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# Effects of Sex and Mating Status on Who Initiates Contact in the Parasitoid Wasp *Spalangia endius* (Hymenoptera: Pteromalidae)

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**Abstract** In species without obvious aggression, individuals may still vary in how likely they are to initiate contact with a conspecific. In the parasitoid wasp *Spalangia endius*, who was more likely to initiate contact during pair wise interactions depended on sex and mating status. Specifically, more contacts between the sexes were initiated by the male than by the female both when the female was still virgin and when the female had already mated with a different male. After a male mated with a given female, he still sometimes initiated contacts with her, but no longer more often than she did. A male was more likely to initiate contact when he was with a female than when he was with another male, and he was more likely to retreat from a mated female than from a male.

**Keywords** Ardent, approach, interaction, parasitoid wasp, pteromalid, virgin

## Introduction

Much of what is known about intraspecific interactions in insects is based on very obvious behaviors like courtship, chases and aggression (e.g., Thornhill and Alcock 1983; O'Donnell 1999; Goubault et al. 2006). However, focusing on just showy behaviors or species may provide an incomplete or misleading picture. For example, in species with showy aggressive males, the influence of more subtle female behavior may be overlooked.

In many species males have been described as ardent and females as coy or passive (e.g., Darwin 1871; Dewsbury 2005). Such a difference between the sexes could evolve because of eggs being expensive to produce relative to sperm (Bateman 1948; Trivers 1972). One mate is often sufficient to fertilize all of the eggs that a female can produce or raise, so a female's fitness is limited by the number of offspring that she can produce rather than by the number of mates that she can obtain. In contrast, a male's fitness will be limited by the number of mates that he can obtain. Even though male pursuit of mating may be more visible than female pursuit, females are clearly not as passive as once thought (e.g., Bonduriansky 2001; Arnqvist and Rowe 2005; Tang-Martinez and Ryder 2005).

The present study examines a species that lacks obvious aggressive behavior by either sex, the parasitoid wasp *Spalangia endius*, asking whether who initiates contacts and who retreats is dependent on sex and/or mating status. *S. endius* is a widely distributed, 2-3 mm long wasp that parasitizes the pupae of certain fly species that are found in manure and rotting organic matter (Rueda and Axtell 1985). Generally, only one wasp develops per host. However, the hosts are frequently clumped in distribution (personal observation), and males emerge before females and wait at the clump of hosts for females to emerge (King, personal observation). There is no obvious aggression among adults, e.g., no wing flicking, lunging, kicking, or biting; and males and females noticeably respond to each other only at fairly close range, a few wasp lengths or less. Females are larger than males (Napoleon and King 1999; King 2000).

In a typical mating, upon contact, a male chases after a female, often briefly fanning his wings in the process. There may be multiple chases before a male mounts a female. He mounts her dorsally and then begins vibrating and continues doing so through and even after copulation (King et al. 2005). Males do not appear to provide females with any nutrition during mating (King 2002). Males readily mate more than once; females rarely do (King et al. 2005). Specifically, regardless of male mating status, within 6 min about 90% of virgin females copulate, whereas only about 2% of mated females copulate. However, males readily chase and contact both virgin and mated females.

Do males initiate intersexual interactions more often than females and retreat less often, and do they do so only when it comes to interactions likely to lead to mating, i.e., with virgin females, or do they do so regardless of either sex’s mating status? That mating status may affect which sex more often retreats seems plausible because the timing of a male's first retreat is known to be affected by both male and female mating status. Males retreat sooner from mated females than from virgins, and mated males retreat sooner than do virgin males (King et al. 2005).

## Methods

The *S. endius* were from a colony established from wasps collected in Zephyr Hills, Florida and maintained using a natural host, *Musca domestica* pupae (King 1988). To obtain virgin wasps, parasitized hosts were isolated in glass test tubes (12 X 75 mm) and checked daily for wasp emergence. Mated wasps were generated by placing a virgin male and a virgin female into a test tube and watching for mating to occur. Wasps were less than 1 d old when tested.

Each of 99 male-female pairs of wasps was videotaped for 6 min each as described in King et al. (2005). The treatments were virgin male with virgin female (VV, n = 26), mated male with virgin female (MV, n = 23), virgin male with mated female (VM, n = 25), and mated female with mated male (MM, n = 25). Wasps were assigned to treatment at random rather than being self selected, i.e., the virgin wasps were not simply those that failed to mate. A wasp was never paired with the same wasp with which it had just mated.

The videotapes were subsequently viewed in order to record all contacts except during the time that a male was mounted on a female. A contact was when either wasp touched the other wasp, or nearly touched and the other retreated. I recorded whether the contact occurred premating or postmating, who initiated each contact and who retreated from each contact. A retreat involved jumping away, quickly increasing running speed while moving away, or curling into a submissive posture with antennae tucked in.

The proportion of contacts initiated by the female and the proportion initiated by the male were calculated, as were the proportion of contacts from which the female retreated and the proportion from which the male retreated. Within both initiation and retreat, the male and female proportions do not add to one because there were also mutual initiations and sometimes neither wasp retreated, e.g., both wasps just continued on their way. The contact initiation data and the retreat data were both analyzed by repeated measures analysis, but separately.

For the two treatments with virgin females (VV and MV), the repeated measures were sex (male, female) and mating (premating, postmating), so only pairs that had interactions both before and after mating were included. The between subjects factor was male mating status. This initial repeated measures analysis clearly revealed only one significant interaction, a 2-way interaction between sex and mating for the analyses of both contact initiations ($F_{1,37} = 26.93$, $P < 0.001$) and retreats ($F_{1,37} = 34.76$, $P < 0.001$). This interaction means that differences between the sexes in who initiated and who retreated were not the same before and after mating. Thus, subsequent analyses examined before and after mating separately.

The two treatments with mated females (VM and MM) were also analyzed by repeated measures analysis but with only sex, not mating, as a repeated measure. (In only one pair with a mated female was there remating; for that pair, only data prior to the remating were included.)

To provide a sense of variation among pairs, in addition to presenting data on the average proportion of contacts that each sex initiated and retreated from, I also present the percent of pairs for which the female was the one who initiated more of the contacts and the percent for which the male was. I did the same for retreats. For these calculations I combined virgin and mated males because the previous analyses showed no significant effect of male mating status.

In addition to the 99 male-female pairs, 20 pairs of males were videotaped under the same conditions and during the same time, and the same type of data was collected. The males in these pairs were all mated. I compared the behavior of these males to that of males in male-female pairs. For the male-male pairs, the proportion of contacts initiated and the proportion of contacts retreated were each averaged between the two males. In comparing male-male pairs to male-female pairs, I again ignored initial male mating status because in previous tests its effect was nonsignificant (see Results). Comparisons between male-male pairs and male-female pairs were by unequal variance t-tests as recommended by Ruxton (2006).

**Table I. The proportion of contacts initiated by each sex in treatments with virgin females**

| Treatment | Premating<br>Mean ± SE (Range) | Postmating<br>Mean ± SE (Range) | N |
|---|---|---|---|
| Initiated by Females | | | |
| Virgin male | 0.12 ± 0.05 (0.00- 0.80) | 0.40 ± 0.05 (0.00 - 0.82) | 21 |
| Mated male | 0.12 ± 0.05 (0.00- 0.67) | 0.43 ± 0.05 (0.00 - 0.86) | 18 |
| Initiated by Males | | | |
| Virgin male | 0.69 ± 0.09 (0.00 - 1.00) | 0.46 ± 0.05 (0.05 - 1.00) | 21 |
| Mated male | 0.51 ± 0.09 (0.00 - 1.00) | 0.39 ± 0.05 (0.09 - 0.90) | 18 |
| Sex by Male Status: | $F_{1,46} = 2.03$, P = 0.16 | $F_{1,38} = 0.75$, P = 0.39 | |
| Sex: | $F_{1,46} = 26.53$, P < 0.001 | $F_{1,38} = 0.035$, P = 0.85 | |
| Male Status: | $F_{1,46} = 1.82$, P = 0.18 | $F_{1,38} = 1.64$, P = 0.21 | |

## Results

Among pairs that copulated, the median time until copulation was 23 s, range: 7 – 352 s, N = 44. In the treatments with virgin females (VV, MV), before mating, the average proportion of contacts that males initiated was substantially greater than that of females (Table I), and the proportion of contacts from which females retreated was significantly greater than that of males (Table II). In contrast, after mating, the average proportion of contacts that each sex initiated was similar (Table I), and the proportion of contacts from which males retreated was significantly greater than that of females (Table II). The male's mating status had no significant effect on who initiated contacts or who retreated, and this was true regardless of female mating status (Tables I, II).

In the treatments with mated females (VM, MM), males both initiated and retreated from contacts significantly more often than females. There was no significant effect of male mating status and no interaction between male mating status and sex for either initiations or retreats (Tables III and IV).

Although in all treatments the male initiated, on average, either a greater or equal proportion of contacts as the female, nevertheless, there were still a substantial proportion of pairs in which it was the female who initiated a greater proportion of the contacts (Table V).

**Table II. The proportion of contacts from which each sex retreated in treatments with virgin females**

| Treatment | Premating<br>Mean ± SE (Range) | Postmating<br>Mean ± SE (Range) | N |
|---|---|---|---|
| Female Retreated | | | |
| Virgin male | 0.45 ± 0.09 (0.00 - 1.00) | 0.22 ± 0.04 (0.00 - 0.50) | 21 |
| Mated male | 0.51 ± 0.10 (0.00 - 1.00) | 0.25 ± 0.04 (0.06 - 0.62) | 18 |
| Male Retreated | | | |
| Virgin male | 0.13 ± 0.05 (0.00 - 0.80) | 0.45 ± 0.05 (0.00 - 1.00) | 21 |
| Mated male | 0.12 ± 0.05 (0.00 - 0.50) | 0.47 ± 0.06 (0.14 - 0.82) | 18 |
| Sex by Male Status: | $F_{1,46} = 0.03$, P = 0.87 | $F_{1,38} = 0.00$, P = 1.00 | |
| Sex: | $F_{1,46} = 10.39$, P = 0.002 | $F_{1,38} = 15.54$, P < 0.001 | |
| Male Status: | $F_{1,46} = 0.01$, P = 0.91 | $F_{1,38} = 0.70$, P = 0.41 | |

**Table III. The proportion of contacts initiated by each sex in treatments with mated females**

| Treatment | Mean ± SE (Range) | N |
|---|---|---|
| Proportion of Contacts Initiated by Females | | |
| Virgin male | 0.38 ± 0.04 (0.08 - 0.96) | 24 |
| Mated male | 0.33 ± 0.03 (0.00 - 0.59) | 25 |
| Proportion of Contacts Initiated by Males | | |
| Virgin male | 0.43 ± 0.04 (0.00 - 0.80) | 24 |
| Mated male | 0.51 ± 0.04 (0.17 - 1.00) | 25 |

Sex by Male Status: $F_{1,47} = 1.57$, P = 0.22
Sex: $F_{1,47} = 5.35$, P = 0.025
Male Status: $F_{1,47} = 0.67$, P = 0.42

Males initiated a lesser proportion of contacts with males than they did either with mated females or with virgin females premating (Table VI). Males retreated from a greater proportion of contacts with mated females than with mated males (Table VI). However, males retreated from the same proportion of contacts with other males as they did with virgin females (Table VI).

## Discussion

Upon casual observation of *S. endius*, that males chase females prior to mating gives the impression that males are more interested in females than vice versa. However, males were more likely than females to initiate contact in only some situations and in only some pairs within each situation. During the encounters that were most likely to lead to mating, i.e., during interactions with virgin females, the male was usually both the one that initiated contact and the one that did not retreat, consistent with sexual selection theory. However, females still initiated some of the intersexual contact even then. In contrast, after a male mated with a virgin female, the male initiated roughly as many contacts as the female and was usually the one that retreated. That males initiated a lesser proportion of the contacts between the sexes after mating than before is probably because of males' overall decrease in sexual responsiveness after mating (King et al. 2005). This decrease in male response to females right after mating may help keep males from immediately trying to remate the same female (Fischer and King 2008).

**Table IV. The proportion of contacts from which each sex retreated in treatments with mated females**

| Treatment | Mean ± SE (Range) | N |
|---|---|---|
| Female Retreated | | |
| Virgin male | 0.28 ± 0.03 (0.00 - 0.64) | 24 |
| Mated male | 0.27 ± 0.03 (0.00 - 0.56) | 25 |
| Male Retreated | | |
| Virgin male | 0.38 ± 0.03 (0.14 - 0.81) | 24 |
| Mated male | 0.33 ± 0.03 (0.00 - 0.62) | 24 |

Sex by Male Status: $F_{1,47} = 0.44$, $P = 0.51$
Sex: $F_{1,47} = 4.30$, $P = 0.044$
Male Status: $F_{1,47} = 1.37$, $P = 0.25$

**Table V. The percent of male-female pairs in which a given sex initiated, or retreated from, more of the contacts**

| Who Initiated More Contacts: | Female | Male | N |
|---|---|---|---|
| Virgin female treatments | | | |
| Pre-mating | 17% | 65% | 48 |
| Post-mating | 43% | 50% | 40 |
| Mated female treatments | 35% | 63% | 49 |
| **Who Retreated from More Contacts:** | **Female** | **Male** | **N** |
| Virgin female treatments | | | |
| Pre-mating | 52% | 21% | 48 |
| Post-mating | 30% | 65% | 40 |
| Mated female treatments | 35% | 55% | 49 |

Females and males do not add to 100% because of ties.

Unlike *S. endius*, in the bethylid parasitoid wasp *Cephalonomia tarsalis*, approaches are reported only for males, not at all for females; and females always flee when they encounter a male (Cheng et al. 2004). In the field cricket *Gryllus bimaculatus*, contact is initiated equally by males and females (Adamo and Hoy 1994). However, *G. bimaculatus* is an aggressive species, and females play a fairly active role throughout mating. Males and females will chase and bite each other, and females follow males and mount them, although it is the male that presents his rump to the female and performs calling and courtship songs.

In *Drosophila melanogaster*, *D. hydei*, and one of two studies with *D. pseudoobscura*, males tend to be more interested in females than vice versa (Gowaty et al. 2002, 2003). Interest was defined as the proportion of changes in direction that were toward the opposite sex and no distinction was made between before and after mating. Males fight each other in the two of these *Drosophila* species with the biggest difference between the sexes in interest (Spieth 1952). As in *S. endius*, for all three species of *Drosophila* there was considerable variation among pairs, including some pairs in which the female was more interested in the male (Gowaty et al. 2003).

**Table VI. The proportion of contacts initiated by the male and the proportion of contacts from which the male retreated**

| | Mean ± SE | N | |
|---|---|---|---|
| Male initiated when with: | | | |
| Another male | 0.40 ± 0.02 | 19 | |
| Virgin female | 0.57 ± 0.06 | 48 | t = 3.01, df = 54.08, P = 0.004 |
| Mated female | 0.47 ± 0.03 | 49 | t = 2.32, df = 65.98, P = 0.02 |
| Male retreated when with: | | | |
| Another male | 0.26 ± 0.02 | 20 | |
| Virgin female | 0.19 ± 0.04 | 48 | t = 1.48, df = 65.94, P = 0.14 |
| Mated female | 0.35 ± 0.02 | 49 | t = 2.86, df = 53.29, P = 0.006 |

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## References

Adamo, S. A., and Hoy R. R. (1994). Mating behaviour of the field cricket *Gryllus bimaculatus* and its dependence on social and environmental cues. Anim. Behav. 47:857-868.

Arnqvist, G., and Rowe, L. (2005). Sexual Conflict, Princeton University Press, Princeton, New Jersey.

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. Heredity 2: 349-368.

Bonduriansky, R. (2001). The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol. Rev. 76: 305-339.

Cheng, L., Howard, R. W. Campbell, J. F., Charlton, R. E., Nechols, J. R., and Ramaswamy, S. B. (2004). Mating behavior of *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) and the effect of female mating frequency on offspring production. J. Insect Behav. 17: 227-245.

Darwin, C. (1871). The Descent of Man and Selection in Relation to Sex, John Murray, London.

Dewsbury, D. A. (2005). The Darwin-Bateman paradigm in historical context. Integr. Comp. Biol. 45:831-837.

Fischer, C. R., and King, B. H. (2008). Sexual inhibition in *Spalangia endius* males after mating and time for ejaculate replenishment. J. Insect Behav. 21: 1-8.

Goubault, M. Batchelor, T. P., Linforth, R. S. T., Taylor, A. J., and Hardy, I. C. W. (2006). Volatile emission by contest losers revealed by real-time chemical analysis. Proc. R. Soc. B. 273: 2853-2859.

Gowaty, P. A., Steinichen, R., and Anderson, W. W. (2002). Mutual interest between the sexes and reproductive success in *Drosophila pseudoobscura*. Evolution 56: 2537-2540.

Gowaty, P. A., Steinichen, R., and Anderson, W. W. (2003). Indiscriminate females and choosy males: within- and between-species variation in *Drosophila*. Evolution 57: 2037-2045.

King, B. H. (2000). Sex ratio and oviposition responses to host age and the fitness consequences to mother and offspring in the parasitoid wasp *Spalangia endius*. Behav. Ecol. Sociobiol. 48: 316-320.

King, B. H. (1988). Sex ratio manipulation in response to host size by the parasitoid wasp *Spalangia cameroni*: a laboratory study. Evolution 42: 1190-1198.

King, B. H. (2002). Breeding strategies in females of the parasitoid wasp *Spalangia endius*: effects of mating status and body size. J. Insect Behav. 15: 181-193.

King, B. H., Saporito, K. B., Ellison, J. H., and Bratzke, R. M. (2005). Unattractiveness of mated females to males in the parasitoid wasp *Spalangia endius*. Behav. Ecol. Sociobiol. 57: 350-356.

Napoleon, M. E., and King, B. H. (1999). Offspring sex ratio response to host size in the parasitoid wasp *Spalangia endius*. Behav. Ecol. Sociobiol. 46: 325-332.

O'Donnell, S. (1999). The function of male dominance in the eusocial wasp, *Mischocyttarus mastigophorus* (Hymenoptera: Vespidae). Ethology 105: 273- 282.

Rueda, L. M., and Axtell, R. C. (1985). Guide to common species of pupal parasites (Hymenoptera: Pteromalidae) of the house fly and other muscoid flies associated with poultry and livestock manure, Technical Bulletin 278. North Carolina State University: North Carolina Agricultural Research Service.

Ruxton, G. D. (2006). The unequal variance t-test is an underused alternative to Student's t-test and the Mann-Whitney U test. Behav. Ecol. 17: 688-690.

Spieth, H. (1952). Mating behavior within the genus *Drosophila* (Diptera). Bull. Am. Mus. Nat. Hist. 99: 395-474.

Tang-Martinez, Z., and Ryder, T. B. 2005. The problem with paradigms: Bateman's worldview as a case study. Integr. Comp. Biol. 45: 821-830.

Thornhill, R., and Alcock, J. (1983). The Evolution of Insect Mating Systems, Harvard University Press, Cambridge, MA.

Trivers, R. L. (1972). Parental investment and sexual selection. In Campbell, B. G. (ed.), Sexual Selection and the Descent of Man, 1871-1971, Aldine, Chicago, pp. 136-179.