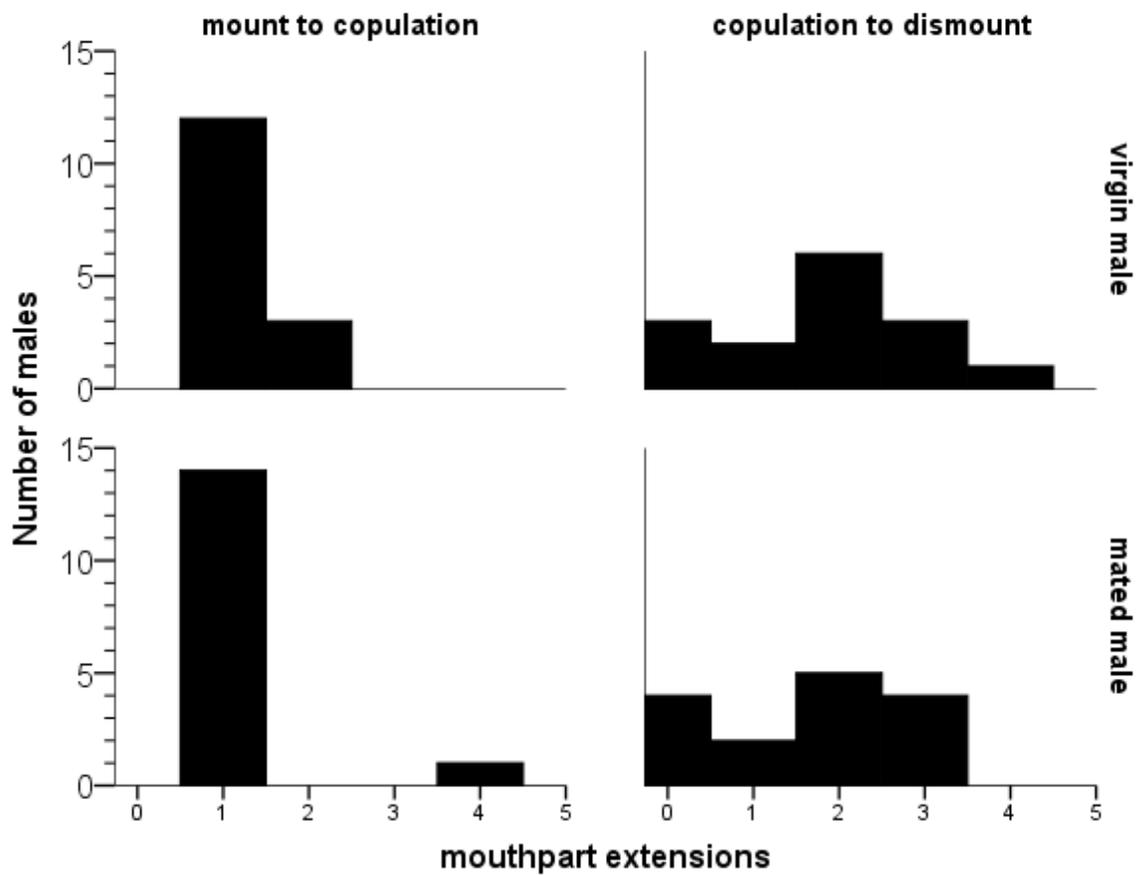


Fig. 3. Number of mouthpart extensions by virgin males and by mated males

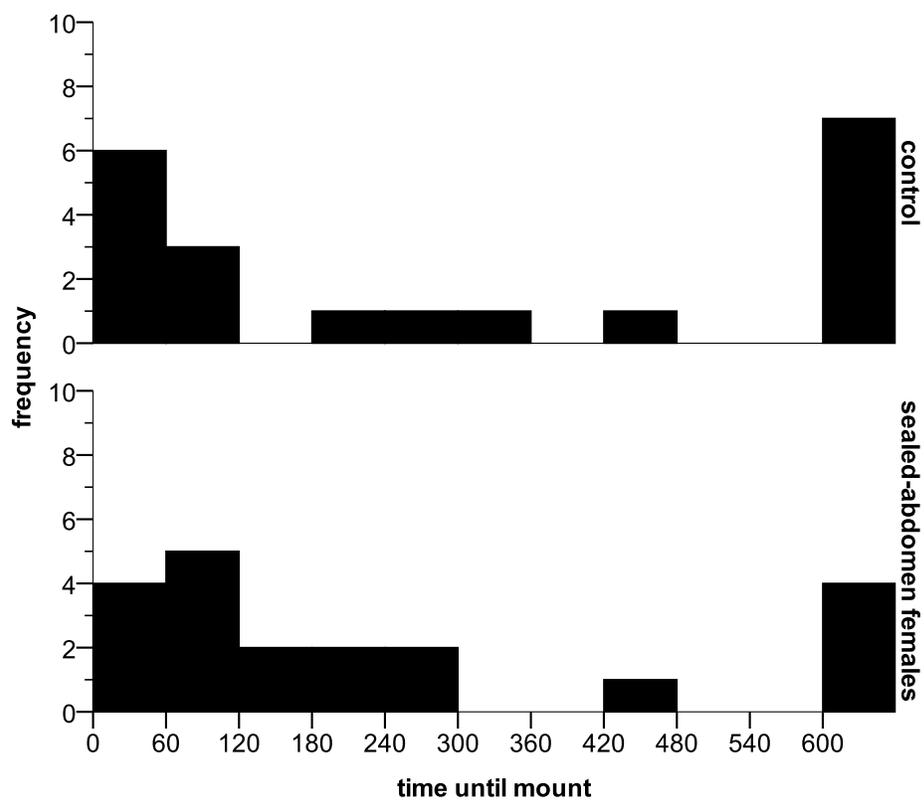


Fig. 4. Time (s) until males first mounted the control females and the sealed-abdomen females