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Unattractiveness of mated females to males in the parasitoid wasp *Spalangia endius*

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Abstract Despite common stereotypes, males are not always indiscriminate and eager when it comes to mating. In the parasitoid wasp *Spalangia endius*, the initial response of males to females was almost always one of apparent excitement; however, this was followed by a clear preference for virgin females over mated females in both no-choice and choice situations. The no-choice data were collected from videotapes of male-female pairs of all possible combinations of mated and virgin individuals. Neither female nor male mating status had a significant effect on likelihood of, or time until, contact or male courtship fanning. However, a male's first retreat was sooner when the female was mated than when she was virgin; mated males exhibited their first retreat sooner than did virgin males; and mated females were less likely to be mounted than were virgin females. In addition to the videotapes, male-choice experiments were performed. When given a choice of a virgin and a mated female, both virgin and mated males were more likely to mount and copulate with the virgin. The difference in response to virgin versus mated females seemed to be less in virgin males than in mated males, perhaps due to virgin males' greater eagerness to mate: when a virgin male and a mated male were presented with a dead virgin female, the virgin male was usually the first to respond to the female. That males preferentially retreated from and avoided mounting mated females appears to be adaptive given that mated females rarely copulated.

Keywords Mating · Mate choice · Mating status · Parasitoid wasp · *Spalangia endius*

Introduction

Mate choice has been defined as “any pattern of behaviour, shown by members of one sex, that leads to their being more likely to mate with certain members of the opposite sex than with others” (Halliday 1983) or as a differential sexual response to different types of reproductively mature conspecifics of the opposite sex (Bonduriansky 2001). It is defined in terms of observable behavior, not in terms of an internal state or conscious thought, in contrast to what the word choice implies in everyday usage (Halliday 1983). As can be seen from the definitions, it is not defined by which sex is “in charge” or has ultimate control. For example, in sensory exploitation, a female's preference is explained as the male exploiting some preexisting feature of the female's sensory system (Ryan 1998). Thus, in a sense, the female choice results from the male “making” the female choose him. Choice can occur at any stage in the mating process, from mate attraction until even after copulation (e.g., Brown 1999).

After some initial reluctance to accept mate choice (Dewar and Finn 1909), mate choice has now been well documented in a wide range of taxa. However, most studies of mate choice have focused on female choice of males (Jennions and Petrie 2000; Bonduriansky 2001). The biological rationale has been that females usually invest more in offspring than males and so should be choosier (Trivers 1972). However, this does not preclude male choice (Parker 1983), and male choice has been reported for species from a wide array of taxa, although few hymenopterans (references below; reviewed in Bonduriansky 2001).

This study examines mate choice in relation to mating status in both females and males of the parasitoid wasp *Spalangia endius* (Hymenoptera: Pteromalidae). Tests for mate choice in both sexes of a single species are uncommon (Bonduriansky 2001). What is most striking about mating in *S. endius* is that a male will sometimes excitedly approach a female only to quickly back away from her upon contact or near contact. Here we investi-

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gate the effect of mating status (virgin vs already mated) on this and other aspects of mate choice.

S. endius parasitizes the pupal stage of certain fly species, e.g., muscids and calliphorids (Rueda and Axtell 1985). These fly hosts are found both on and down in manure and decaying organic matter. *S. endius* is described as quasigregarious because it produces one offspring per host, but its hosts occur in groups such that multiple wasps may emerge in temporal and spatial synchrony. A group of hosts may consist of thousands of hosts, but more often less (King 1990). Emergence sex ratios are female-biased, and males emerge before females and wait at the host site for females to emerge (B.H. King, personal observation). Both males and females are sexually mature at emergence (Gerling and Legner 1968). Regardless of a male's presence, the only interactions between females are explorations of each other with their antennae if they happen to pass near each other. Males behave similarly except they will mount another male that is mounted on a female and then will attempt copulation. There is no evidence that *S. endius* males provide females with any nutrition during mating (King 2002).

In a typical mating between virgin *S. endius*, the male fans his wings very briefly and runs toward the female (R.M. Bratzke and B.H. King, unpublished work). She runs away, but eventually he mounts her. She becomes still as he begins to vibrate his whole body rapidly up and down on her. When she then opens her genital orifice, he backs up slightly and they copulate. He then returns to his previous position on the female, still vibrating. The female closes her genital orifice and then begins stroking her hind legs between her body and his until he dismounts.

Mate choice can be adaptive because potential mates vary in their resources, e.g., in their genes, their number of gametes, their disease status and the nutrition or protection they can provide (Halliday 1983; Paul 2002). There may be selection on both females and males to prefer mating with virgins (e.g., Wedell et al. 2002; Elgar et al. 2003; see also Discussion). From the female's perspective, virgin males, or at least males that have not mated very recently, may offer a larger supply of sperm, as appears to be the case in *Spalangia* (e.g., Nadel and Luck 1985; King and King 1994). From the male's perspective, virgin females may offer a larger number of not yet fertilized eggs. For both sexes, virgins are less likely than mated individuals to be a source of sexually transmitted diseases. Selection on one sex to prefer virgins may be reinforced by selection on the other to avoid remating. For example, already-mated females may gain more by investing in the gametes they have already received than by mating again, and already-mated males may gain more by waiting for their semen components to replenish before mating again. If already-mated individuals are unwilling to remate, attempting to mate with them may be a waste of time and energy, and thus there may be selection to avoid them.

In the present study, first, the effects of mating status on mating behavior were examined by collecting detailed behavioral data from videotapes of male-female pairs, in which one, both or neither sex had previously mated (but not with each other). Male mate choice against mated females might manifest as the male being quicker to retreat from mated females, or slower or less likely to mount and court, or courting for a shorter period of time. Female choice might manifest as females being quicker in retreating from mated than from virgin males. To test whether males might be avoiding mated females because they represent low fecundity through an unwillingness to remate, we first tested whether mated females were quicker than virgin females in retreating from males, and we examined whether mated females were receptive (willing to copulate). Then mating behavior was examined in choice experiments in which a single male, either virgin or mated, was given a simultaneous choice of a virgin versus a mated female. Finally, we examined whether a difference between mated and virgin males in the strength of their discrimination against mated females might be related to a difference in eagerness to mate.

Methods

The *S. endius* were from a colony that was started with F₁s whose parents were collected from a poultry farm in Zephyr Hills, Florida in March 1996. The colony was maintained using a natural host, *Musca domestica* pupae (following King 1988). Parasitized host pupae were individually isolated in glass test tubes prior to the wasps' emergence in order to obtain virgin wasps.

Females and males were assigned to the mated versus virgin treatments at random. Mated wasps were produced by placing a virgin male and a virgin female into a test tube and watching for copulation to occur. Each pair mated only once. Males were tested within a few minutes or more of mating, and they were not tested with the same females with which they had mated.

Experiments were performed in sand dishes. The dish was a small blue plastic cap (1.5 cm diameter, 1 cm height) except in the Mated Male Choice experiment. It was filled about three-quarters of the way with off-white sand that had been wetted with water to keep the wasps from burrowing and to keep humidity high, which reduces static. The dish was covered with a glass cover slip. Sample-size discrepancies among behaviors resulted from some behaviors not being observable in every replicate.

Statistical tests were two-tailed. Alpha was set at 0.05 for each response variable within experiments. Alpha was controlled separately for each variable because preliminary observations had suggested that mating status would have different effects on different response variables, e.g., fanning versus mounting. Whether alpha should be adjusted for multiple tests within an experiment is unclear (e.g., Rice 1989; Fiese 2002). Nevertheless, our conclusions regarding statistical significance would not have been affected by using sequential Bonferroni to control the experiment-wise alpha at 0.05 (Rice 1989), except in the first male eagerness experiment. The patterns of the first male eagerness experiment were replicated and statistically significant in a second experiment (see below).

Videotaped interactions

Ninety-nine pairs of 0-day-old wasps were videotaped for 6 min each. (Initial observations indicated that 6 min provided pairs with ample opportunity to interact.) The treatments were male-female pairs in all possible combinations of virgin and mated (Table 1).

During videotaping, illumination was from fiber-optic lights, which gave off no noticeable heat.

The following data were collected from the videotapes:

1. contacts: when either wasp touched the other wasp, or nearly touched and the other retreated;
2. who initiated each contact (male, female, or mutual);
3. who retreated from each contact: a retreat was when one wasp jumped back away from the other, quickly increased its running speed as it moved away, or curled into a submissive posture with its antennae tucked in;
4. fan: the male (never observed in females) briefly moved his wings up and down, usually rapidly, often running toward the female at the same time; this did not include simply lifting the wings during grooming;
5. mount: when more than half of one wasp's body was on the other's (frontward or backward); this did not include just walking over the other wasp as if it was not there;
6. copulate: the female opened her genital aperture, and the male inserted his aedeagus;
7. brush off: a wasp stroked its hind legs up and over its dorsal abdomen while another wasp was mounted on it;

The effects of male and female mating status on whether or not there was fanning, mounting, or copulation were analyzed by log linear analyses. A two-way analysis of variance (ANOVA) on log transformed data was used to examine the effects of male and female mating status on time from the start of taping until a behavior (contact, fan, retreat, mount, and copulate) first occurred, as well as for time from copulation to brush off. Confidence intervals are presented in place of standard errors for backtransformed variables (Zar 1984).

Virgin-male choice experiment

A virgin female and a mated female were placed in a sand dish ($n=61$) and then a virgin male was immediately added. Whether the virgin or mated female was placed in the dish first did not affect which female the male contacted first (chi-square test: $\chi^2_1=0.40$, $P=0.53$). The wasps were 0–3 days old.

The wasps were observed for 10 min or until the male had copulated with one of the females, whichever came first. Data recorded were which female was first to be contacted by the male, completely mounted by the male, courted by the male, and copulated with. Whether males exhibited any preference between virgin versus mated females was tested using chi-square tests for each variable.

Mated-male choice experiment

This experiment was similar to the previous one except mated males ($n=63$) were tested rather than virgin males, the wasps were

0–1 days old, and the sand dish was a small petri dish (3.4 cm diameter by 1.1 cm high).

Male eagerness experiments

One virgin freeze-killed female was placed in a sand dish, near the edge ($n=58$). Then a live mated male and live virgin male were placed into the dish, opposite the female. We were able to keep track of which male was which without marking them. The female was freeze-killed in order to examine male eagerness to mate in the absence of effects of female behavior. In the first male eagerness experiment, which male first contacted, first mounted, first courted, and first attempted to copulate with the freeze-killed female was recorded. The results were analyzed with four separate chi-square tests. However, if adjusted for simultaneous inference with a sequential Bonferroni test (Rice 1989), the tests change from significant to nonsignificant. Thus, a second experiment testing the same pattern is presented.

The setup of the second experiment was the same as the first except that the sand dish was a small petri dish (3.4 cm diameter by 1.1 cm high), and the trials were videotaped and the data collected from the videotapes. In addition, the virgin and mated males were entered one right after the other (alternating which was first). Only data on mounting were collected because the first male eagerness experiment showed that the male that was first to contact the female was also the first to mount, court, and attempt copulation in most (54 of 57) cases, meaning that the 4 measures gave almost identical results.

Results

Videotaped interactions

Contact and fanning were not significantly affected by mating status. Contact occurred in all male-female pairs. Most males fanned in the presence of the female, and the likelihood of fanning was unaffected by both the male's and the female's mating status (Table 1). Time until first contact was not significantly related to the male's status ($F_{1,95}=0.89$, $P=0.35$). If it was related to the female's status, the effect was small (about 1 s sooner for mated females v virgin females; $F_{1,95}=2.89$, $P=0.09$). The interaction between the effect of the male's and the female's mating status was not significant ($F_{1,95}=0.22$, $P=0.64$). Mean time until first contact was 2.66 s, range: 0–28, $n=99$. Likewise, time until first fan was not significantly related to mating status of the male ($F_{1,90}=0.34$, $P=0.56$) or that of the female ($F_{1,90}=1.29$, $P=0.26$), and

Table 1 When one male was with one female for 6 min, percent of males that fanned, mounted, and copulated (V virgin, M mated)

Treatment	Fanned ^a		Mounted ^b		Copulated ^c	
	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>
VV=V male, V female	96.2	26	96.2	26	92.3	26
VM=V male, M female	96.0	25	72.0	25	0.0	25
MV=M male, V female	95.7	23	95.7	23	86.4	22
MM=M male, M female	92.0	25	32.0	25	4.2	24

^a Fanned: male mating status $G_1=0.27$, $P>0.50$; female mating status $G_1=0.19$, $P>0.50$; interaction $G_1=0.10$, $P=0.75$

^b Mounted: male mating status $G_1=7.30$, $P<0.01$; female mating status $G_1=29.18$, $P<0.001$; interaction $G_1=0.95$, $P=0.33$

^c Copulated: male mating status $G_1=0.029$, $P>0.75$; female mating status $G_1=91.70$, $P<0.001$; interaction $G_1=1.87$, $P=0.17$

Table 2 When one male was with one female for 6 min, time until first mount (s) among pairs where mounting occurred. Treatments defined in Table 1. Means and confidence intervals backtransformed from natural logs. Female mating status: $F_{1,69}=5.02$, $P=0.028$; male mating status: $F_{1,69}=0.77$, $P=0.39$; interaction: $F_{1,69}=1.27$, $P=0.26$

Treatment	n	Mean	Range	95% Confidence Interval
VM	18	10.57	0–74	5.43–19.84
MV	22	16.98	1–153	9.56–29.61
MM	8	5.07	1–37	0.74–20.14

Table 3 When one male was with one female for 6 min, time until first male retreat (s). Treatments defined in Table 1. Means and confidence intervals backtransformed from natural logs. Male mating status: $F_{1,93}=4.71$, $P=0.032$; female mating status: $F_{1,93}=7.65$, $P=0.007$; interaction: $F_{1,93}=0.39$, $P=0.53$

Treatment	n	Mean	Range	95% Confidence Interval
VM	25	23.79	2–126	15.31–36.68
MV	23	27.15	3–197	17.20–42.56
MM	24	12.62	0–86	7.88–19.88

the interaction between the effect of the male's and the female's mating status was not significant ($F_{1,90}=0.31$, $P=0.58$). Mean time until first fan was 9.28 s, range: 0–219, $n=94$.

Mounting, both the proportion that mounted and time until mounting, was affected by mating status. More virgin than mated females were mounted, and virgin males were more likely than mated males to mount the female (Table 1). The ANOVA revealed no significant interaction between the effect of the female's and the male's mating status (Table 1); however, virgin males were more than twice as likely as mated males to mount a mated female, and this effect was statistically significant ($\chi^2_1=8.01$, $P=0.005$) when just the treatments with mated females were analyzed.

Although mated females were less likely than virgin females to be mounted (Table 1), when they were mounted, it was sooner (Table 2). Among pairs in which mounting occurred, time until mounting was not significantly related to the male's own mating status, and the interaction between the effect of the male's and the female's mating status was not significant (Table 2).

Copulation was significantly affected by female, but not male, mating status. Considerably more virgin than mated females copulated (Table 1). Mated females rarely copulated. Virgin and mated males did not differ significantly in their likelihood of copulating. The interaction between male and female mating status on likelihood of copulation was not significant. Time until copulation with a virgin female was not significantly different between virgin and mated males ($t_{41}=0.04$, $P=0.97$). Mean time until copulation was 26.64 s, range: 7–352, $n=44$.

The time until the male's first retreat from the female was significantly affected by both his and the female's mating status. The male retreated sooner when he was

Table 4 When one male, either a virgin male or a mated male, was with one virgin and one mated female, the number of males that first contacted, mounted, courted and copulated with the virgin (V) versus the mated female (M)

	Female		
	V	M	
Virgin males			
Contacted first	27	33	$\chi^2_1=0.60$, $P=0.44$
Mounted first	44	17	$\chi^2_1=11.95$, $P=0.001$
Courted first	49	12	$\chi^2_1=22.44$, $P<0.001$
Copulated first	59	1	$\chi^2_1=56.07$, $P<0.001$
Mated males			
Contacted first	31	32	$\chi^2_1=0.016$, $P=0.9$
Mounted first	55	8	$\chi^2_1=35.06$, $P<0.001$
Copulated first	61	2	$\chi^2_1=55.25$, $P<0.001$

mated (Table 3) and when the female was mated. The interaction between the effect of the male's and the female's mating status was not significant.

The time until the female's first retreat from the male was not significantly related to her mating status ($F_{1,92}=1.12$, $P=0.29$) or that of the male ($F_{1,92}=2.08$, $P=0.29$), and the interaction between the effect of the male's and the female's mating status was not significant ($F_{1,92}=0.031$, $P=0.90$). Mean time until the first female retreat was 13.09 s, range: 0–334 s, $n=96$.

After mounting a virgin female, virgin males did not copulate sooner than mated males, i.e., precopulatory courtship was not shorter (Mann-Whitney $U=198.00$, $n_1=24$, $n_2=19$, $P=0.45$), and after copulation, females did not try to dislodge mated males sooner than virgin males ($t_{41}=0.46$, $P=0.65$). Mean first-mount-to-first-copulation time was 11.16 s, range: 1–109, $n=43$. Mean copulation-to-brush-off time was 14.58 s, range: 5–35, $n=43$.

Virgin-male choice experiment

A virgin male's first contact was equally likely to be a virgin female as a mated female (Table 4). In contrast, significantly more males mounted, courted, and copulated with the virgin female than with the mated female. The number of males responding to the mated female decreased from contact to mount to court to copulation. All 44 males that mounted the virgin female also copulated with her. Of the 17 males that mounted the mated female, only 1 (5.9%) proceeded to copulate with her.

Mated-male choice experiment

A mated male's first contact was equally likely to be a virgin female as a mated female (Table 4). (This was true even when first contact of a female's abdomen was examined ($\chi^2_1=0.40$, $P=0.53$.) For 21 of 63 males, the first contact was with the female's abdomen. Most males mounted and copulated with the virgin female rather than with the mated female (Table 4).

Table 5 The frequency with which the first male to contact, mount, court, and attempt copulation with a virgin freeze-killed female was a virgin male (V) or a mated male (M)

	Male		
	V	M	
First male eagerness experiment			
Contacted first	37	20	$\chi^2_1=5.07, P=0.02$
Mounted first	38	21	$\chi^2_1=4.90, P=0.03$
Courted first	38	20	$\chi^2_1=5.59, P=0.02$
Copulated first	38	20	$\chi^2_1=5.59, P=0.02$
Second male eagerness experiment			
Mounted first	52	15	$\chi^2_1=20.43, P=<0.001$

As in the previous experiment, all males (55 of 55) that mounted the virgin female then copulated with her as well, whereas only 2 of the 8 males that mounted the mated female then subsequently copulated with her. Most (52 of 61=85%) males that copulated with the virgin female had already contacted the mated female. The two males that copulated with the mated female had previously retreated from the virgin female. Some males that copulated with the virgin had previously retreated from her. Females exhibited no obvious aggression, although they did sometimes back away from each other.

Casual observations of mating suggested that mated males exhibit greater discrimination against mated females than do virgin males, so the two choice experiments were compared (Table 4). There was no significant difference between virgin and mated males in which female they first contacted or copulated with ($G_1=0.22, P>0.50$; $G_1=0.30, P>0.50$). However, a greater percentage of virgin males than mated males mounted the mated female ($G_1=4.51, P<0.05$) even when the analysis was restricted to wasps of age 0 to control for age differences between the experiments (40% vs 13%; $G_1=9.31, P<0.05$).

Male eagerness experiments

The first male to respond to the freeze-killed virgin female was the virgin male more often than the mated male (Table 5).

Discussion

The existence of mate preference must be considered in relation to particular traits and particular steps in the mating sequence. In *S. endius*, both virgin and mated males showed no preference for virgin over mated females in terms of which they fanned in the presence of or contacted first. However, males did show a preference for virgins in terms of which they mounted. The preference for virgins at mounting was seen both in the videotapes, where males had no choice between females, and in the choice experiments. Besides mounting virgins preferentially, males also avoided mated females preferentially: a male's first retreat occurred sooner when the female was mated than when she was virgin.

As noted in the introduction, mate choice has been defined as "any pattern of behaviour, shown by members

of one sex, that leads to their being more likely to mate with certain members of the opposite sex than with others" (Halliday 1983). Whether one concludes that *S. endius* males are exhibiting mate choice depends on how one interprets "leads to" in Halliday's definition. Greater receptivity of virgin versus mated females clearly contributes to males preferentially copulating with virgins because when mounted, virgin females usually copulated whereas mated females rarely did. However, even in the absence of female receptivity differences, differential male mounting should lead to fewer copulations with mated females because copulation occurs only after the male mounts the female. Thus, both male mounting behavior and female receptivity contribute to males preferentially copulating with virgins. Another contributor to males preferentially copulating with virgins may be female behavior prior to male mounting. Females do not exhibit any observable behaviors that precipitate male retreats or prevent male mounting (B.H. King and R.M. Bratzke, unpublished data). However, females may be releasing volatile antiaphrodisiacs. Even if they do, the male behavioral response of retreating and not mounting contributes to males preferentially mating with virgins. Another factor besides male discrimination that may contribute to mated females receiving fewer mating attempts than virgins is the fact that females burrow in search of hosts more when they are mated, and males do not usually burrow (King 2002).

That males contacted and fanned mated females as readily as virgins suggests that the pheromones or other cues that are involved in initial attraction differ from those used in later discrimination.

Male discrimination against mated females may benefit both the male and the female in *S. endius*. Because mated females so seldom copulate again, there may be strong selection on males not to waste time and energy by courting them. In addition, even if mated females would copulate again, they might provide low fitness to a male if there is first-male sperm precedence. Sperm precedence has not been examined in *S. endius*, but there appears to be some tendency towards first-male precedence among the few pteromalids that have been examined (Wilkes 1966; Beukeboom 1994; Baker et al. 1998; Do Thi Khanh et al. 2004). That an *S. endius* female must open her genital orifice in order for copulation to take place may strengthen selection on males to avoid mounting already-mated females because it reduces a male's options for forcing copulation.

Mated *S. endius* females may benefit by preventing males from mounting and courting them because it might interfere with burrowing for hosts. Furthermore, there may be no benefit to a female of remating if she receives sufficient sperm from a single copulation. Remating leads to greater offspring production by females in some animals (Arnqvist and Nilsson 2000); however, in the confamilial *Nasonia vitripennis*, females that mate twice do not produce more daughters (Beukeboom 1994). Decreased female longevity after remating further selects against females remating in some animals (reviewed in Arnqvist and Nilsson 2000). In *S. endius*, the effect of a second mating on female longevity has not been examined; however, mating does not appear to decrease longevity relative to not mating (King 2002).

Mate choice has been studied very little in parasitoids (Godfray 1994), but the presence of male preference for virgins is variable among the few confamilials of *S. endius* that have been examined. Preference is seen in *Lariophagus distinguendus* (Ruther et al. 2000), but not in *Muscidifurax* species (van den Assem and Povel 1973). The discrimination against mated females in *S. endius* and *L. distinguendus* seems to make sense because mated females in these species are unlikely to be receptive (this study; Ruther et al. 2000). However, mated females also tend to be unreceptive in *Muscidifurax* species, yet males lack discrimination. Perhaps there is some other mechanism in *Muscidifurax* species by which males avoid wasting time on unreceptive females. Preference for virgin females has also been documented in a few parasitoid wasps in other families (Allen et al. 1994; McNeil and Brodeur 1995; Schworer et al. 1999; but see Nong 1982), a relatively small number of other insects (references in Bonduriansky 2001), a few spiders (Suter 1990; Riechert and Singer 1995; Bukowski et al. 2001), and a few vertebrates (Whitfield 1990; Horan 1997; Saether et al. 2001).

The strength of discrimination against mated females in *S. endius* seemed to differ with male mating status. Both virgin and mated males exhibited all elements of courtship from wing fanning to postcopulatory courtship. However, virgin males appeared to be more eager and less discriminating. In both the choice and no-choice experiments, mounting of mated females relative to virgin females was more than twice as great among virgin males as among mated males. Also, virgin males were slower than mated males to retreat from females, irrespective of the female's mating status.

These differences between virgin and mated males appear to reflect differences in their motivation, not just differences in how females behave toward them. This conclusion is supported by two lines of evidence. First, in contrast to the clear evidence of male discrimination in relation to mating status, females were not significantly more likely to choose virgin versus mated males in terms of which they were less likely to retreat from or copulate with. Second, greater eagerness by virgin than mated males was seen even with dead females, i.e., even when female behavior could be ruled out. After mounting, time

until, and likelihood of, copulation were not significantly different between virgin versus mated males: copulation was very likely if the female was virgin and extremely unlikely if she was mated.

Lower sperm loads in mated males than in virgin males may provide both an ultimate and a proximate explanation for their decreased eagerness. Males emerge with their full complement of sperm, i.e., they do not continue sperm production as adults (Gerling and Legner 1968). However, after a male mates, it may take time for accessory-gland fluid to be replenished and for additional sperm to move to that part of the male reproductive tract where it is available for ejaculation. A male probably gains less by attempting to mate before this reloading occurs than he gains after. Furthermore, less pressure or less chemicals from the sperm or the accessory-gland fluids may stimulate less interest in mating. Whether the duration of a mated male's decreased eagerness coincides with his increased discrimination and with the duration of this reloading remains to be tested.

Another proximate explanation for the decreased eagerness of mated males is learning. This occurs in *Drosophila melanogaster* (Tompkins et al. 1983). If mated females still provide the same attractive cue that virgin females do but simply coupled with a repulsive cue (e.g., brushing off the male after copulation or an antiaphrodisiac pheromone), then any previous exposure to a mated female may cause a male to subsequently associate the attractive cue with the repulsive cue and so avoid even virgin females. Such conditioning may occur even with just one exposure to a mated female (Tompkins et al. 1983), e.g., when a male becomes a mated male.

This study demonstrates that *S. endius* males preferentially mount virgin females over mated females and preferentially retreat from mated females, and that this may be adaptive due to the low probability that a mated female will copulate. That males are more likely to mount virgin than mated females contributes to males being more likely to complete mating with virgins because copulation does not occur in the absence of mounting. This study also demonstrates that males exhibit a decline in eagerness to mount after themselves mating. Additional studies should examine the fitness advantages of monandry and of the mated males' decreased eagerness, as well as the proximal mechanisms involved.

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