

King, B. H. 2010. Which sex controls the duration of postcopulatory courtship and to what effect in the parasitoid wasp *Spalangia endius*. Behaviour 147:993-1007.

This version matches the text but not the publisher's layout of the final publication, which is available at <http://brill.publisher.ingentaconnect.com/content/brill/beh> or by emailing bking@niu.edu

Which sex controls the duration of postcopulatory courtship and to what effect in the parasitoid wasp *Spalangia endius*

Who controls postcopulatory courtship duration

B. H. King

(Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, USA)

email: bking@niu.edu

Summary

Resistance by females during a reproductive interaction is thought to reflect conflict between the sexes over the optimal outcome. In the parasitoid wasp *Spalangia endius*, the female attempts to brush the male off her back with her hind legs after a few seconds of postcopulatory courtship. Although males do not dismount immediately, this signal is effective. Removal of female hind legs resulted in postcopulatory courtship that was longer, although a normal duration was sufficient to turn off a female's receptivity permanently. Responding immediately to the signal was not advantageous to males. When postcopulatory courtship was experimentally terminated as soon as females began signalling, they sometimes remained attractive and receptive to subsequent males. However, earlier studies suggest that for most if not all females, mating a second time would not increase the production of daughters or of total offspring, and being mounted interferes with oviposition. Thus the normal duration of postcopulatory courtship is determined by the behaviour of both the male and the female, and currently this may usually be better for both partners than a duration determined by just one partner. Coevolution between the sexes may have mitigated previous conflict, and remaining resistance may reflect the ghost of conflict past.

Keywords: mate guarding; postcopulatory courtship; receptivity; sexual conflict; signalling

Introduction

Sexual conflict occurs when the optimal trait value differs between the sexes, and it includes fitness conflict over the outcome of interaction between a male and a female (Chapman et al., 2003; Arnqvist & Rowe, 2005; Wedell et al., 2006). Although sexual conflict is defined in terms of fitness effects, sexual conflict is often inferred by behaviour, specifically by persistence by one sex and resistance by the other (e.g. Lauer et al., 1996; Edward & Gilburn, 2007). Such behaviour may reflect the ghost of conflict past and may or may not reflect current conflict. This is because the sex that suffers a significant cost will experience selection for counter adaptations to reduce that cost.

Using the parasitoid wasp *Spalangia endius*, the present study examines how the behaviour of each sex influences the duration of postcopulatory courtship and how the duration affects female remating and, potentially, fitness. Whether there is sexual conflict over postcopulatory courtship has not previously been examined experimentally in any other species. However, males frequently stay with females beyond sperm transfer, and the potential for conflict from doing so has long been recognized (e.g. Parker, 1970; Wilber, 1989; Alcock, 1994; Birkhead & Møller, 1998; Sato & Kohama, 2007). Observations of mating behaviour in a diversity of animals suggest that sexual conflict over the duration of mating in general is common (reviewed in Arnqvist & Rowe, 2005).

Spalangia endius parasitizes the pupal stage of a range of fly species that are found in manure and decaying organic matter (Rueda & Axtell, 1985). The hosts are often in groups, and male *S. endius* emerge before females and wait at the hosts for females to emerge (King, 2006). Generally, only one wasp emerges per host. Both sexes are reproductively mature at emergence, and *Spalangia* males do not produce sperm as adults, but rather have all of their sperm when they emerge as adults from their hosts (Gerling & Legner, 1968; King & King, 1994). *S. endius* is largely monandrous and polygynous (King et al., 2005).

To mate, an *S. endius* male mounts a female dorsally, usually after briefly fanning his wings as he runs towards her. Soon after mounting, his wings now flat against his body, the male's whole body begins to vibrate. The vibration appears to trigger the female to open her genital aperture, which is necessary for copulation to take place. The vibration continues until the male dismounts, and sometimes for a brief time after he dismounts. Postcopulatory courtship is defined as the period of vibration after copulation and prior to dismount. Sometime during postcopulatory courtship, the female begins a termination signal in which she appears to be trying to dislodge the male. Specifically, the female begins to brush her hind legs, one leg at a time, over the dorsal side of her abdomen in between herself and the ventral surface of the male. There is no evidence that males suffer any damage from this.

This study compares the duration of postcopulatory courtship when the male is in behavioural control, when the female is and when they both have some control. This study also examines how duration affects subsequent intersexual interactions, e.g., whether female willingness to remate is affected; and then uses results of previous studies on the effects of remating on fitness (see Discussion) to assess the effects of duration on fitness.

This study asked the following: Is the female's termination signal effective in limiting the duration of male postcopulatory courtship, i.e., would the male continue postcopulatory courtship for even longer if the female did not signal? If the male dismounted as soon as the female began signalling, would the female's genital aperture still be open, allowing her to remate? How does the duration of postcopulatory courtship (as opposed to just its presence or

absence (King & Fischer, 2005) affect the chance that a female will subsequently be mounted and copulate? Do males reduce the duration of their postcopulatory courtship when the ratio of females to males is greater, i.e., when the opportunity for additional matings is greater (Parker, 1974)?

Methods

The *S. endius* were from a Florida strain (King et al., 2005) that was maintained using a natural host, *Musca domestica* pupae (King, 1988). To obtain newly emerged virgin wasps, parasitized hosts were individually isolated in test tubes. When a mated wasp was needed, virgins were paired and observed until mating was complete. Wasps had emerged from their hosts within the last 1 d when used in experiments.

Wasps were assigned to treatments at random. Within an experiment, each replicate consisted of one trial of each treatment performed at nearly the same time. The statistical analyses ignore these treatment pairings for simplicity, but including them had no effect on conclusions. Statistical tests were two-tailed. Previous studies of this species show that mating status can have different effects on different variables, e.g., fanning versus mounting (King et al., 2005), so type I error was controlled separately at $\alpha = 0.05$ for each aspect of mating within each experiment, i.e., no multiple-comparison correction was utilized. Sample size discrepancies among behaviours resulted from some behaviours not being observable in every replicate. Tests of independence of categorical data were by G tests (Zar, 1999); conclusions would have been the same with Pearson's chi-square tests. Analyses of duration of postcopulatory courtship by independent t-test, analysis of variance (ANOVA) and regression are on untransformed data; however, log-transformation had no effect on conclusions.

For ease of discussion, the duration of postcopulatory courtship of unmanipulated pairs is referred to as "normal duration", the duration when males were removed as soon as the female signalled is referred to as "female duration" and the duration when females were prevented from signalling is referred to as "male duration". Male duration is equivalent to there being no female signal or to males not responding to the female's signal.

Normal duration of mating behaviours

This experiment examined the duration of the different elements of mating in the absence of any manipulation. For example, how soon after copulation ends does the female begin her termination signal, and how soon after she begins it before the male finally dismounts? A virgin male and a virgin female were put in a glass vial (12 mm in diameter x 75 mm in height) with a cotton plug and videotaped at 30X magnification until mating was completed ($N = 20$ pairs). Vials were moved as necessary to keep wasps in view. Durations of behaviours (Table 1) were measured using the time recorder on the video cassette recorder. Whether and when the female began walking after copulation but while the male was still mounted was also recorded. When it was clearly visible, I also examined whether the female's genital aperture had closed by the time that she began the termination signal ($N = 17$) and by the time that the male dismounted ($N = 12$). Temperature was mean \pm SE: $22.1 \pm 0.1^\circ\text{C}$ (range: $21.5 - 23.0^\circ\text{C}$) and relative humidity was $23.4 \pm 0.1\%$ (range: $22.5 - 24.0\%$).

Table 1. Normal duration (s) of mating behaviours.

Event	<i>N</i>	Mean ± SE	range
Start of mount to Start of copulation	18	10.61 ± 1.30	5 - 27
Copulation	20	6.20 ± 0.33	4 - 9
End of copulation to Start of brush off	17	5.06 ± 0.63	1 - 10
End of copulation to Complete dismount*	19	21.89 ± 2.09	9 - 49
Start of signal to Complete dismount	17	16.53 ± 1.91	5 - 40
Start of mount to Complete dismount	18	38.50 ± 2.88	24 - 64
Start of mount to Start of vibration	9	2.67 ± 0.44	1 - 5

*Postcopulatory courtship

Female-duration of postcopulatory courtship

This experiment examined the effect of stopping postcopulatory courtship as soon as the female began the termination signal. Specifically, I ask whether a subsequent male suitor will mount a female if she received a female-duration of postcopulatory courtship; and whether she is receptive, i.e., if he mounts, will she copulate? This experiment included two treatments, both of which involved a virgin male with a virgin female. In the interruption treatment, the male was removed with forceps as soon as the female began signalling. Females were included in this treatment even if they did not signal ($N = 3$ of 52) because the goal was to examine the female-determined duration of postcopulatory courtship. In the control treatment, the male dismounted without assistance, but forceps hovered nearby as in the interruption treatment.

After treatment, each female was tested with either a mated male or a virgin male, and his first reaction was recorded. Each mated male had just mated with a different virgin female prior to the test. The reason that some tests were with mated males and some with virgin males is that each type of male provides different information. Mated males are more discriminating about mounting than virgin males are (King et al., 2005). However, because mated males usually do not mount mated females, using mated males makes it difficult to address whether mated females are receptive once mounted. In contrast, virgin males frequently mount even mated females, making it possible to examine receptivity. Temperature was $22.6 \pm 0.2^\circ\text{C}$ (range: 18 - 25°C) and relative humidity was $24.6\% \pm 0.8$ (range: 15-43%).

Male-duration of postcopulatory courtship

This experiment examined whether the female's termination signal is effective in limiting the duration of the male's postcopulatory courtship. Females were prevented from signalling by removal of the hind legs. This experiment included two treatments, virgin females whose hind legs had been ablated (hind-legless females) and virgin females whose middle legs had been ablated (control females). The legs were ablated using a sharpened insect pin after

immobilization by cold with a cryolizer (a 9 cm diameter polypropylene cylinder enclosing a cold-retaining medium that holds a temperature of about -18°C , BioQuip Products, Inc.) (following Edvardsson & Tregenza, 2005; Edvardsson & Canal, 2006). The middle legs were ablated below the coxa, but it was possible to ablate the hind legs above the coxa. Once a female recovered, as evidenced by her walking, a virgin male was introduced and the duration of the postcopulatory courtship was determined with a stopwatch and compared between treatments ($N = 15$ females per treatment). I also compared a single female that used her middle legs for the termination signal with all the females that used their hind legs. For this I used a modified t-test designed to compare a single observation to the mean of a sample (Sokal and Rohlf, 1969, p. 224). Temperature was about 23°C during testing.

Male mating opportunity

This experiment examined whether males reduce the opportunity cost of staying on the female after copulation by reducing the duration of postcopulatory courtship when other mating opportunities are available. A pair of males was put in a test tube with either a single virgin female ($N = 31$) or five virgin females ($N = 31$). Postcopulatory guarding, i.e., how long the male stayed on the female following copulation, was determined with a stopwatch for the first male that mounted a female. Postcopulatory guarding was the same as the duration of postcopulatory courtship except for some pairs in which the other male mounted the mating pair. When the other male mounted the pair, the first male often stopped vibrating at least temporarily in order to hold on to his position. The effect of number of females and the effect of whether or not the other male mounted the mating pair was examined by a two-way ANOVA with both treatment (one female, five females) and mounted (mounted, not mounted) as fixed effects. Temperature was $21.8 \pm 0.2^{\circ}\text{C}$ ($20 - 24^{\circ}\text{C}$) and relative humidity was 21.3 ± 0.9 ($16 - 27\%$).

Results

Normal duration of mating behaviours

18 of 18 females gave the termination signal. Males began to vibrate within a few seconds of mounting (Table 1). They continued vibrating even after the female's signal. They vibrated until dismounting or shortly after dismounting. The period of precopulatory vibration lasted approximately 8 s, copulation was another 6 s and postcopulatory courtship was about 22 s. Adding these shows that females received roughly 36 s total of vibration from males. If males had dismounted as soon as females began the signal, postcopulatory courtship would have lasted only about 5 s, not 22 s, so females would have received only about 19 s of vibration in total, not 36 s.

Males did not dismount at a fixed number of seconds after females began signalling (Figure 1). Instead, females that waited longer before signalling had males that waited longer after the signal began before dismounting. Thus in pairs in which the female was quicker to begin signalling, total postcopulatory courtship was considerably shorter ($R^2 = 0.54$, $F_{1,15} = 17.75$, $P = 0.001$; $y = 2.71x + 7.87$). A role of walking in terminating postcopulatory courtship

was not apparent. Postcopulatory courtship was longer, not shorter, among females that walked while the male was mounted compared to females that did not (mean \pm SE, range: 29 ± 4 s, 9 - 25 s, $N = 7$ versus 16 ± 1 s, 18 - 49 s, $N = 10$; $t_{15} = 3.57$, $P = 0.003$). Among females that walked, walking sooner after copulation did not result in shorter postcopulatory courtship ($R^2 = 0.28$, $F_{1,5} = 1.98$, $P = 0.22$).

The female's genital aperture had only just begun closing and in all cases was still mostly open when she began signalling. In contrast, at the time that the male dismounted, 11 of 12 females were closed or so close to being closed that another male almost certainly would have been unable to insert his aedeagus.

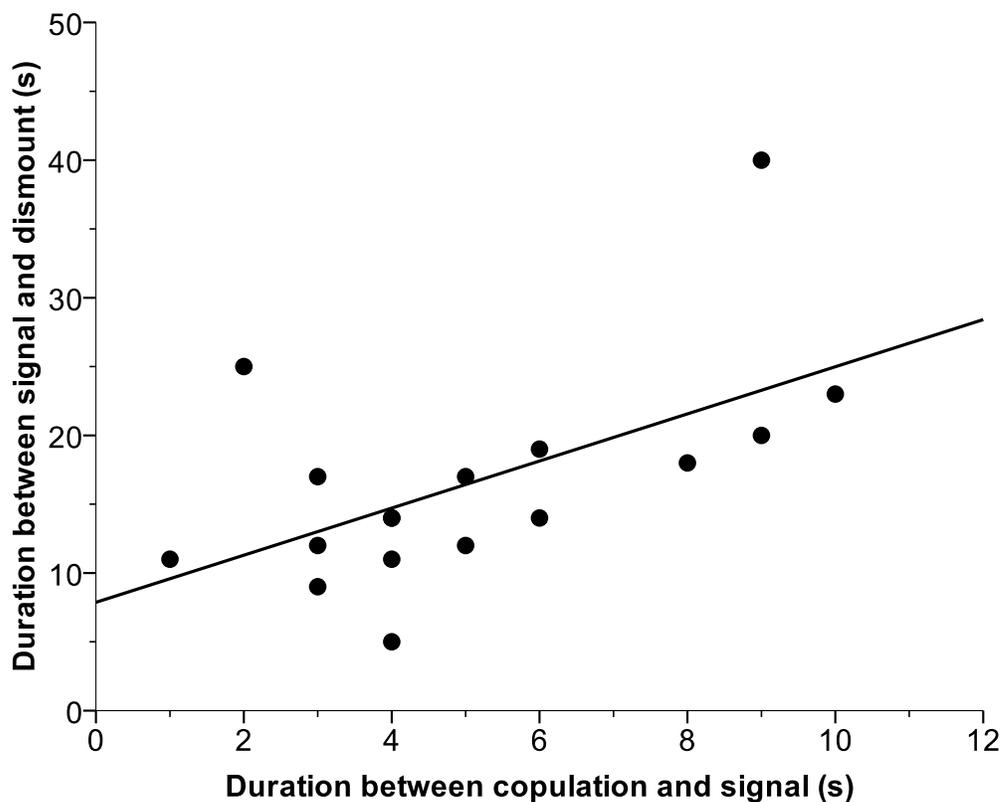


Figure 1. Relationship between the duration (s) from the start of female signalling to male dismount and the duration (s) from copulation to the start of female signalling ($R^2 = 0.32$, $F_{1,15} = 7.07$, $P = 0.018$; $y = 1.71x + 7.87$)

Female-duration of Postcopulatory Courtship

Females from the interruption treatment were just as likely to be fanned as control females were, and this was true regardless of the male's mating status (Table 2). However, females from the interruption treatment were significantly more likely to be mounted and courted than

control females, regardless of the male's mating status. Among females that were mounted by virgin males, females from the interruption treatment were significantly more likely than control females to be receptive, i.e., to copulate (38% versus 0%, $G_1 = 5.23$, $P = 0.02$). The pattern was the same with mated males, but too few mated males mounted to make a formal statistical test meaningful (29% versus 0%, $N = 7$, $N = 1$). No control females copulated a second time.

Table 2. Percent of females to which the male responded when tested with a female that had previously received a normal-duration (control female, $N = 20$ for mated males, $N = 32$ for virgin males) or a female-duration of postcopulatory courtship (experimental female, $N = 20$ for mated males, $N = 32$ for virgin males).

	Control female	Experimental female	Likelihood Ratio test	
			G_1	P
Mated male				
Fanned	85	95	1.16	0.28
Mounted	5	35	6.19	0.013
Courted	0	30	9.38	0.002
Copulated	0	10	2.88	0.09
Virgin male				
Fanned	97	97	0.00	1.00
Mounted	22	50	5.61	0.018
Courted	22	47	4.51	0.034
Copulated	0	19	8.9	0.003

Male-duration of Postcopulatory Courtship

Hind-legless females tended to receive longer postcopulatory courtship than control females (Figure 2). No middle-legless females received postcopulatory courtship longer than 25 s, whereas 73% of hind-legless females did ($G_1 = 22.03$, $P < 0.001$). Only 1 of 13 hind-legless females gave the termination signal, and that female did so with her middle legs; whereas 10 of 13 control females gave the termination signal, and they did so with their hind legs ($G_1 = 14.33$, $P < 0.001$). In the control treatment, females that gave the termination signal had much shorter postcopulatory courtship than female that did not (mean \pm SE, range: 11 ± 1 s, 5 - 14 s, $N = 10$ versus 23 ± 2 s, 19 - 25 s, $N = 3$; $t_{11} = 5.00$, $P < 0.001$). The single hind-legless female that tried to brush off the male with her middle legs was not very effective. She still received a postcopulatory courtship that was longer than that received by any of the control females (53 s versus 14 ± 2 s, 5 - 25 s, $N = 15$; $t_{14} = 6.16$, $P < 0.001$).

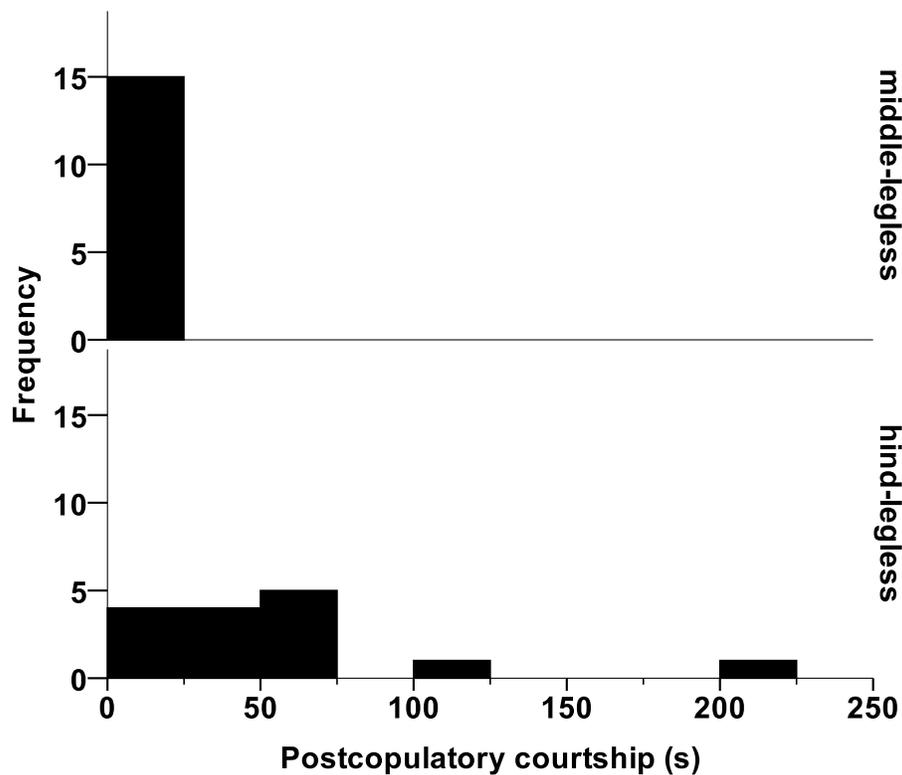


Figure 2. Duration of postcopulatory courtship (s) on females with middle legs removed versus females with hind legs removed

Male Mating Opportunity

The duration of postcopulatory guarding was not significantly different when one versus five females were present (Table 3; $F_{1,58} = 0.15$, $P = 0.70$). However, the duration was about twice as long when another male mounted during the mating ($F_{1,58} = 36.03$, $P < 0.001$). There was no significant interaction between the main effects (number of females, whether the other male mounted) ($F_{1,58} = 0.20$, $P = 0.66$). Despite a lack of obvious aggression between males, a male's presence on the female's back appeared to make it harder for other males to mount and to reach her genital opening. Across treatments, both males inserted their aedeagi into the female in about 5% of all vials ($N = 3$ of 62), which was 21% of the vials with double mountings ($N = 3$ of 14).

Table 3. The duration of postcopulatory mate guarding when a male was in the presence of either one or five virgin females and when another male was not or was mounted on the same female.

Number of Females	Other male not mounted		Other male mounted	
	<i>N</i>	Mean \pm SE (range)	<i>N</i>	Mean \pm SE (range)
1	21	14.5 \pm 1.7 (5.9 – 37.3)	10	31.6 \pm 5.2 (12.6 – 68.1)
5	27	14.3 \pm 1.3 (0.9 – 26.6)	4	34.1 \pm 4.4 (22.2– 41.1)

Discussion

Both male and female behaviour influenced the duration of postcopulatory courtship. Males normally prolonged postcopulatory courtship beyond the start of the female's termination signal, but not as long as in the absence of the signal. In the absence of the signal, postcopulatory courtship was also more variable. Contrary to expectations based on behavioural observations, there appears to be little or no current sexual conflict. The normal duration appears to be better for males and most, if not all, females than either a male duration or a female duration, even though females appear to attempt to decrease the duration.

Normal-duration appears to benefit a male relative to female-duration by reducing to almost zero the chance that his mate will copulate with another male immediately or later. This is important because females in this family of wasps can use sperm from both males when they mate multiply (Holmes, 1974; van den Assem and Feuth-De Bruijn, 1977; Beukeboom, 1994; Khanh et al., 2005). With normal duration, female genital closure was almost 100% (*Normal duration of mating behaviours* experiment), and loss of receptivity was

100% (control females in Table 2) and permanent (King & Fischer, 2005). In contrast, at the time that a female signalled, her genital aperture was still open so that a concurrent competitor would have been able to insert his aedeagus, and about one-fourth of females remained receptive to subsequent males. Male duration is not expected to provide any additional benefit to males given that normal duration is sufficient to completely turn off female receptivity.

Normal-duration may also benefit females, by making them less attractive for mounting by subsequent males. With a female duration, the time gained from shorter mating might be lost to subsequent harassment from other males mounting. Being mounted interferes with her ability to bend her body into the position used for oviposition (personal observation). Being mounted should also make it more difficult for her to burrow in search of buried hosts (Rueda & Axtell, 1985). A female duration would increase female remating, but at present there is no evidence that remating currently benefits females. Daughter production requires sperm, whereas sons are produced parthenogenetically. Experimentally inducing females to remate results in sperm transfer yet has no significant effect on daughter production, total offspring number or female longevity (King & Bressac, 2010). This is true even when the remating takes place after a week of oviposition, at which time a female's daughter production has dropped dramatically. These results are based on females that mated with virgin males. A female might at least be expected to benefit from remating when her original mate had fewer sperm from having mated previously. However, in *S. endius*, a male's mating history has no detectable effect on daughter production until his fourth or fifth mating; and sex ratios and female choice suggest that in nature being a fourth, fifth or later female is probably uncommon (King & Fischer 2010). Although fifth females rarely remate, whether they would benefit from remating remains to be tested. For a female duration to provide a net benefit for females, not only would there need to be a benefit of remating for these later females, but it would also need to exceed the cost of harassment that all females would experience from not losing their mounting-attractiveness.

Postcopulatory courtship's current function of making a female less attractive for mounting by subsequent males may be recent. This function has seldom been tested for in related species, but the function is absent in a congener (King, 2000) and a confamilial (unpublished data), as well as in another parasitoid wasp of the same superfamily (Allen et al., 1994). If this function is recent, in the past an *S. endius* male could still have benefited from postcopulatory courtship, due to mate guarding benefits while he was physically present on the female. In contrast, the postcopulatory courtship would only have cost a female, due to that male's physical presence interfering with oviposition and host searching.

Similar to *S. endius*'s termination signal, in a biting midge and a bean weevil, a female's kicking with her hind legs limits the duration of copulation (Linley & Mook, 1975; Crudgington & Siva-Jothy, 2000). In the bean weevil, limiting the duration of copulation decreases egg production (Edvardsson & Tregenza, 2005; Edvardsson & Canal, 2006); and the benefit of kicking seems to be through reduced damage to the female's genital tract from the male's spiny genitalia (Crudgington & Siva-Jothy, 2000).

The duration of various mating elements may not always be what is currently best for both sexes. In silkworm moths a shorter copulation would probably benefit females, whereas the normal 6 – 12 h duration of copulation appears to be roughly optimal for males (reviewed in Arnqvist & Rowe, 2005).

The present study examined the role of the female's termination signal in limiting postcopulatory courtship during mating. The same signal is also used by already mated

females to effectively limit the duration of subsequent mating attempts by males (unpublished data). At first glance, it also seems plausible that the termination signal, specifically the female brushing her legs across the male's abdomen, may play a role in the female's postmating unattractiveness (King et al., 2005). A female becomes less attractive to subsequent male mounting sometime during postcopulatory courtship with her first mate (King & Fischer, 2005), which is also when she signals. However, despite the concurrence, the signal does not appear to cause the unattractiveness: virgin females with ablated hind legs fail to brush off their first mate yet still become unattractive to subsequent male mounting (unpublished data).

The present study shows that a female's brush-off behaviour limits the duration of male postcopulatory courtship. Whether chemicals released by females contribute to limits on the duration of postcopulatory courtship remains to be determined. Female *S. endius* sometimes release a pheromone that reduces the chance that a male will copulate with them (King & Dickenson, 2008; I.C.W. Hardy, personal communication).

Contrary to expectations (Parker, 1974), males did not reduce their postcopulatory guarding when additional mating opportunities were present in the form of additional virgin females. The number of mating opportunities should depend not only on female density, but also on male density (Yamamura, 1986). In many, but not all, other organisms, males guard longer in the presence of greater male density, as expected (Jormalainen, 1998; Bateman and MacFadyen, 1999; Saecki et al., 2005; Knox & Scott, 2006). However, there should be little to no advantage of increasing the duration of postcopulatory courtship with increasing male density for *S. endius*. This is because the duration that a lone male provides is already long enough that by the time he dismounts, the female has almost always closed her genital orifice and become unreceptive (King et al. 2005; this study). *Spalangia endius* males postcopulatory guarded for longer when another male mounted them. However, guarding time and courting time were not synonymous when there was double mounting, so double-mounted males may not have courted for any longer. In addition, the causal direction between duration and double mounting is unclear. When a first male's postcopulatory guarding was longer, there would have been a longer opportunity for double mounting.

The present study has demonstrated that the duration of postcopulatory courtship in *S. endius* depends on the behaviour of both of the mating partners but not on the density of females. Evaluating whether there is sexual conflict over the normal duration is a more difficult task, but available evidence suggests no current conflict between males and most, if not all, females.

Acknowledgements

Thanks to C. Geden for providing starter *S. endius*, R. Dickenson for laboratory assistance, and C. R. Fischer, I. C. W. Hardy and J. Ruther for feedback on the manuscript. This research was supported by Northern Illinois University's Department of Biological Sciences.

References

Alcock, J. (1994). Post insemination associations between males and females in insects: the mate guarding hypothesis. — *Ann. Rev. Entomol.* 39: 1–21.

- Allen, G. R., Kazmer, D. J. & Luck, R. F. (1994). Post-copulatory male behaviour, sperm precedence and multiple mating in a solitary parasitoid wasp. — *Anim. Behav.* 48: 635-644.
- Arnqvist, G. & Rowe, L. (2005). *Sexual Conflict*. — Princeton University Press, Princeton, New Jersey.
- Bateman, P. W. & MacFadyen, D. N. (1999). Mate guarding in the cricket *Grylloides sigillatus*: Influence of multiple potential partners. — *Ethology* 105: 949-957.
- Beukeboom, L. W. (1994). Phenotypic fitness effects of the selfish B chromosome, paternal sex ratio (PSR) in the parasitic wasp *Nasonia vitripennis*. — *Evol. Ecol.* 8: 1-24.
- Birkhead, T. R. & Møller, A. P. (1998). *Sperm competition and sexual selection*. — Academic Press, London.
- Chapman, T., Arnqvist, G., Bangham, J., Rowe, L. (2003). Sexual conflict. — *Trends Ecol. Evol.* 18: 41-47.
- Crudgington, H., Siva-Jothy, M. T. (2000). Genital damage, kicking and early death. — *Nature* 407: 855-856.
- Edvardsson, M. & Canal, D. (2006). The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*. — *Behav. Ecol.* 17: 430-434.
- Edvardsson, M. & Tregenza, T. (2005). Why do male *Callosobruchus maculatus* harm their mates? — *Behav. Ecol.* 16: 788-793.
- Edward, D. A. & Gilburn, A. S. (2007). The effect of habitat composition on sexual conflict in the seaweed flies *Coelopa frigida* and *C. pilipes*. — *Anim. Behav.* 74: 343-348.
- Gerling, D. A. & Legner, E.F. (1968). Developmental history and reproduction of *Spalangia cameroni*, parasite of synanthropic flies. *Ann. Entomol. Soc. Amer.* 61: 1436-1443.
- Holmes, H. B. (1974). Patterns of sperm competition in *Nasonia vitripennis*. — *Can. J. Genet. Cytol.* 16: 789-795.
- Jormalainen, V. (1998). Precopulatory mate guarding in crustaceans: Male competitive strategy and intersexual conflict. — *Q. Rev. Biol.* 73: 275-304.
- Khanh, D. T. H., Bressac, C. & Chevrier, C. (2005). Male sperm donation consequences in single and double matings in *Anisopteromalus calandrae*. — *Physiol. Entomol.* 30: 29-35.
- 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. (2000). Sperm depletion and mating behavior in the parasitoid wasp *Spalangia cameroni* (Hymenoptera: Pteromalidae). — *Great Lakes Entomol.* 33: 117-127.
- King, B. H. (2006). Mate location and the onset of sexual responsiveness in the parasitoid wasp *Spalangia endius* (Hymenoptera: Pteromalidae). — *Environ. Entomol.* 35: 1390-1395.
- King, B. H. & Bressac, C. (2010). No fitness consequence of experimentally induced polyandry in a monandrous wasp. — *Behaviour* 147: 85-102.
- King, B. H. & Dickenson, R. M. (2008). A behavioral study of the proximal mechanisms of male recognition of female mating status in the parasitoid wasp *Spalangia endius* (Hymenoptera: Pteromalidae). — *Ann. Entomol. Soc. Am.* 101: 229-234.
- King, B. H. & Fischer, C. R. (2005). Males mate guard in absentia through extended effects of postcopulatory courtship in the parasitoid wasp *Spalangia endius*. — *J. Insect Physiol.* 51: 1340-1345.

- King, B. H. & Fischer, C. R. (2010). Male mating history: effects on female sexual responsiveness and reproductive success in the parasitoid wasp *Spalangia endius*. — Behav. Ecol. Sociobiol. 64: 607-615.
- King, B.H. & King, R.B. (1994). Sex ratio manipulation in response to host size in the parasitoid wasp *Spalangia cameroni*: is it adaptive? — Behav. Ecol. 5: 448-454.
- King, B. H., Burnell, K. B., Ellison, J. H. & Bratzke, R. M. (2005). Unattractiveness of mated females to males in the parasitoid wasp *Spalangia endius*. — Behav. Ecol. Sociobiol. 57, 350-356.
- Knox, T.T. & Scott, M. P. (2006). Size, operational sex ratio, and mate-guarding success of the carrion beetle, *Necrophila americana*. — Behav. Ecol. 17: 88-96.
- Lauer, M. J., Sih, A. & Krupa, J. J. (1996). Male density, female density and inter-sexual conflict in a stream-dwelling insect. — Anim. Behav. 52: 929-939.
- Linley, J. R. & Mook, M. S. (1975). Behavioural interaction between sexually experienced *Culicoides melleus* (Coquillett) (Diptera: Ceratopogonidae). — Behaviour 54: 97-110.
- Parker, G. A. (1970). Sperm competition and its evolutionary consequence in the insects. — Biol. Rev. 45: 525-567.
- Parker, G. A. (1974). Courtship persistence and female-guarding as male time investment strategies. — Behaviour 48: 157-184.
- Rueda, L. M. & 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.
- Saeki, Y., Kruse, K. C. & Switzer, P. V. (2005). The social environment affects mate guarding behavior in Japanese beetles, *Popillia japonica*. J. Ins. Sci. 5: 18-23.
- Sato, Y. & Kohama, T. (2007). Post-copulatory mounting behavior of the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae). — Ethology 113: 183-189.
- Sokal, R. R. & Rohlf, F. J. (1969). Biometry. — W. H. Freeman and Company, San Francisco, California.
- van den Assem, J. & Feuth-De Bruijn, E. (1977). Second matings and their effect on the sex ratio of the offspring in *Nasonia vitripennis* (Hymenoptera: Pteromalidae). — Entomol. Exp. Appl. 21: 23-28.
- Wedell, N., Kvarnemo, C., Lessells, C. K. M. & Tregenza, T. (2006). Sexual conflict and life histories. — Anim. Behav. 71: 999-1011.
- Wilber, D. H. (1989). The influence of sexual selection and predation on the mating and postcopulatory guarding behavior of stone crabs (Xanthidae, *Menippe*). — Behav. Ecol. Sociobiol. 24: 445-451.
- Yamamura, N. (1986). An evolutionarily stable strategy (ESS) model of postcopulatory guarding in insects. — Theor. Pop. Biol. 29: 438-455.
- Zar, J. H. (1999). Biostatistical analysis, 4th edn — Prentice Hall, Upper Saddle River, New Jersey.