Male preference and female cues: males assess female sexual maturity and mating status in a web-building spider

Cristina Tuni\textsuperscript{a} and Reut Berger-Tal\textsuperscript{b}

\textsuperscript{a}Department of Bioscience, Aarhus University, Ny Munkegade 116, DK-8000 Aarhus C, Denmark and
\textsuperscript{b}Mitrani Department of Desert Ecology, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Midreshet Ben-Gurion, Israel

Reproductive success relies on communication signals used by females to exert mate attraction and assessment of female reproductive value by males. During mate search males of the spider, Stegodyphus lineatus experience high mortality and low female encounter rate. Females vary in sexual maturity and mating status (virgin and mated) and hence in reproductive value for males, which should influence male mating effort. Due to high costs of remating, only virgin females are expected to signal mate attraction. Males would benefit from mating preferentially with virgins due to the costs of overcoming mated females’ resistance, although they gain paternity share. Males should avoid immature females, unless guarding precopulatory to access females on maturation. Low encounter rates predict males to invest also in females of inferior reproductive value. We investigated male ability to discriminate immature, virgin, and mated females by assessing: the number of females males visit in the field; male mating effort and male discrimination of females based on silk cues in laboratory trials. In the field, males were found most frequently with virgin females. Male mating effort, copulation success, and preference based on silk cues were higher with mature compared with immature females. Our data suggest that females signal sexual receptivity and that males are able to discriminate both sexual maturity and mating status, however, males do not exert strong preference for virgins. The combination of high costs of mate search and low encounter rate likely exerts selection on males to mate indiscriminately and overcome female resistance to remating.

Key words: Eresiidae, male mate choice, mate assessment, reproductive state, sperm competition, Stegodyphus lineatus.

INTRODUCTION

Reproductive success for both sexes relies on informative signals that can be used in mate choice for assessing the reproductive value of a mate and for subsequent decision-making processes (Maynard-Smith and Harper 2003). Females are expected to release visual, chemical, or auditory cues to signal their receptivity and attract males (Andersson 1994), whereas males should evolve mate choice when costs associated with mating (i.e., sperm production, courtship, and mate guarding) are high and there is sufficient variation in female quality (Bonduriansky 2001; Kokko and Wong 2007). Sexual maturity and mating status (virgin or mated) may represent important indicators of female reproductive value for males and may influence the investment of males into mating.

Males would benefit from mating with females carrying mature eggs, whereas they would incur high energetic and reproductive costs by courting nonfecund immature females (Ridley 1989; Bonduriansky 2001). Similarly, in species that store sperm female mating status (virgin vs. mated) is crucial for male reproductive success due to the risk of sperm competition that arises when females mate polyandrously (Parker 1970; Simmons 2001; Wedell et al. 2002). Sperm priority patterns that confer advantage to the first male to mate predict preferential mating with virgin females, which increases fertilization success and lowers the risk of infection by sexually transmitted diseases (Bonduriansky 2001; Simmons 2001; Wedell et al. 2002; Knell and Webberley 2004). In contrast, under last-male sperm precedence, males should evolve traits to protect paternity against later arriving males (Bonduriansky 2001; Simmons 2001; Wedell et al. 2002). Male preference may, however, be constrained by factors, such as costs of mate search, male encounter rates, and the accuracy of assessment mechanisms of potential mating partners (Bonduriansky 2001).

From the female perspective, immature females are not expected to invest in mate attraction and should not signal their presence to avoid male harassment (Ridley 1989). In contrast, sexually mature females should advertise to attract males to ensure sperm supply and avoid the risk of remaining unmated (Andersson 1994). Once insemination has occurred, the arrival of additional males may be a disadvantage to females because of loss of foraging opportunities, risks of predation, disease transmission, or sexual harassment (Arnqvist and Rowe 2005). Therefore, if the costs of remating are high, selection should act on mated females to reduce their attractiveness (Ridley 1989). Alternatively, mated females could benefit from attracting multiple partners to acquire direct (material) benefits of polyandry (i.e., food, sperm) from males (Arnqvist and Nilsson 2000) or to exert postmating sexual selection for indirect (genetic) benefits (Jennions and Petrie 2000; Simmons 2005).

Several studies on invertebrates show that males are able to distinguish between mated and virgin females and adjust the
intensity of courtship, ejaculate size, and content accordingly (Martin and Hosken 2002; Siva-Jothy and Stutt 2003; Carazo et al. 2004; Friberg 2006; Thomas and Simmons 2007). However, very little is known about the cues males use to assess female mating status. Sex pheromones released by females are an important mode of intersexual communication for advertising sex and receptivity and are known to influence mate choice (Johansson and Jones 2007). In spiders, the composition and/or concentration of chemical compounds deposited on silk threads or released onto body cuticle, may change with female age or reproductive state (Trabalon et al. 1997; Papke et al. 2001). Males are known to exploit state-dependent variation in chemical cues to discriminate female mating status (Andrade and Kasumovic 2005; Roberts and Uetz 2005; Stoltz et al. 2007; Baruffaldi and Costa 2010).

In this study, we investigated the ability of male Stegodyphus lineatus (Araneae: Eresidae) spiders to assess female reproductive value in relation to sexual maturity and mating status. Field studies show that S. lineatus males encounter relatively few females during their lifetime (an average of 1.8 females) (Schneider and Lubin 1997; Berger-Tal and Lubin 2011) and experience high mortality once they abandon their nest in search for a mate (Bilde et al. 2005; Berger-Tal and Lubin 2011). Males are protandrous and mature earlier than females over a wide time range that overlaps with female maturation time (Schneider 1997; Maklakov et al. 2004). Therefore, during the breeding season, males encounter both immature and mature females, and males may also encounter females that have already mated or produced an egg sac. Males have been observed to cohabit with both mature and immature females prior to, between and after matings (Schneider 1997). During cohabitation, males feed on prey captured by the female web, which negatively affects female body condition (Erez et al. 2005) and consequently female fecundity.

Virgin females mate indiscriminately with the first male arriving at their nest, whereas mated females are reluctant to remate and often engage in physical combat with additional males (Schneider and Lubin 1998; Maklakov and Lubin 2004). Females are semelparous, and when males encounter females that guard their eggs, they engage in fights and on winning the aggressive interaction they commit infanticide by removing the egg sac and force the female to remate and produce a replacement egg sac (Schneider and Lubin 1996). Such males gain parentage at very high costs to females including energy loss of delaying, delayed oviposition, smaller replacement clutches, and higher brood failure (Schneider and Lubin 1997, 1998; Maklakov et al. 2005). There is no clear sperm priority patterns in this species; 2 males that mate with a female have an equal probability of fathering the offspring, although patterns of parentage within broods can vary considerably (Schneider and Lubin 1996).

We investigated male discrimination of female sexual maturity (immature vs. mature) and mating status (virgin vs. mated) in S. lineatus by 1) assessing the frequency of male visits to nests of immature, virgin, and mated females in a natural population from the Negev Desert (Israel); 2) analyzing male mating effort, copulation success, and copulation duration with immature, virgin, and mated females, respectively, in staged laboratory mating trials; and 3) testing male ability to discriminate mating partners via silk-bound cues in choice assays where males were exposed simultaneously to silk threads released by immature versus mature and virgin versus mated females. The costs of polyandry in this system predict mated females to reduce their attractiveness and reduce postmating receptivity. Males pay a high cost of overcoming female resistance and would benefit from discriminating and preferentially mating with virgin females. However, the high costs of mate search, the relatively infrequent encounters with potential mating partners, and potential to acquire parental representation in broods of mated females imply that males should invest in mating also with mated females. Immature females are not expected to exert mate attraction due to lack of fertilization benefits. Immature females that were observed to mate during their penultimate instar stage do not preserve male sperm as they lose their sperm storage organ on molting to adulthood. Hence, males should avoid mating interactions with immature females. Cohabitation with immature females could instead provide food, shelter, and future mating opportunities.

MATERIALS AND METHODS

Study species

Stegodyphus lineatus (Latreille 1817) is a subsocial web-building spider found in arid and semiarid areas around the Mediterranean basin (Kraus O and Kraus M 1988; Schneider 1997). Spiders are patchily distributed within populations and occupy thick silk cone-shaped retreats located on bushes and shrubs. The mating season starts in March to April when spiders molt to adulthood and become sexually active (Schneider 1997; Schneider and Lubin 1997). Mated females produce one egg sac and care for it by tending the sac, releasing the young and feeding them by regurgitation (Kullmann et al. 1971). Females are semelparous and produce only a single brood as they are inevitably killed and eaten by their young approximately 2 weeks after hatching. However, females may produce a replacement egg sac if the first one is damaged, removed by a predator or by an infantidal male (Schneider 1997; Schneider and Lubin 1997).

Field survey

A field survey was conducted along Wadi Zipporim (2000 m²) in the Negev Desert of Israel between March and June 2006, where S. lineatus nests were found on shrubs and low vegetation (dry annual plants) of a seasonally dry stream bed. All nests were marked and monitored every second day. We recorded the sex of the inhabiting spider, the presence of a molt to indicate developmental state, visits of males to a female nest, and the occurrence of egg sacs. Due to the difficulty of assessing maturity in this species, female sexual maturity was determined a posteriori: Immature females (subadults in their penultimate maturity) were those that did not molt during the field observations prior or after a males’ visit, whereas mature females were those for which we recorded a molt. Although copulations were not directly observed, we considered mature females that were visited by a male as mated. In this species, male encounter rate is a reliable proxy for female mating rate, as males that gain access to a females’ nest also mate with her (Maklakov and Lubin 2004; Bilde et al. 2005). Adult males that were found in the area were marked individually with a specific combination of water-based colors on the dorsal side of the abdomen (Schneider 1997). The frequency of male visits to immature and mature females was calculated in relation to the availability of mature females (number of mature females/total number of females) in the field at the time of the visit, as the proportion of mature females increased as the season progressed.

Collecting and rearing

We collected approximately 100 juvenile spiders in February 2008 from the Judean Desert (Israel) and brought them to Sede Boquer Campus of the Ben-Gurion University of the Negev. Spiders were kept in their natal nests and were housed individually in plastic cups (3 cm diameter × 10 cm high) with mesh lids and reared in the laboratory. Individuals were maintained at
room temperature (25–27 °C) with stable humidity and 13:11 h light:dark cycle. Spiders were fed once a week with a varied diet consisting of house flies (Musca domestica), crickets (Acheta domestica), and early instar grasshoppers (Locusta sp.).

Male mating behaviors

As spiders gradually reached maturity (March to May), they were assigned to behavioral assays. Each male was presented to an immature female of penultimate instar (N = 47), a virgin (N = 53), and a mated (N = 42) female in random order. Female sexual maturity was determined a posteriori (see above). Thus, immature females were those that molted after being exposed to a male; virgin females were those that had molted in the laboratory and did not molt after exposure to males. Females were tested 2 weeks after molting. Mated females were those virgin females that had copulated once and were presented to a second male 1–2 days after mating.

Mating trials were conducted on female nests. In order to allow clear visibility to the observer, we clipped a wooden clothes peg at the end of the nest so that females could not sit deep inside their retreat. We introduced a male on the web surrounding the nest entrance by gently inducing him to walk onto it with a paintbrush. The trial started (t = 0 s) when the male reached the female’s web. On encounter with the web, males usually perform a stereotyped searching behavior consisting of a “spiral walk” toward the female and vibration bouts (Maklakov et al. 2003). Searching behavior ended when a male made physical contact with a female, its front legs touching the female’s cephalothorax. However, the sequence of such behaviors (spiral walk, vibrations, and physical contact) was often repeated more than once. For each trial, we recorded the following: 1) male courtship behaviors: occurrence of searching behavior (spiral walk performed on the web), occurrence of vibrations on female web, physical contact (male touching the female with first pair of legs), and latency to first contact (time from start of trial to first physical contact with the female); 2) copulation success, latency to copulation (time from start of trial to copulation), copulation duration, and frequency of mating interruption (number of times males abandon the mating position). Trials were ended after 60 min if males showed no response to females and positioned themselves motionless on the web.

Silk choice assays

To examine female signaling with silk, we tested male response to female silk threads in the absence of the female, in order to exclude the effect of tactile cues from the female. We constructed a Y-shaped apparatus by attaching 2 wooden skewers (30 cm long) by their ends at an oblique (45°) angle to a central wooden skewer fixed to the inside bottom of a plastic cup (Figure 1). Each oblique arm was wrapped with silk threads drawn from webs of females differing in their sexual maturity and mating status. Males were tested in 3 different choice assays: 1) immature versus mature (virgin) female silk (N = 27 males) to test for discrimination of sexual maturity, 2) virgin versus mated female silk (N = 23 males) to test for discrimination of mating status, and 3) immature versus no silk (N = 27 males) as a control of silk preference. All immature females molted after we conducted the assays, whereas virgins did not (see above). Mated females were mated in the laboratory, and their silk was used approximately 5 days after mating to obtain post-mating silk.

The silk from a female’s web was placed on 1 of the 2 oblique arms, and the order was randomized to control for potential side bias. Assays started when a male was introduced at the base of the central arm. Males could climb the arm, reach the intersection, and move toward 1 of the 2 arms of the apparatus. Only males that touched both oblique arms with their front legs before moving toward one of the arms were included in the analysis (N = 12 males were excluded). A choice was considered made when the male had climbed to the upper end of one of the oblique arms. Each male and each female silk was used only once. The used wooden skewers were replaced with new ones in each assay. Trials were run after sunset in a darkened room, during the spiders’ natural activity hours.

Statistical analysis

All statistical analyses were performed using JMP 7 software (SAS Institute). Variables were transformed if they did not conform to assumptions of normal distribution of residuals and homogeneity of variances. For comparing mean values, we used analysis of variance for parametric data and Wilcoxon test for nonparametric data. We used chi-square test (with Bonferroni correction) to compare frequencies. A logistic regression was performed to test for frequency dependence of male visits to mature females. Results are given as mean ± standard deviation.

RESULTS

Field survey

We found a total of 171 spiders, including individuals in their natal nest and males visiting females. The first male that molted to adulthood was recorded on the 30th of March. By the 3rd of June, all males had left their natal nests, no males were found in the population, and mated females started to produce egg sacs. Out of the total number of males (N = 54), 30 males were initially found in their own nest, and the remaining (N = 24 males) were found on female nests. In total, we recorded 78 male visits to female nests, but in only 38 cases, females occupied the nest for at least 6 days (3 consecutive observations) after the male had left allowing us to correctly identify her reproductive state. These data suggest high female mortality. No male was found visiting a female during her molt to adulthood. Males visited significantly more virgins (N = 20) than mated (N = 6) or immature (N = 10) females or empty nests (N = 2) (chi-square test: $\chi^2_{3, 38} = 18.8$, $P = 0.0003$; Figure 2). The higher proportion of male visits to mature (virgin and mated) females did not depend on their availability in the field (logistic regression: $F_{1, 34} = 0.765$, $R^2 = 0.022$, $P = 0.38$). We found no significant difference in the duration of time a male spent visiting immature, virgin, or mated females or empty nests in the field (Wilcoxon test $\chi^2_{3, 38} = 1.8$, $P = 0.64$; immature females 3.9 ± 1.6 days, virgin
male mating behaviors
A total number of 142 mating trials were observed. Male courtship behaviors differed significantly toward females of different sexual maturity. Male searching behavior, occurrence of vibratory courtship, and establishment of physical contact occurred in higher proportions toward mature (virgin and mated) compared with immature females (Table 1). Copulation occurred exclusively with mature females (Table 1).

Male differed in vibratory performance in response to females of different mating status. Vibratory behavior occurred in greater frequency toward virgins compared with mated females (Table 1). No significant difference was found in male searching behavior and establishment of physical contact (Table 1). Similarly, there were no significant differences in copulation success, copulation duration, and latency to copulation between virgin and mated females (Table 1). Silk choice assays

Data from the choice test are shown in Figure 3. Males that were exposed simultaneously to silk of immature and mature virgin females showed significant preference for silk of mature females (chi-square test: $\chi^2_{1,26} = 14.7, P = 0.001$). No significant difference in male preference was found in choice assays between silk of virgin and mated females (chi-square test: $\chi^2_{1,23} = 1.09, P = 0.3$). When exposed to silk of immature females versus no silk, males preferred silk (chi-square test: $\chi^2_{1,26} = 11.55, P = 0.0007$).

DISCUSSION

Our results show that males are able to discriminate between immature and sexually mature females and that they exert an overall preference for mature (virgin and adult) females. This was supported by the larger number of males visiting mature females in the field, by the greater courtship effort and mating success with mature females in mating trials, and by male preference for silk of mature over that of immature females during silk choice assays. These results suggest that females advertise sexual maturity, and males have evolved the ability to exploit female chemical cues for acquiring information on female receptivity to mating and female reproductive value.

Our data provide some evidence for the ability of males to discriminate virgin females, as predicted by theory: First, males were found most frequently in the nests of virgin females in the field survey. Males visited significantly more virgin females. *denotes significance.

females $4.5 \pm 1.03$ days, mated females $4.5 \pm 1.2$ days, and empty nests $3.5 \pm 0.5$ days).

Table 1

<table>
<thead>
<tr>
<th>Male behavioral responses toward immature, virgin, and mated females during controlled laboratory mating trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male responses</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Searching behavior %</td>
</tr>
<tr>
<td>Vibrations %</td>
</tr>
<tr>
<td>Physical contact %</td>
</tr>
<tr>
<td>Latency to first contact min, median (range), mean ± SE (N)</td>
</tr>
<tr>
<td>Copulation success %</td>
</tr>
<tr>
<td>Latency to copulation min, median (range), mean ± SE (N)</td>
</tr>
<tr>
<td>Copulation duration min, median (range), mean ± SE (N)</td>
</tr>
</tbody>
</table>

*Denotes significant differences among groups ($P < 0.025$).
Discrimination between virgin and mated females

Studies on other spider species show that males are more inclined to court and mate with virgins rather than mated females and that males are likely to exploit silk-bound chemicals to discriminate female mating status (Riechert and Singer 1995; Rypstra et al. 2003; Stolz et al. 2007; Baruffaldi and Costa 2010). While we have evidence that S. lineatus males can discriminate virgin from mated females, they did not exert strong preference for virgins over mated females. Vibration performance occurred in significantly higher proportions toward virgin compared with mated females, suggesting that there are differences in the signals produced by females of different mating status which are detected by males. A similar result was found in a previous study (Maklakov et al. 2003), which further tested whether male vibration is triggered by silk-bound cues (sex pheromones) alone by presenting a male to an empty female nest. They found that males initiated courtship also in the absence of direct encounter with the female and that vibrations decreased with time, suggesting that male courtship behavior is elicited by female silk-bound cues that need to be renewed by the female (Maklakov et al. 2003).

Given the heavy fecundity costs of polyandry in this system (Schneider and Lubin 1997, 1998; Maklakov and Lubin 2004; Erez et al. 2005; Maklakov et al. 2005), mated females should avoid the costs of remating, for example, by interrupting the production of pheromones or by modifying blends of sex pheromone compounds to repel males (Herberstein et al. 2002; Gaskett 2007). Indeed, our field data could support this prediction, as 15% of the males were found in mated females nests compared with 50% in virgin nests. Results from our mating trials were less clear as males experienced similar mating success with virgin and mated females, although we expected a lower mating success with mated females (Schneider and Lubin 1996; Maklakov et al. 2004, 2005; Maklakov and Lubin 2004). In previous studies, females were exposed to additional males 10 days after mating (Maklakov et al. 2004, 2005; Maklakov and Lubin 2004), whereas we tested females 1–2 days after mating. This result suggests that changes in female post-mating signaling of receptivity occur over a period of time. Indeed, postmatting receptivity advertised by female silk is known to be subject to large variation in time. For example, in the case of female sierra dome spiders, Linyphia litigiosa silk-bound sex pheromones may persist for 1 week postmating (Watson 1986). By contrast, a study on the Australian redback spider Latrodectus hasselti shows that the change in female chemicals may be very rapid and extracts from female web fail to attract males immediately after mating (Stolz et al. 2007). Variation in the timing of loss of advertisement for males could reflect the costs and benefits of intersexual communication.

It is likely that ecological constraints favor male investment in all encountered females regardless of their mating status. Moderate remating frequencies for females (Schneider 1997; Berger-Tal and Lubin 2011; Tuni et al. 2012) suggest low remating competition risks for males. Moreover, males achieve a 50% success of fertilizing eggs of already mated females (Schneider and Lubin 1996; Tuni et al. 2012). The benefits of fertilizing a portion of the females’ eggs might overcome the costs of searching for a virgin female due to high male mortality during mate search. Furthermore, the chance of encountering a virgin female decreases as the season progresses (Berger-Tal and Lubin 2011). Low encounter rates and relatively high paternity gain from mating with mated females because levels of polyandry are low, is likely to exert strong selection on males to overcome female resistance to remating. Hence, females and males are in conflict over optimal mating rates, which may result in suboptimal
mating rates for *S. lineatus* females (Schneider and Lubin 1996; Maklakov and Lubin 2004; Arnqvist and Rowe 2005).

**FUNDING**

C.T. was supported by the European Union Sixth Framework Transnational Access Dryland Research Specific Support Action. This is publication number 757 of the Mitrani Department of Desert Research.

We would like to thank Iris Musli for helping collecting and rearing spiders, Yael Lubin and Trine Bilde together with anonymous referees for valuable comments on the manuscript, and the members of the spider-labs from Denmark and Israel for constructive discussion.

**REFERENCES**


