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Male mating history: effects on female sexual responsiveness and reproductive success in the parasitoid wasp *Spalangia endius*

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Abstract Males frequently mate multiply, but are there negative fitness consequences for their later mates? Potential costs include less sperm and less nutrition. In most hymenopterans, daughters, but not sons, are produced sexually. This means that effects of being a later mate on sperm received versus on nutrients received should be distinguishable. If later mates receive less sperm, it should manifest as a reduction in daughter production, whereas a reduction in nutrients should affect production of both sexes. Any cost to being a later mate may in turn select for polyandry or for female choice of virgin males. Males of the parasitoid wasp *Spalangia endius* were presented with up to five females in succession. Offspring production was compared among first, third and fifth females; and it did not differ. However, about half of fifth females had begun producing only sons by their tenth day, whereas first and third females rarely had. Despite the reduction in daughter production, even fifth females rarely remated. However, females tended to mate with virgin males rather than mated males when given a choice. This tendency was dependent on male, not female, behavior, but should benefit the female nevertheless. Sex ratios in this species are one male for every one and a half to three females. Thus the number of times that males could mate before daughter production was reduced coincided roughly with the mean number of times that males likely mate in this species. Nevertheless, some females are likely to experience the cost of being a fifth female because of skewed mating success among males.

Keywords Male mating history; Mating system; Parasitoid wasp; Receptivity; Sex ratio; Virgin

Introduction

Multiple mating is well accepted as an important aspect, if not the most important aspect, of male reproductive success (Trivers 1972); and in most animals, males are polygynous. However, multiple mating by males may have important fitness consequences not only for the males themselves, but also for females. For example, mated males may deliver fewer sperm than virgin males or smaller spermatophores or nuptial gifts, as has been especially well-documented among lepidopterans (Torres-Vila and Jennions 2005; Lauwers and van Dyck 2006; Marcotte et al. 2007). As a result, females that mate with mated males may produce fewer offspring, as has been shown, for example, in lemon tetra (Nakatsuru and Kramer 1982). Besides affecting offspring production directly, mating may also affect a female's subsequent mating behavior or longevity (Savalli and Fox 1999; Wolfner 2002). These effects are often mediated by seminal components from male accessory glands and/or the presence of sperm in a female's spermatheca. The extent of these effects may vary with male mating status because a male that has already mated may have less ejaculate available to transfer to subsequent females.

Although a male's mating history can clearly have important fitness consequences for a female, she may be able to avoid or minimize those consequences. She may do this by remating to increase whatever was in short supply from the first mate, e.g., sperm, nutrition, or seminal components, or by preferentially mating with virgin males in the first place (e.g., Nakatsuru and Kramer 1982). Effects of male mating history on females have been especially well-studied in Lepidoptera (reviewed in Torres-Vila and Jennions 2005), with many fewer studies in other insect taxa (but see e.g., Savalli and Fox 1999; Wedell and Ritchie 2004; Rönn et al. 2008).

The present study examines fitness costs of mating with a previously mated male in a hymenopteran, the parasitoid wasp *Spalangia endius*. This study also examines whether females avoid costs of mating with already mated males by avoiding such males in the first place or by remating. In *S. endius* and most other hymenopterans, the only obvious benefit that males provide females is sperm. In contrast to nutrient benefits, sperm number is expected to affect the number of daughters but not the total number of offspring because daughters are produced sexually but sons are produced parthenogenetically. Although females can produce some offspring, namely sons, even without sperm, being able to produce daughters as well should increase a female's fitness by allowing her to adjust the sex of her offspring in response to environmental conditions (Charnov 1982; Steiner and Ruther 2009).

As noted, polyandry is one way that females can limit any costs of being a later mate; and most hymenopterans, like most animals generally, are polyandrous (Ridley 1993; Arnqvist and Nilsson 2000). However, there is a group of hymenopterans that appears to be an exception. These are parasitoid wasps, such as *S. endius*, that produce only a single offspring from each host. At least when mated to virgins, females of these species are largely monandrous, and in the case of *S. endius*, highly so (King et al. 2005; King and Bressac in press). However, are females still monandrous when their first mates are not virgin?

Although laboratory studies of mating in hymenopterans generally use virgin males, in nature, some females will mate with already mated males because sex ratios are typically female biased and males readily mate many times (Ridley 1993). In *S. endius*, sex ratios are female biased; and males emerge before females and linger in the host patch, waiting for females to emerge from nearby hosts (Napoleon and King 1999; King 2006). *Spalangia* males do not produce sperm as adults but rather emerge from their hosts with a full complement of sperm (Gerling and Legner 1968).

The reason that *S. endius* females are monandrous when mating has been with virgin males is because certain aspects of mating make females both unattractive and unreceptive to subsequent males (King et al. 2005). Are multiply-mated males as successful at turning off attractiveness and receptivity of females? In the present study, attractiveness refers to the mounting stage, i.e., mounting-attractiveness, because female mating status does not affect earlier male responses. Males still fan and run towards mated females, contacting them as quickly as they do virgin females (King et al. 2005). Once mounted, a male almost always begins to vibrate his body, but a female *S. endius* must open her genital orifice for copulation to occur. Receptivity is used here to refer to copulation once mounted. Males never dismount immediately after copulation, but rather remain mounted and vibrating for about half a minute more. This period from the end of copulation until the male completely dismounts is defined as postcopulatory courtship. Proximally, one of the ways that a male's mating status might influence a female's attractiveness and receptivity in *S. endius* is by a change in the duration of his postcopulatory courtship. For example, mated males may provide less postcopulatory courtship, which may cause their mates to be more likely to remate. Completely preventing a virgin male from providing his mate with postcopulatory courtship increases the chance that she will remain mounting-attractive and receptive to subsequent males (King and Fischer 2005). However, it is unclear whether more subtle changes in the duration of postcopulatory courtship also affect her attractiveness and receptivity and whether mated males differ from virgins in the amount of postcopulatory courtship that they provide.

The present study tests the following predictions: 1) Later females experience a decreased ability to produce daughters. 2) Later females compensate by remating, and the willingness to remate occurs because later females fail to lose their attractiveness and receptivity during mating. 3) The failure to lose their attractiveness and receptivity results from males performing shorter postcopulatory courtship on later mates. 4) Females minimize the costs of being a later female by choosing virgin males over mated males when given a choice.

Materials and methods

S. endius offspring develop on pupae of various dipteran species that develop in carrion, manure, or rotting vegetation (Rueda and Axtell 1985). The *S. endius* were from a colony established from wasps collected in 1996 from Zephyr Hills, Florida, U.S.A. and were maintained using a natural host, *Musca domestica* pupae (King 1988). In order to obtain newly emerged virgin wasps, parasitized host pupae were individually isolated

in glass test tubes prior to the wasps' emergence. Wasps were provided honey upon emergence. They were 0-d-old at testing and were randomly assigned to a treatment. Testing was done in a small plastic dish (35 mm diameter x 10 mm height) that was about two-thirds full of sand that had been dampened to reduce static electricity. Clean dishes, glass covers, and sand were used for each trial.

Experiment 1

This experiment tested 1) whether the duration of a male's postcopulatory courtship differed depending on whether he was virgin or had mated recently and 2) whether the attractiveness and receptivity of a mated female to a virgin male was affected by whether her previous mate had been a virgin male (first females) or a recently mated male (second females).

We started with an initial 40 pairs of a virgin male with a virgin female. These and all subsequent pairs were videotaped. The initial 40 pairs were used to determine the duration of postcopulatory courtship of virgin males and to generate the first females (Fig. 1 top left). In other words, after this mating, this initial female then became a first female whose attractiveness and receptivity to a new virgin male could then be tested (Fig. 1 top right). The initial male, which had now mated once, was paired with a second virgin female in order to measure the duration of postcopulatory courtship of a mated male (Fig. 1 bottom left). When this male remated, the resulting second female was paired with a new virgin male to determine her attractiveness and receptivity (Fig. 1 bottom right). There were fewer second females than first females because not all mated males remated. "Now mated males" (Fig. 1 bottom left) were presented with a virgin female roughly within a minute after their first mating. This was meant to simulate a high female density situation and to maximize the chance of detecting an effect of male remating (see also Discussion).

Each test of attractiveness and receptivity was for 5 min or until copulation occurred, whichever came first. That is, we determined whether the female was fully mounted and, if she was mounted, whether they copulated. 5 min was considered sufficient because in a previous no choice experiment with 6 min trials, all copulations occurred within 3 min (King and Fischer 2005). Duration until the first retreat from the female was also recorded. The first retreat was defined as the first time that the male walked up to and contacted or nearly contacted the female, but instead of mounting, backed away (King et al. 2005). Retreats are sometimes very abrupt and can occur before or after a completed mating. Retreats are never immediately preceded by being mounted, which distinguishes them from dismounting.

Recorded: postcopulatory courtship

Recorded: duration until retreat, mounting, copulation

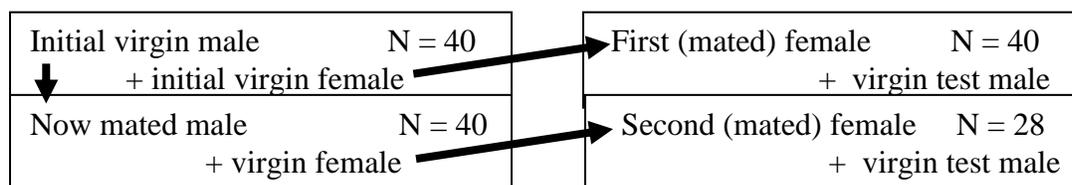


Fig. 1 Flowchart of first experiment

Experiment 2

This experiment tested the effect on a female of mating with a male that had mated more than just once, as the sex ratios of *S. endius* suggest is likely (see Discussion). We tested the effect on a female's subsequent attractiveness and receptivity as in the previous experiment, but also the effect on her daughter production. Thus, this experiment followed the same protocol as the previous one except that 1) each male was mated up to five times, not just twice; 2) offspring production was assessed for a subset of first, third and fifth females; and 3) pairs were not videotaped and duration until first retreat was not analyzed.

A male was placed in a clean container with a virgin female. After mating, he was then transferred to a clean container with another virgin female. This process was continued until he had mated with five females in succession or until he failed to mount a female within 5 min, whichever came first. Attractiveness and

receptivity were tested for first, third and fifth females. We also noted whether the test male performed courtship once mounted and whether the female opened her genital orifice.

For a subset of females, daughter production was also tested ($n = 28$ first females, $n = 10$ third females, $n = 22$ fifth females). A female was given 20 hosts daily for 10 d, along with ad lib honey. The hosts had pupated within the last 2 d when they were presented to females. 10 d of hosts was chosen because even when females mate with virgin males, offspring production, especially daughter production, drops dramatically in the first week (King and Bressac in press; Results). Offspring were allowed to develop and emerge and were subsequently counted.

Experiment 3

This experiment tested whether females were more likely to mate with a virgin than a mated male when given a choice. A virgin male and a mated male were introduced simultaneously into the container with a live virgin female ($N = 36$ pairs of males). The first male to contact, mount, court, and copulate with the female within 10 min was recorded. 10 min was chosen to match previously published male choice experiments on this species (King et al. 2005). Rather than marking males, a separate observer kept track of each male. A single trial in which neither male mated within 10 min was excluded from analyses. Which male copulated first could not be determined for eight additional trials where both males were on the female simultaneously.

Statistical Analyses

Two-tailed P values are presented. In comparing multiple matings of the same male, using male as the sampling unit, e.g., with a paired t -test, takes into account the possibility that a male's behavior during his first and subsequent matings may be correlated. For example, the duration of the postcopulatory courtship that he provides his first mate may be correlated with what he provides his second mate, likewise for duration until first retreat and for his effect on his mates (her attractiveness and receptivity, her offspring production and sex ratio). On the other hand, treating each mating event as an independent event, e.g., with an independent t -test, has the advantage of increasing sample size and thus statistical power, by including males that did not mate multiply. In addition, when events are independent, the latter type of test can provide greater statistical power even without including males that did not remate (Zar 1999, p. 164). Therefore, we included males that did not remate, except when there was a correlation, e.g., between a male's first and second mate, and ignoring the correlation changed the conclusions. In the results, a paired t -test or sign test indicates that we kept the pairing in the analysis, i.e., that a lack of independence mattered.

Prior to analyses, the duration of postcopulatory courtship was square root transformed to improve normality. Duration until the first retreat was analyzed in two different ways. First we used a paired t -test to compare first females to second females using only the 28 males for which we had data on both females, and we assigned nonretreaters a value of 5 min, which was the duration of the test. Second we used survival analysis, specifically Cox's regression, to compare first and second females. Survival analysis allowed us to add 12 males that failed to remate and took into account that nonretreaters might or might not have retreated if the test period had been longer (review of survival analysis in van Alphen et al. 2003). However, the survival analysis ignored the correlation between the duration until first retreat of first female - second female pairs that were generated using the same male (Pearson correlation: $r_{27} = 0.47$, $P = 0.011$).

In analyzing attractiveness, trends in the relationship between attractiveness (mounting) and female order were the same for both experiments, and there was no significant difference between experiments in the proportion of first females ($n=40$, $n=51$) that were subsequently mounted by the virgin test males (likelihood ratio $\chi^2_1 = 0.01$, $P = 0.91$). Therefore, we combined the results prior to additional analyses. For 57 males we had mounting data on all females tested, i.e., first and second females in the first experiment and first, third, and fifth females in the second experiment. We categorized each male as exhibiting an increased tendency for his mate to be mounted going from the first to subsequent mates, a decreased tendency, or no consistent increase or decrease. For example, in the second experiment, for each of a male's females that was tested, 0 represents "not mounted" and 1 represents "mounted". A male was said to show an increase if he was 001 or 011, to show a decrease if he was 100 or 110 and to show no consistent increase or decrease if he was 000, 111, 101, or 010. Whether there were more increases than decreases was tested with a sign test.

For total number of offspring and proportion of sons, mean values of third and fifth females were compared to first females using Dunnett's test, i.e., using first females as a control (Zar 1999, p. 217). The Dunnett's test was on arcsine transformed proportion of sons, in order to improve homogeneity of variance. Variances were also compared, using Levene's tests for equality of variance.

Results

Postcopulatory Courtship - Experiments 1 and 2

A male's duration of postcopulatory courtship did not change with successive mates. In experiment 1, virgin males did not differ from mated males (independent t -test: $t_{66}=0.49$, $P=0.63$; back transformed means, ranges: 25.61, 8 – 80 s, $n = 40$ versus 24.16, 2 -51 s, $n = 28$); and in experiment 2, the duration did not change from the first to the fifth female (analysis of variance with female order as a covariate ($F_{1,143}=0.006$, $P=0.94$) and male ID as a random effect ($F_{49,143}=2.12$, $P<0.001$)).

When mated females were subsequently exposed to virgin test males to test their mounting-attractiveness, whether the female was mounted was not related to the amount of postcopulatory courtship that she had received during her first mating. In other words, females that were mounted had not received significantly less postcopulatory courtship than females that were not mounted (independent t -tests: experiment 1: $t_{66}=1.01$, $P=0.32$; experiment 2: $t_{72}=0.42$, $P=0.68$).

Duration until Retreat - Experiment 1

Duration until first retreat did not differ significantly between first and second females, regardless of analysis. Retreats were not significantly quicker from first females compared to second females among the 28 males for which we had data on both females (paired t -test: 143.93 ± 21.06 s for first females versus 185.79 ± 21.97 for second females; $t_{27}=-1.90$, $P=0.069$). Duration also did not differ significantly between first and second females with survival analysis (Cox's regression: $\chi^2_1=1.72$, $P=0.19$).

Attractiveness – Experiments 1 and 2

Going from the first to subsequent mates, for 32% of males ($n=57$) there was an increased tendency for their mate to be mounted; for 9% there was a decreased tendency; and for 60% of males, there was not a consistent increase or decrease (sign test $P=0.011$). More than 40% of all females were not mounted (Fig. 2).

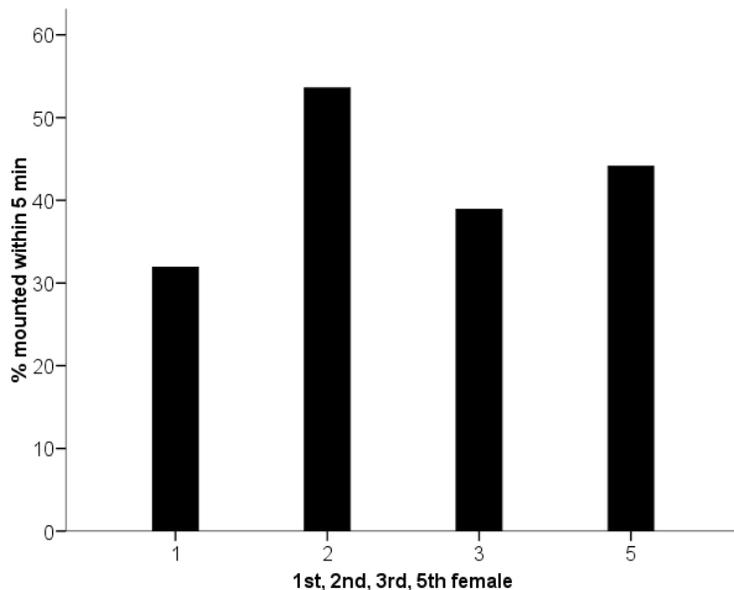


Fig. 2 Percent of virgin males that mounted a female that had previously mated with a different virgin male and so had been a first female ($n = 91$) or had previously mated a mated male and was his second ($n = 28$), third ($n = 36$) or fifth female ($n = 34$)

Receptivity – Experiments 1 and 2

In experiment 1, receptivity to virgin test males was about 14% and did not differ between first ($n=13$) and second ($n=15$) females (likelihood ratio $\chi^2_1=0.024$, $P=0.88$). In experiment 2, no females that were mounted subsequently copulated, regardless of whether they were the first female ($n=16$), third female ($n=14$) or fifth female ($n=15$). Females failed to open their genital orifices even though, regardless of female order, 94% of males that mounted also courted.

Offspring Production - Experiment 2

Number of total offspring from third and from fifth females did not differ from first females (Table 1; third vs. first females $P=0.36$; fifth vs. first females $P=0.61$). Fifth females, but not third females, produced a greater proportion of sons than first females (Table 1; fifth vs. first females $P=0.005$; third vs. first females $P=0.72$). Fifth females produced a greater proportion of sons as a result of producing both more sons and fewer daughters (Fig 3). For all females, the mean number of sons was fairly steady, whereas the number of daughters decreased rapidly (Fig. 3). The proportion of mothers that produced only sons by their tenth day did not differ between third and first females (Fig. 4; 1 of 10 versus 2 of 28; likelihood ratio $\chi^2_1=0.08$, $P=0.78$), whereas more fifth females than first females had begun producing only sons (10 of 22 versus 2 of 28; likelihood ratio $\chi^2_1=10.38$, $P=0.001$). However, some fifth females still produced very female-biased sex ratios. Fifth females, but not third females, exhibited greater variance in overall sex ratio than first females (Table 1; $W_{1,48}=24.49$, $P<0.001$; $W_{1,36}=0.023$, $P=0.88$). Neither fifth nor third females differed from first females in variance in number of offspring (Table 1; $W_{1,48}=0.16$, $P=0.69$; $W_{1,36}=0.57$, $P=0.45$).

Table 1 Mean \pm s.e. (minimum – maximum) number of offspring and proportion of sons produced over 10 d by first females ($n=28$), third females ($n=10$) and fifth females ($n=22$).

Total offspring	Proportion of sons*			
First females	94.75 \pm 4.78	(33 - 135)	0.24 \pm 0.02	(0.09 - 0.40)
Third females	84.10 \pm 5.83	(45 - 108)	0.29 \pm 0.03	(0.16 - 0.43)
Fifth females	89.18 \pm 4.34	(50 - 119)	0.41 \pm 0.06	(0.09 - 0.98)

*Proportions of sons are presented as untransformed values.

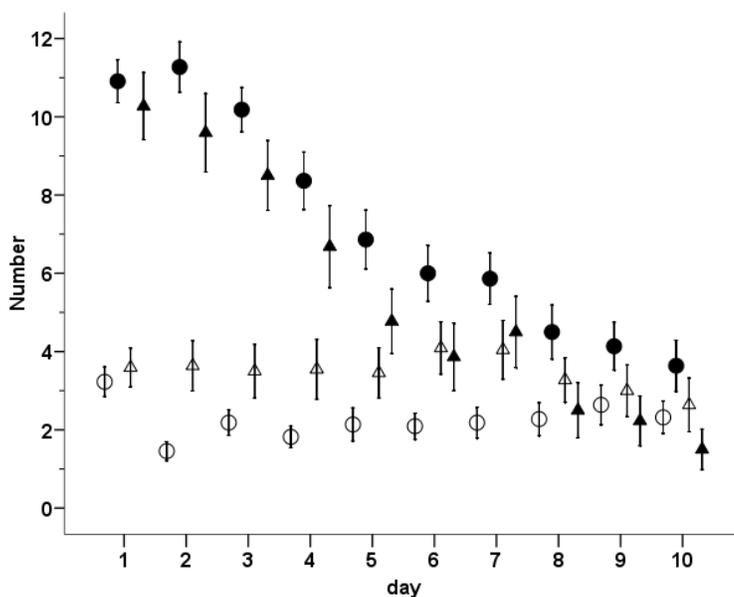


Fig. 3 Mean \pm s.e. number of sons and number of daughters during each of 10 d of oviposition; for clarity, shown here for just first females (\circ sons, \bullet daughters) and fifth females (Δ sons, \blacktriangle daughters)

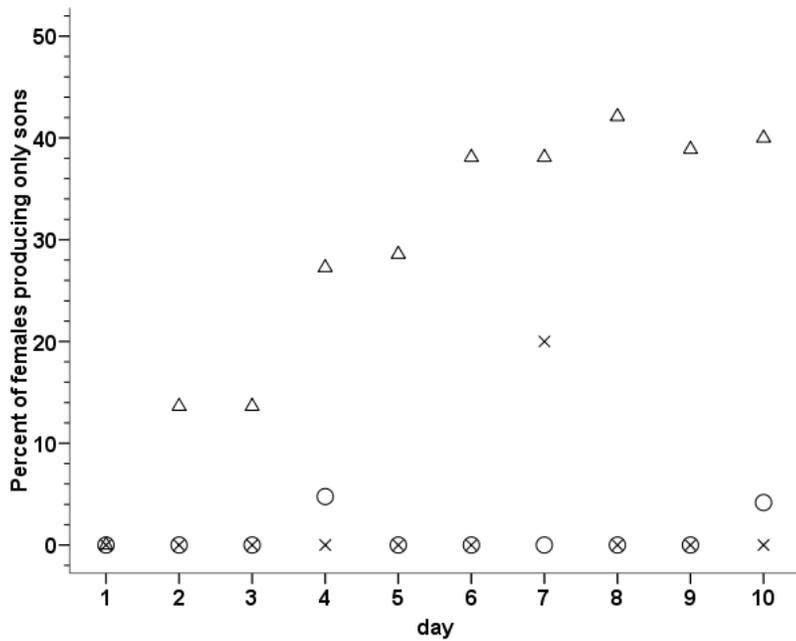


Fig. 4 Percent of first (○), third (x) and fifth (Δ) females that produced only sons during each of 10 d of oviposition

Experiment 3

Generally, the first male to mount a female then proceeded to vibrate on her and copulate with her. Significantly more virgin males copulated first than mated males (Fig. 5; likelihood ratio $\chi^2_1=9.23$, $P=0.002$). The first contact was equally likely to have been with the virgin as the mated male (likelihood ratio $\chi^2_1=0.57$, $P=0.81$).

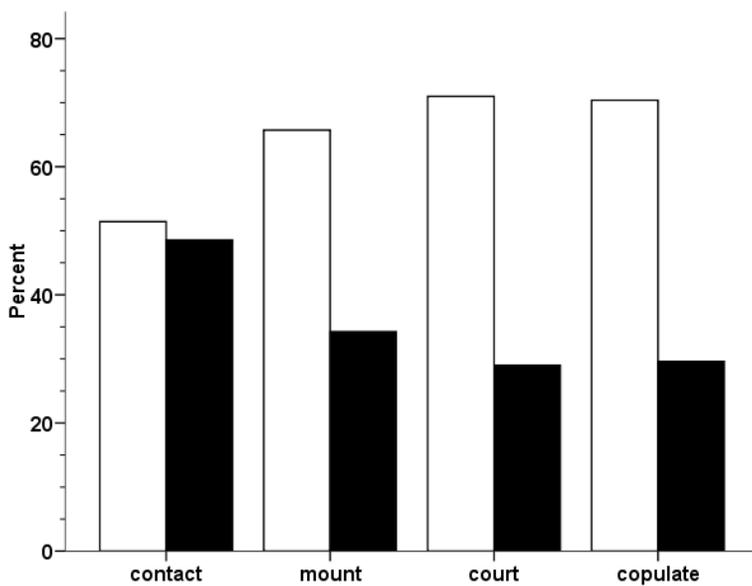


Fig. 5 Percent of virgin males (□) and mated males (■) to first contact, mount, court, and copulate with a live virgin female

Discussion

S. endius females did not appear to suffer a cost from mating with an already mated male unless he had already mated four times, and even then, females almost never remated.

Currently there is no evidence in parasitoid wasps of females facultatively remating in response to the reproductive capacity of their mate. However, the number of studies available is few (Steiner et al. 2008; this study). In contrast, in many other insects, females that mate with already mated males compensate by being more likely to remate or by remating sooner than females mated to virgins; examples include lepidopterans (e.g., Foster and Ayers 1996; Marcotte et al. 2007), beetles (e.g., Savalli and Fox 1999), flies (e.g., Luck and Joly 2005; Perez-Staples et al. 2008), and hemipterans (e.g., Bailey and Nuhardiyati 2005). However, in the cockroach *Nauphoeta cinerea*, females are not more likely to remate if their first mate was sperm-depleted from having mated multiply (Montrose et al. 2004).

The reason that later *S. endius* females seldom remated was a combination of unattractiveness and lack of receptivity. Regardless of whether a female had mated with a virgin male or a male that had mated up to four times previously, she was often unattractive for subsequent mounting by other males (Fig. 2). Once mounted, mated females were almost always unreceptive; and whether they were receptive was not affected by whether they had mated with a virgin or an already mated male. Likewise, in the cockroach *N. cinerea* even sperm depleted males cause females to become unreceptive (Montrose et al. 2004).

Preventing an *S. endius* female from receiving postcopulatory courtship by removing her mate immediately after copulation clearly increases her subsequent attractiveness and receptivity (King and Fischer 2005). However, in the present study, there was no evidence that the duration of postcopulatory courtship that a female received was related to her subsequent attractiveness. Perhaps even the shortest duration was sufficient or perhaps duration is not the aspect of postcopulatory courtship that matters. There was some tendency for later females to be more attractive despite having received as much postcopulatory courtship as early females. One explanation is that a female's decrease in attractiveness after mating may result from a pheromone that her mate releases during postcopulatory courtship, and mated males may have less pheromone left to release than virgins. In support of the latter part of this hypothesis, in the confamilial *Nasonia vitripennis*, males that have mated seven or more times produce less of a pheromone that attracts females (Ruther et al. 2009). However, in Caribbean fruit flies, mated males release twice as much sex pheromone as virgin males (Teal et al. 2000). Alternatively, how vigorous postcopulatory courtship is, not just its presence or duration, may affect attractiveness; and mated males may be less vigorous than virgins, e.g., through fatigue. There is no evidence for this hypothesis yet; and in side blotched lizards, fatigue has little effect on the vigorousness of courtship (Brandt and Allen 2004).

Being a fifth female, but not a third female, increased a female's proportion of sons. These results are important in providing an idea of the reproductive capacity of males. However, do males mate as many as five times in natural situations? Sex ratios of *S. endius* in the field range from 61-75% females (Donaldson and Walter 1984; King 1991). This translates to an average of roughly one male for every one and a half to three females. If mating success is not evenly distributed among males, some males may still mate five times. Field data on male reproductive success is available for only one parasitoid wasp species, *Trichogramma pretiosum* (Kazmer and Luck 1995). Some skew is present, as it is in many animals (e.g., Stein and Uy 2006; Nsubuga et al. 2008; Kelly 2008). In *T. pretiosum* the relative fitness of the most successful (largest) males was about two, i.e., twice that of an average male (Kazmer and Luck 1995). If this pattern is also true in *S. endius*, given that an average male mates with one and a half to three females, the most successful males may mate six times, although this may be true of relatively few males.

If the number of *S. endius* males that mate five times in nature is small, this could explain why fifth females never remated. This, together with the lack of a fitness decrease from being a third female, would mean that selection is weak for mechanisms that facilitate female remating and for mechanisms that make females avoid mating with already mated males in the first place. Furthermore, regardless of how many times a male has already mated, it is to his advantage to prevent his mate from remating in order to reduce sperm and offspring competition (Damiens and Boivin 2005). Whether later females are more likely than first females to remate in a given species may also be related to the stimulus that causes loss of female receptivity. Some stimuli may be easier than others for males to maintain at a near constant level through multiple matings. For example, a behavior, such as postcopulatory courtship, may be easier to maintain than the amount of seminal fluids or nutrients transferred.

The close correspondence in *S. endius* between sex ratio and the number of times that males can mate

before daughter production is reduced may not be coincidental. Experimental evidence that sex ratio may affect selection on male ejaculate transfer comes from a study of *Drosophila melanogaster* that had been maintained at different sex ratios (Linklater et al. 2007). The female-biased lines had been maintained at an adult sex ratio of three females for every one male, and the male-biased lines were maintained at the inverse. The number of offspring produced by males from the female-biased lines and the male-biased lines did not differ when their first through third mates were examined. However, with fourth and fifth mates, the males from the female-biased lines produced more offspring than the males from the male-biased lines.

In contrast to the close correspondence between sex ratio and insemination ability in *S. endius*, Martel and Boivin (2007) report that “males of several parasitoid species have an insemination capacity higher than that necessary to inseminate females from their brood”. Martel and Boivin (2007) include partial insemination in their definition of male insemination capacity, whereas we did not. However, as Martel and Boivin (2007) note, in *T. turkestanica* there is excess capacity even when considering only fully inseminated females (Damians and Boivin 2005). Whether this results from greater variation in male mating success in *T. turkestanica* than in *S. endius* remains to be tested.

That daughter production but not overall offspring production was affected by mating order in *S. endius* is consistent with differences in sperm transfer being responsible rather than differences in nutrient transfer (e.g., nutrients in the ejaculate; there is no traditional nuptial gift in *S. endius*). However, sperm transfer is not always less for later mates in parasitic hymenopterans. In *Anisopteromalus calandrae*, females contain the same amount of sperm regardless of whether they mate with a virgin male or a male that already mated five times, despite the latter having less sperm in their seminal vesicles (Bressac et al. 2009).

In contrast to *S. endius* and other parasitic Hymenoptera (e.g., Ruther et al. 2009), among insects generally, a female’s overall offspring production frequently decreases with the number of previous matings by her mate (e.g., a leafroller moth (Jimenez-Perez and Wang 2004); seed beetles (Rönn et al. 2008); a cockroach (Edvardsson et al. 2008)), although not always (e.g., lepidopteran references in Torres-Vila and Jennions 2005 and McNamara et al. 2007). In the blowfly *Lucilia cuprina*, mating with a male that has already mated multiply may affect not only the number of offspring that a female produces, but also whether she oviposits at all (Barton Browne et al. 1990).

Theoretically, in nature, later *S. endius* females might produce fewer offspring overall due to their being mounted more and that interfering with their ability to search for hosts. However, the frequency of harassment will be reduced by the female-biased sex ratio and because after mating, females tend to burrow in search of hosts and away from males (King 2002), although whether being a later female also reduces burrowing remains to be tested. Also, even if a virgin male mounts and courts an unreceptive female, he is usually on her for only about half a minute, which is probably a very small proportion of a female’s total time budget (King unpublished data).

One factor that will tend to reduce multiple mating by male *S. endius* is the fact that females were more likely to mate with a virgin male than with a once-mated male when presented with both. This outcome is independent of female behavior because virgin males are also quicker than mated males to attempt copulation when the female is dead. This conclusion is based on a comparison of results of experiment 3 to a similar experiment with dead females (King et al. 2005) (likelihood ratio $\chi^2_1 = 0.20$, $P = 0.66$). This sexual inhibition of mated males is thought to have resulted from selection on males to avoid immediately trying to remate the same female (Fischer and King 2008). However, females may also benefit because it should reduce their chances of being later females. There have been relatively few studies of female preference for virgin males among insects. In sagebrush crickets, a species in which the female feeds on the male’s wings during mating, females prefer males whose wings have not been consumed, as would be true of virgin males (Johnson et al. 1999). In *D. melanogaster*, females avoid mating with recently mated males (Markow et al. 1978); whereas in *D. bifurca*, females do not, despite a cost in lower sperm transfer (Luck and Joly 2005). However, *D. bifurca* females are more likely to remate after mating with already mated males. Green veined white butterfly females also do not discriminate against mated males (Kaitala and Wiklund 1995). In European corn borers, females preferentially mate with males that have already mated three times over virgin males. Females appear to be responding to the fact that males that are able to obtain multiple matings are a nonrandom, high quality subset of males (Schlaepfer and McNeil 2000). In *S. endius*, male quality, as measured by daughter production, decreased by

the fifth mating even within males.

Whether *S. endius* females are also attracted to virgin males over mated males independent of male behavior remains to be determined. Females of the confamilial *Nasonia vitripennis* are less attracted to multiply mated males as a result of such males producing less sex pheromone (Ruther et al. 2009). Although the sexual inhibition of mated males in *S. endius* and the reduced attractiveness of mated males in *N. vitripennis* are both likely to reduce females mating with mated males, neither is likely to completely eliminate it. Females are still expected to mate with mated males because sex ratios are female-biased. Furthermore, in *S. endius* the decreased sexual responsiveness of mated males is short-lived (Fischer and King 2008).

In the present study, males encountered females in fairly quick succession, such as might occur at high densities. In some species, a greater delay in male remating reduces any disadvantage of mating with an already mated male by allowing time for sperm replenishment (e.g., Nadel and Luck 1985; Marcotte et al. 2007). However, sperm transfer appears to be independent of how quickly males remate in *S. endius* (Fischer and King 2008).

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