



Mate Location and the Onset of Sexual Responsiveness in the Parasitoid Wasp *Spalangia endius* (Hymenoptera: Pteromalidae)

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ABSTRACT In some animals, transformation to the adult stage occurs in a hidden location, such as a burrow or a host. Males that can locate hidden females sooner, e.g., before they emerge, may have a mating advantage, particularly if the females are ready to mate. Whether males locate pre-emergent females and whether pre-emergent females will mate was examined in the parasitoid wasp *Spalangia endius* Walker. *S. endius* parasitize fly pupae. A single wasp offspring feeds, pupates, and transforms into an adult within the fly puparium (an outer shell around the fly pupa), and males emerge a day or more before females. Whether pre-emergent wasps are ready to mate was examined by dissecting them out of their hosts and presenting them with naturally emerged adults of the opposite sex. Many of the pre-emergent wasps were ready to mate. Nevertheless, males did not distinguish between hosts containing a pre-emergent female versus a pre-emergent male, or even between parasitized and unparasitized hosts. In contrast, males were able to differentiate between hosts from which a female versus a male had recently emerged. Although females are ready to mate before emergence, there may be little advantage to recognizing and staying with a host that contains a pre-emergent female because emergence takes so long, which raises the cost of missed mating opportunities elsewhere.

KEY WORDS mate location, parasitoid wasp, receptivity, sexual development, *Spalangia*

Most animals, including most insects, reproduce sexually, necessitating that the sexes locate one another (Thornhill and Alcock 1983). However, in some species, each individual develops within a separate burrow or host. In these species, mate location generally does not take place until at least one sex has emerged, and in many insects, the first sex to emerge is males (Wiklund and Fagerström 1977, Thornhill and Alcock 1983). In such situations, a male may open up a host or burrow to reach a female, may guard an unemerged female, or may locate females as or after they emerge. Competition from other males may select for males that reach the female earlier, provided that females either are monandrous or are polyandrous with first male sperm precedence and provided that females are ready to mate that early (Thornhill and Alcock 1983). If the female is not ready to mate, the male faces costs of waiting, such as lost opportunities to mate with other females, and these costs could exceed the benefits of having first access.

This study examines when adults become ready to mate and whether this corresponds to when males locate females in the parasitoid wasp *Spalangia endius* Walker. *S. endius* is a solitary species (one offspring develops per host) that parasitizes the pupae of certain fly species that are found in manure and rotting organic matter (Rueda and Axtell 1985). The fly host

consists of a shell-like puparium around the live fly pupa. *S. endius* develops from egg to adult within the puparium and so are hidden, at least visually. Approximately 3 wk after being oviposited, the wasp chews out of the puparium. Because hosts have a clumped distribution, multiple wasps are likely to emerge with some temporal and spatial synchrony.

Male *S. endius* begin emerging from their hosts a day or two before females (King 2000). Males do not immediately disperse from the group of parasitized hosts from which they emerged, but rather stay with them for more than a day (unpublished data). There is no overt aggression among adults. Both males and females mate readily when paired at 0–2 d after emergence. Whether they are ready to mate sooner was studied here.

A male's sexual response to a female begins when he is less than a few centimeters away from her. He chases her and usually briefly wing fans (rapidly moves his wings up and down) (King et al. 2005). Males wing fan only to females, not to males, probably in response to sex-specific pheromones. The male may mount the female and perform courtship. None of these aspects of his sexual response require any active solicitation by the female (i.e., even dead females elicit it; unpublished data), whereas copulation requires that the female open her genital orifice. Males readily remate, but females rarely do so (King et al. 2005).

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This study examined (1) when during development males and females begin to perform or elicit sexual behavior, (2) whether males recognize and stay with a host when it contains a female, and (3) whether males are attracted to a host when a female has recently emerged from it. Hosts from which females have recently emerged could help males identify a host patch as being one from which females are emerging.

Materials and Methods

General Methods. The *S. endius* were from a colony established from wasps collected in 1996 from Zephyr Hills, FL, and maintained using a natural host, *Musca domestica* L. pupae (King 1988). Vouchers are at the Illinois Natural History Survey Center for Biodiversity, catalog numbers "Insect Collection 6035 through 6054." Parasitized hosts were individually isolated in glass test tubes before the wasps' emergence to obtain virgin wasps. Mated wasps were produced by placing a virgin male and a virgin female into a test tube and watching for copulation to occur. No wasp was tested more than once.

Onset of Sexual Responsiveness. In these experiments, I examined the response of recently emerged adults to younger wasps, specifically to pupae and to pre-emergent adults. Any sexual behavior was recorded (fanning, mounting, courting, attempts at copulation). Recently emerged adults were 0-d-old virgins (i.e., <24 h from emergence) and had chewed their way out of their hosts on their own.

First, I examined the response of recently emerged males to the pupal stage of females and of males. The wasp pupae were dissected out of hosts. The pupae were black, and sometimes the tarsi could be seen moving under the pupal skin. Each recently emerged male was tested with a male or a female pupa but not both. He was nudged into contact with a pupa three times and observed with the pupa for 5 min in a test tube.

Second, the response of recently emerged adults of each sex to pre-emergent adults of the opposite sex was examined. Each male-female pair was observed together for 5 min in a test tube. The pre-emergent adults had eclosed from their wasp pupal skins but had not yet chewed out of their hosts. They were obtained by dissecting hosts.

Choice of Hosts Containing Pre-emergent Wasps. Each recently emerged adult virgin male was presented simultaneously with a host that had previously been exposed to virgin females for parasitization and a host that had previously been exposed to mated females for parasitization. The hosts had been parasitized ≈ 3 wk previously so that the wasps were usually within a few days of emerging (see Results). One of the reasons for testing males with hosts parasitized by virgin versus mated females was simply to test males with parasitized hosts containing different sexes. Hosts parasitized by virgin females can contain males but not females, whereas hosts parasitized by mated

females can contain either sex. This is because males are produced parthenogenetically, whereas females are produced sexually in *S. endius*. Another reason for testing males with hosts parasitized by virgin females versus by mated females was that an ability to distinguish the two types of hosts was plausible. Males can distinguish between virgin and mated females; e.g., they retreat from mated females more often than from virgin females (King et al. 2005). The mechanism by which males distinguish is still under study. However, if the distinction is pheromonal, some of the pheromone might transfer onto hosts during oviposition.

The hosts were presented to the males in small plastic dishes (15 mm in diameter by 11 mm in height) that were approximately one-half full of sand and covered with glass coverslips. The sand was dampened to reduce static electricity and prevent wasps from burrowing. The hosts were placed parallel to each other, were oriented the same direction (ventral side up), and were far enough from the sides of the dish that the test wasp would not contact them just by walking the dish's perimeter. Which host was on the left was alternated between trials. The test male had emerged within 2 d. How long he spent in contact with each host was recorded over a 6-min period.

The hosts were subsequently isolated. For each wasp that emerged, sex and day of emergence were determined. The time spent on a host was compared between (1) hosts that had been parasitized by virgin versus mated females, (2) hosts from which a wasp versus no wasp later emerged, and (3) hosts from which a male versus a female wasp later emerged. In this experiment and the next, these comparisons of time spent between host types was by paired *t*-test or by sign test if the paired *t*-test's assumption of normality of the difference (difference between time on the two host types) was violated (SPSS 2000). Which host was contacted first was also compared between host types by binomial tests.

Choice of Hosts from Which Females Versus Males Emerged. This experiment was similar to the previous one except that the test wasp was presented with a host from which a female had emerged and a host from which a male had emerged. The emergence had been within 1 d of presentation. The hosts were presented with the emergence hole facing up.

The tests wasps included virgin males, mated males, and virgin females. In addition to recording how long the test wasp spent in contact with each host, the following data were collected: (1) which host was contacted first, (2) whether the wasp fanned its wings at the emergence hole, (3) which host the wasp first began to enter (as evidenced by placing a body part into the emergence hole), and (4) which host the wasp first entered completely. The latter was recorded because it may indicate a stronger attraction than just beginning to enter. Besides comparing the responses of test wasps to the two types of hosts as in the previous experiment, the responses of virgin males were compared with those of mated males and to those of virgin

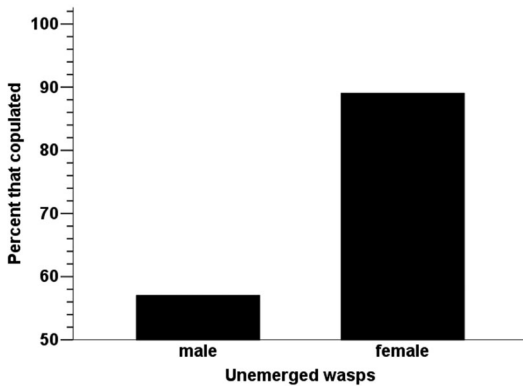


Fig. 1. Comparison of the proportion of pre-emergent adult males ($n = 14$) versus pre-emergent adult females ($n = 28$) that copulated when placed with a naturally emerged adult of the opposite sex ($G = 5.46$, $df = 1$, $P = 0.02$).

females. These latter comparisons were by log-likelihood ratio tests of independence when the comparisons were of categorical data (SPSS 2000, Zar 2000). The comparisons of contact time were by independent t -tests or by Mann-Whitney U tests when the assumption of normality was violated.

Results

Onset of Sexual Responsiveness. Males did not exhibit any sexual behavior toward female pupae: no wing fanning, mounting, courting, or attempts at copulation ($n = 20$ different males and 20 different pupae). When a male made contact with a female pupa, he did not even usually antennate it, and when he walked over it, there was no noticeable change in his speed or direction. Males exhibited the same lack of reaction to male pupae ($n = 17$ different males and 17 different pupae).

In contrast, pre-emergent adult females were attractive to males and were receptive. Almost 90% copulated within a few minutes of being placed with a male (Fig. 1). Females that copulated included some that had finished eclosing from their pupal cuticle immediately before testing. Mounting was usually preceded by the male wing fanning. The males that did not copulate did fan repeatedly but without mounting, despite ample opportunity. When the pre-emergent adult females were given hosts shortly after copulating, 83% ($n = 23$) produced no offspring, 13% produced at least one daughter, and 4% produced only sons.

About one half of the pre-emergent adult males copulated, which is significantly less than among the pre-emergent adult females (Fig. 1). When the mates of males that copulated were then given hosts, some of them (57%, $n = 7$) produced at least one daughter, indicating sperm transfer. Most of the males that did not copulate also did not even wing fan.

Choice of Hosts Containing Pre-emergent Wasps. Males showed no evidence of any host preferences in this experiment. They were not significantly more likely to first contact hosts that had been exposed to parasitization by mated females than hosts that had been exposed to parasitization by virgin females (binomial test: $n = 88$, $P = 0.30$), nor did they spend more time on hosts that had been exposed to mated females (sign test: $n = 91$ pairs of hosts, $P = 0.67$). When the same analysis was restricted to hosts from which wasps emerged, i.e., hosts that were successfully parasitized, males still did not prefer hosts parasitized by mated females over hosts parasitized by virgin females in terms of time spent on each host (sign test: $n = 33$ pairs of hosts, $P = 1.00$) or in terms of which host was contacted first (binomial test: $n = 31$ pairs of hosts, $P = 0.24$).

Likewise, males were not significantly more likely to first contact or to spend more time with successfully parasitized hosts versus hosts that were not successfully parasitized (first contact: binomial test, $n = 39$, $P = 0.37$; time spent: sign test, $n = 40$ pairs of hosts, $P = 1.00$). The wasps emerged an average of 4 ± 0.2 d (range: 1–11 d) after testing, but these results held even when the analysis was restricted to wasps that emerged in the next 1–2 d. (The purpose of this restriction was in case males can recognize parasitized hosts only when the wasps within are closer to emergence.) Males also were not significantly more likely to first contact or to spend more time with a host from which a female later emerged than a host from which a male later emerged (first contact: binomial test, $n = 23$, $P = 0.34$; time spent: $t = 0.078$, $df = 23$, $P = 0.94$).

Choice of Hosts from Which Females Versus Males Emerged. Virgin males differed from both mated males and virgin females in their responses to hosts from which wasps had recently emerged. Virgin males tended to contact the female's host before the male's host, whereas mated males showed no significant preference, and virgin females tended to contact the male's host before the female's host (Fig. 2). Virgin males were more likely than mated males to fan at the emergence hole of the female's host (35%, $n = 20$ versus 7%, $n = 29$; $G = 6.29$, $df = 1$, $P = 0.012$). No males fanned in response to the male's host, and no females fanned in response to any hosts.

Both virgin males and mated males spent more time in contact with the female's host than the male's host, whereas virgin females showed no significant preference (Table 1). Virgin males also spent more total time in contact with both hosts combined than did mated males ($t = 2.15$, $df = 48$, $P = 0.037$) or virgin females (Mann-Whitney $U = 49.0$, $P < 0.001$). This was because virgin males spent more time with the female's host than did either mated males or virgin females ($t = 2.23$, $df = 48$, $P = 0.031$; Mann-Whitney $U = 53.5$, $P < 0.001$). Virgin males did not spend more time with the male's host than did mated males or virgin females (Mann-Whitney $U = 297.0$, $P = 0.88$; Mann-Whitney $U = 49.0$, $P < 0.81$).

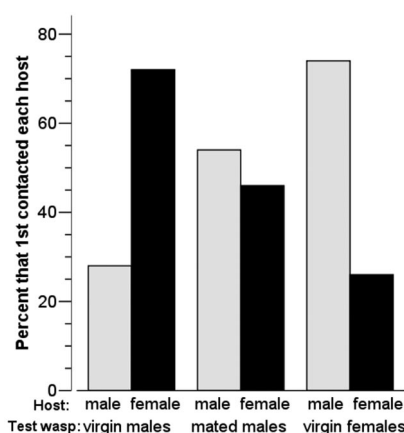


Fig. 2. Percent of test wasps that first contacted a host from which a male had recently emerged versus a host from which a female had recently emerged when both hosts were presented simultaneously and the test wasps were virgin males (binomial test: $n = 18$, $P < 0.001$), mated males (binomial test: $n = 26$, $P = 0.48$), and virgin females (binomial test: $n = 19$, $P < 0.001$).

Virgin males were more likely to begin to enter the female's host first than the male's host (19 versus 1; binomial test: $P < 0.001$). Mated males showed the same pattern, but it was of borderline statistical significance (17 versus 8; binomial test: $P = 0.054$), and the preference of mated males was not as strong as that of virgin males ($G = 5.75$, $df = 1$, $P = 0.016$). Fewer virgin females began to enter a host than did males (33%, $n = 20$ virgin females versus 95%, $n = 21$ virgin males, and 86%, $n = 29$ mated males). Virgin males were more likely to enter a host completely (86%, $n = 21$) than were mated males (52%, $n = 29$; $G = 6.71$, $df = 1$, $P = 0.010$) or virgin females (20%, $n = 20$; $G = 19.38$, $df = 1$, $P < 0.001$). Across all wasps that completely entered a host, most (95%, $n = 37$) entered the female's host first, perhaps because emergence holes were larger on female hosts than on male hosts (9 of 10 pairs, based on visual observation). Location of the hole did not differ (head end:spiracle end = 10:9 for male hosts; 12:8 for female hosts; $G = 0.22$, $df = 1$, $P = 0.64$).

Discussion

Although *S. endius* females were usually receptive before they chewed out, *S. endius* males do not chew

Table 1. Time (of 360 s) spent by virgin males, mated males, and virgin females on a host from which a female had emerged versus on a host from which a male had emerged

Test wasp	n	Female's host	Male's host
Virgin males ^a	21	216 ± 27 (2–346)	19 ± 9 (0–184)
Mated males ^b	29	129 ± 27 (0–356)	23 ± 10 (0–288)
Virgin females ^c	18	47 ± 24 (0–341)	13 ± 5 (0–77)

Values are mean ± SEM (range).

^a $t = 6.49$, $df = 20$, $P < 0.001$.

^b $t = 3.40$, $df = 28$, $P = 0.002$.

^c Sign test, $P = 0.33$.

into hosts in search of females, probably because the toughness of the puparium would make doing so costly and for little benefit, just for one female. Very few parasitoids exhibit within-host mating, and the few that do are gregarious (Drapeau and Werren 1999). Males of gregarious species have access to multiple females, even without having to chew into a host first.

Spalangia endius males did not seem to recognize which hosts contained pre-emergent females specifically or pre-emergent wasps more generally. That males would spend more time with such hosts if they did recognize them is suggested by the experiment with empty hosts. The failure of males to distinguish between hosts parasitized by virgin versus mated females suggests that any pheromonal differences between virgin and mated females either do not transfer to hosts during oviposition or are not long lasting.

In contrast to *S. endius*, males locate hidden females before they emerge in a variety of hymenopterans (Table 3.5 in Thornhill and Alcock 1983, Tooker and Hanks 2004). In the solitary bee, *Centris pallida* Fox, males even dig out 1–2 cm of soil to reach conspecifics that are about to emerge (Thornhill and Alcock 1983). Although *S. endius* males did not spend more time on parasitized hosts than on unparasitized hosts, at least two confamilials do. *Nasonia vitripennis* (Walker) males do so even if the parasitized host has only been parasitized for 1 d (development time is ≈ 2 wk) (King et al. 1969). The situation is similar for *Lariophagus distinguendus* Förster, which parasitizes hosts within grains. Males spend considerably more time on grains containing unemerged conspecifics than on grains containing just their hosts or nothing, and they wing fan only to grains containing conspecifics (Steiner et al. 2005). They do not distinguish between grains containing a female versus a male.

Why do *N. vitripennis* and *L. distinguendus* but not *S. endius* wait on or near a parasitized host for female(s) to emerge? The benefit to waiting may be higher for *N. vitripennis* than for *S. endius*. *N. vitripennis* is gregarious and has female-biased sex ratios, so a parasitized host represents multiple potential mates. *S. endius* also has female-biased sex ratios; however, because it is solitary, a given host represents only one female. *L. distinguendus* is also solitary; however, its cost of waiting may be considerably less than that of *S. endius*. *S. endius* can take more than an hour to chew out of a *M. domestica* host (unpublished data). Preliminary observations of *L. distinguendus* females suggest that they may take considerably less time (J. Ruther, personal communication). That selection to locate pre-emergent females is affected by cost of waiting is also suggested by species in which males preferentially guard those females that will emerge sooner (Matthews 2003, Schroeder 2003).

Costs of waiting for female emergence from a host include the risk of lost opportunity, i.e., while waiting, a male might miss emerged females; and the risk of mistaken identity, i.e., unless males can distinguish between hosts parasitized by conspecifics versus by other species that use the same hosts, they may wait only to have the wrong species emerge. *S. endius*

commonly co-occurs with congeners that parasitize the same host species (Olbrich and King 2003 and references therein).

That males did not respond to conspecifics in the pupal stage was not surprising because they do not normally encounter them, except in the rare instances in which two wasps develop within a single host (unpublished data). That they did not respond suggests that any sexual attractants are not on the pupal skin and that the pupae just being the same size and general form as adults is not sufficient. In contrast, *L. distinguendus* males wing fan to both male and female pupae, even unmelanized ones and even though this species develops within a grain and is also solitary (Steiner et al. 2005).

At least some pre-emergent *S. endius* males have mature sperm, as evidenced by their mates producing daughters. Nevertheless, *S. endius* males did not consistently exhibit sexual behavior toward females at this time. There may be little selection for interest in mating among males that have just eclosed from their pupal skins because the males still have to chew out of the host and because they tend to emerge before females (King 2000).

Spalangia endius males probably detect females as the females chew their emergence hole. Females were attractive for wing fanning by this stage, and the emergence hole should allow female pheromone to reach the male. The attraction of males to hosts from which females had already emerged and left may be directly beneficial. Because hosts are aggregated, a male's observation that a female has emerged from one host may predict for him that females are likely to emerge from the remaining hosts. Alternatively, the attraction to hosts from which females had already emerged may also or instead be a by product of selection to respond to females directly. Female pheromones may simply get on the host as the adult female emerges. Knowing that males sometimes wing fan not only to females but also to the hosts from which they emerged may have practical applications in helping to identify the chemicals involved. Analyses of the wasps themselves will reveal many chemicals, but the subset that are also found on the hosts that they emerged from are the ones that should be presented to males to test for wing fanning response.

Like *S. endius*, in the hyperparasitic wasp *Dendrocerus carpenteri* (Curtis), males find hosts from which females have emerged sooner and more often and stay with them longer than hosts from which males have emerged (Schworer et al. 1999). In *N. vitripennis*, males even prefer visiting hosts from which conspecifics have recently emerged over hosts from which conspecifics will soon emerge (King et al. 1969), presumably because the pheromonal cues are stronger. In *S. endius*, the strength of preference for the hosts of females was particularly strong for virgin males (Fig. 2). Likewise, virgin males are more strongly attracted to virgin female adults than are mated males (King et al. 2005).

Spalangia endius males more actively pursue mating than do females. Like virgin males, virgin females were

quicker to contact hosts from which the opposite sex versus the same sex had emerged. However, these females clearly did not spend more time on the opposite sex's host, whereas males did. In addition, in interactions between male and female virgin adults, males initiate about four to five times as many of the interactions that involve contact as females do (unpublished data).

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