



## Alternative mating tactics in a cannibalistic widow spider: do males prefer the safer option?

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Mating generally occurs with adult females, which undergo a suite of changes in morphology, physiology and behaviour during maturation. In the brown widow spider, *Latrodectus geometricus*, however, males can mate with immature females during a short period before they moult to the adult stage. Mating with immature females seems beneficial for males, because they are not at risk of being cannibalized, whereas cannibalism inevitably occurs in matings with adult females. We conducted choice experiments to elucidate male preference, courtship and mating behaviour with immature and adult females of different ages. We controlled for age of the females' webs to provide males with potential web-borne attractants of similar age. We tested whether males distinguish immature females that are ready to mate (late subadult stage) from adult females and from immature females that do not mate (early subadults), and we examined male response to young versus old adult females. Males approached and mated with adult females more frequently than late subadult females, but there were no differences in the frequencies of approach to early and late subadults or to adult females of different ages. Once on the web, however, males attempted to mate with the late subadults. We suggest that web-borne volatile cues, typical of adult females, may be reduced or lacking in late subadult females, yet less volatile cues may indicate receptivity.

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Traits and processes associated with male reproduction can be costly (Dewsbury, 1982; Scharf, Peter, & Martin, 2013); for example, males often have to search for mates, compete for access to them and perform lengthy and energetically demanding courtship displays in order to mate. Courtship can be risky as it may attract the attention of predators. Moreover, males can be attacked by the female before or during mating (Zuk, 2016). In some species, in which courtship is especially risky, males have evolved alternative tactics by which they may mate without first courting the female. Alternative tactics might include sneaking copulations or mating with females while they are occupied in some other activity and unable to repel or attack the male (Buzatto, Tomkins, & Simmons, 2014; Schradin, 2019; Shuster, 2010; Taborsky, Oliviera & Brockmann, 2008; Thornhill & Alcock, 1983). Sexual cannibalism can be especially pronounced in spiders (Buskirk, Frohlich, & Ross, 1984; Elgar, 1992; Robinson & Robinson, 1980), and the males of

some spiders are known to circumvent the risk of being killed by mating with moulting or freshly moulted females, which are immobile while the cuticle is still soft (Danielson-François, Hou, Cole, & Tso, 2012; Foellmer & Fairbairn, 2003; Lubin, 1986; Uhl, Zimmer, Renner, & Schneider, 2015). Males of two widow spiders, *Latrodectus geometricus* and *Latrodectus hasselti* (Theridiidae), take this tactic a step further: they mate with females in the subadult stage before their final moult to maturity (denoted immature mating in Biaggio, Sandomirsky, Lubin, Harari, & Andrade, 2016). Until recently, it had been considered impossible for male spiders to copulate with and transfer sperm to immature females (Dodson & Beck, 1993; Fahey & Elgar, 1997). However, males of these two *Latrodectus* species do mate with immature females, yielding viable offspring, and the fertility and fecundity of immature-mated females are not distinguishable from those of adult-mated females (Biaggio et al., 2016; Waner, Motro, Lubin, & Harari, 2018).

When mating with adult females, males of these two *Latrodectus* species are limited to a single copulation due to sexual cannibalism. The males actively facilitate cannibalism by somersaulting into the female's mouthparts, which appears to trigger it. The somersault and subsequent cannibalism never occur at any other time during

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mating (Andrade, 1996; Forster, 1992; Segoli, Arieli, Sierwald, Harari, & Lubin, 2008). In *L. hasselti*, the act of cannibalism secures the male's reproductive success: females are less receptive to additional mates after having cannibalized a male during copulation (Andrade, 1996). Moreover, cannibalized males copulate for longer and transfer more sperm than males that do not perform a somersault and survive the copulation (Andrade, 1996). Despite being mobile and capable of foraging, immature females only rarely attack or cannibalize males during copulation and males only rarely perform the copulatory somersault (Biaggio et al., 2016). Males mating with an immature female court briefly or not at all (Biaggio et al., 2016), whereas mating with adult females in widow spiders involves lengthy courtship consisting of body vibrations and web alteration, whereby the male cuts and bundles the female's web and adds his own silk to it (Anava & Lubin, 1993; Harari, Ziv, & Lubin, 2009; Segoli, Harari, & Lubin, 2006).

Female spiders possess paired copulatory openings and sperm storage organs (spermathecae) and males have paired copulatory organs (pedipalps; Foelix, 2011). In immature females of *L. hasselti* and *L. geometricus*, the genitalia seem to be fully formed a few days before the final moult, and only then does immature mating occur (Biaggio et al., 2016). The copulatory openings, however, are covered by the cuticle and must be ripped open by the male before mating (Baruffaldi & Andrade, 2017; Biaggio et al., 2016). During mating, *Latrodectus* males often leave part of the pedipalp in the female's genitalia, which may function as a mating plug, preventing females from remating with another male (Berendonck & Greven, 2000; Snow & Andrade, 2005; Segoli, Arieli, et al., 2008). The plugs are effective only when placed in a specific location and often more than one tip can be found within female spermathecae (Neumann & Schneider, 2011; Segoli, Lubin, & Harari, 2008; Snow, Abdel-Mesih & Andrade, 2006). Male *L. hasselti* and *L. geometricus* that mate with immature females typically inseminate and leave plugs in both spermathecae (Biaggio et al., 2016). By contrast, in mating with an adult female, the male may be cannibalized after inserting a single pedipalp, which would prevent him from inseminating both spermathecae (Andrade, 1996; Segoli, Arieli, et al., 2008). Waner et al. (2018), however, found no difference between immature and adult genitalia in the number of plugs deposited, suggesting that the outcome of mating with an immature or adult female may not necessarily differ.

Male *L. hasselti* and *L. geometricus* thus have two alternative tactics: (1) to mate with an adult female and secure paternity through self-sacrifice or (2) to mate with an immature female, plug both spermathecae and survive to potentially mate again. Considering the benefit of survival, males should prefer to mate with immature females. However, in a previous study, when given a choice, male *L. geometricus* chose adult over immature females as well as older over young adult females (Waner et al., 2018). This begs the question: do males recognize immature females as mating partners?

Many, perhaps all, male web-building spiders locate females by the chemical attractants (pheromones) associated with their webs and, generally, sexual attraction is limited to the stage of sexual maturity (Fischer, Lee, Stewart, & Gries, 2018; Gaskett, 2007; Schneider & Andrade, 2011; Uhl, 2013; Uhl & Elias, 2011). Therefore, in Waner et al.'s (2018) study, male *L. geometricus* might not have recognized immature females as mating partners. Immature females might produce little pheromone or do so only after the final moult. This is suggested by studies of other widow spiders where males responded with courtship behaviour and/or higher activity to silk from adult females but were less active on silk from subadult females (Anava & Lubin, 1993; Ross & Smith, 1979; Stoltz, McNeil, & Andrade, 2007). Old, unmated adult females, by contrast, might produce a higher concentration of these pheromones than young

adult females (Fischer, 2019), a phenomenon seen in the spider *Agelenopsis aperta* (Agelenidae; Riechert & Singer, 1995).

The differential attractiveness of females that increased with age in Waner et al.'s (2018) study might also be explained by the experimental set-up. Males were presented simultaneously to differently aged females (immatures versus young and old adults) whose webs also differed in age. This experimental set-up simulated a natural situation, as widow spider females often inhabit a single web for their entire adult lives (Andrade, 2003; Andrade & Banta, 2002; Lubin, Ellner, & Kotzman, 1993). Webs of older, unmated females potentially accumulate a greater amount of sex pheromone than those of immature or younger adult females. Thus, the signal is likely to be quantitatively different in females of different ages and developmental stages due to the age of the web itself. Consequently, rather than choosing older females specifically, males may simply be more strongly attracted to a stronger signal.

Here, we investigated the mate choice decisions that *L. geometricus* males make in relation to cues provided by females of different ages. In all experiments, we controlled the age of the females' webs, such that any difference in attractiveness would be attributable to the stage of the female alone. We asked whether males distinguish between, and are attracted differentially to, immature versus adult females, immature females of different ages and young versus old adult females. All immature females were in the stage before the final moult to adult; henceforth we refer to this as the subadult stage. Mating with immature females occurs only within a narrow window, up to 4 days before the final moult to adult stage (late subadult stage; Biaggio et al., 2016; Waner et al., 2018). We exposed virgin males to late subadult and adult virgin females to determine whether males express a preference related to female developmental stage. We expected males to prefer the late subadult females as this tactic will allow them to survive and potentially mate again. In choices between early and late subadult females, we suggest two possible outcomes. First, *L. geometricus* males may cohabit with early subadults to be able to mate as soon as the female moults to the late subadult stage. Males of many spider species cohabit with immature females in anticipation of mating as soon as the female matures (Jackson, 1986), and cohabitation with immatures has been observed in *L. hasselti* (Biaggio et al., 2016) as well as in *L. geometricus* (Y. Lubin, personal observation). Nevertheless, cohabitation with an early subadult female can be risky, as they are often aggressive towards the males and may even attack them as prey (Biaggio et al., 2016). Thus, we predicted either no preference or a preference for late subadult females. Additionally, it is possible that early and late subadult stages are indistinguishable if neither stage produces sex pheromones. Finally, Waner et al. (2018) suggested that unmated old females invest more in pheromone production, lest they remain unmated, and consequently are more attractive to males than young virgin females. To test this hypothesis, we exposed males simultaneously to young and old adult females.

## METHODS

### Housing

Females and eggsacs of *L. geometricus* were collected in central Israel (Bat Yam, Beer Yaakov, Rishon Le'Zion, Ramat Gan and Ma'agan Michael). We brought the females to the laboratory at the Sede Boqer Campus of Ben-Gurion University of the Negev, Israel, and housed them separately in plastic boxes in a climate chamber ( $25 \pm 1$  °C, 60% relative humidity and 14:8 h light:dark). They were fed twice a week with flies or grasshopper nymphs to satiation. We placed spiderlings that hatched from the eggsacs into individual plastic containers after they had reached the third instar. The

spiders were transported to the University of Greifswald, Germany, for experiments, and maintained at  $25 \pm 1$  °C in a climate chamber under a reversed 12:12 h light:dark regime and 60% relative humidity. Their webs were sprayed with water twice a week. We recorded the date of the moult to subadult stage and the final moult of each spider. Spiderlings and males were fed twice a week with six fruit flies, *Drosophila hydei*. Subadult females were fed with two *Lucilia* sp. flies, mature females with one *Protophormia* fly or one small cricket, *Acheta domesticus* (7–10 mm) twice a week. We used only virgin individuals in the experiments. All experiments were conducted during the dark phase under a red light since these spiders are nocturnal (Lamoral, 1968).

#### General Experimental Protocol

We experimentally tested whether males are attracted to females of specific developmental stages. Five days prior to testing, females of different stages (see below) were transferred to clean experimental boxes (10 × 10 cm and 6 cm high) with removable lids. The lids were covered on the inside with a thin layer of Vaseline, which prevented the females from attaching their webs to the lid. To control for the age of the web, all tested females were given the same length of time to construct one. The boxes were placed on their side such that the lid was on one side and could be kept open during the trial. The females were fed 1 day before they were transferred into the experimental boxes but then received no food to avoid the presence of confounding food-related cues.

At the beginning of a trial, two boxes were in place in diagonally opposite corners of a square test arena (40 × 40 cm and 12 cm high), which was covered with a glass plate. There was either a female in each box (two-female tests) or one female and an empty box (one-female tests). The lid of each box was removed exposing the female's webs and giving the male access. A virgin male was released into one of the corners of the test arena that did not contain a box, equidistant from the openings of both boxes. During the following 30 min, we recorded (1) the box entered by the male, (2) occurrences of and (3) latencies to first contact with the female's web, (4) adding silk to the female's web, (5) cutting and removal of silk from the female's web, (6) abdomen vibrating and (7) contact with the female's body (see Segoli, Arieli, et al., 2008 for a detailed description of the courtship of *L. geometricus*).

The latency to mate after the initial web contact is more than 3 h with adult females and approximately 45 min with subadults (Biaggio et al., 2016). After a 30 min period of continuous observation had elapsed, males were left to continue courtship and, if successful, mate with the female in the box. During this time, we inspected the spiders at irregular intervals. The female that was not chosen (except for the early subadults, see below) was paired with another male ('control male') to test her attractiveness and receptivity. If the unchosen female did not mate with the control male, the data from the trial were not included in the analysis. Some of the young adult and late subadult females were used for the single female tests (see below). After 24 h, the trials were terminated. In trials with adult females, cannibalism of the male was considered an indication that copulation had occurred. In males, the loss of the tip of the pedipalp was considered as additional evidence of copulation, as was the presence of a mating plug in adult and subadult females and bites in the cuticle covering the genital area of subadult females.

#### Experiments

We performed three sets of choice trials with two females. Virgin males (10–41 days after the moult to adulthood) were exposed simultaneously to (1) one adult (7–25 days after the moult

to adulthood) and one late subadult female (more than 10 days after the moult to the subadult stage, which is approximately 4 days before the moult to adulthood (when subadult copulation can occur) see Biaggio et al., 2016 for details;  $N = 40$ ); (2) one early subadult (less than 8 days after the moult to subadult stage) and one late subadult female (more than 10 days after the moult to subadult stage;  $N = 39$ ); (3) one young adult (10–14 days after the moult to adulthood) and one old adult female (2–3 months after the moult to adulthood;  $N = 39$ ). Late subadult females are distinguished by a dark brown epigynal area, indicating that the already sclerotized genitalia are fully formed beneath the cuticle, which will be shed during the last moult (Biaggio et al., 2016). In contrast, early subadult females have a pale genital area (Biaggio et al., 2016).

By staging encounters of virgin males with single females, we investigated whether the identity of the female affected details of male behaviour. For this, the male was introduced into the arena with one box housing the female, either adult ( $N = 20$ ) or late subadult ( $N = 21$ ), and one empty, control box.

#### Statistical Analyses

Male choice in the experiments was analysed using the binomial test. Whether the occurrence of the courtship components (silk adding, silk cutting and abdomen vibrating) differed with female developmental stage was analysed with proportion tests. Latencies were analysed with the Mann–Whitney *U* test for independent samples when sample sizes allowed. The effect of a male's age on his mate choice was tested by a generalized linear model with a binomial distribution (GLM-b). All statistical analyses were performed in the R environment (The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>), version 3.4.3.

## RESULTS

Taking all tests together (with two females and with one female), males paired with adult females mated in 95% of cases ( $N = 111$ ) and males paired with late subadult females mated in 91% of cases ( $N = 77$ ). Males never mated with early subadult females ( $N = 35$ ). Adult females were cannibalistic only when copulation occurred, cannibalizing males in 88% of cases ( $N = 105$ ). Late subadult females never cannibalized males during copulation ( $N = 70$ ) and only a single case of precopulatory cannibalism was recorded ( $N = 77$  pairings with late subadult females).

#### Tests with Two Females

All but three males entered one of the boxes with a female within 30 min ( $N = 119$ ). After contacting her web, all but one male began to court; one male in the early versus late subadult female treatment only briefly entered the box with the late subadult female, then immediately left, and remained motionless within the arena. All other males ( $N = 115$ ) remained with the chosen female during the 30 min observation period. A male's age did not affect his mate choice in any of the treatments (GLM-b: adult versus late subadult females:  $F_{1,33} = 0.04$ ,  $P = 0.84$ ; early versus late subadult females:  $F_{1,33} = 0.04$ ,  $P = 0.83$ ; young versus old adult females:  $F_{1,33} = 0.26$ ,  $P = 0.61$ ).

#### Adult versus Late Subadult Females

In five of 40 trials (12.5%), the unchosen female (one adult and four late subadults) did not mate with a control male after the experiment and so these trials were not included in the analyses because these females were probably not receptive to mating.

Males presented simultaneously with one adult and one late subadult female chose the box with the adult female more often than the subadult female (binomial test:  $N = 35$ ,  $P = 0.01$ ; Fig. 1).

Adding silk occurred in 96% of trials with adult females and in only 20% of trials with subadult females (proportion test:  $\chi^2 = 17.8$ ,  $P < 0.005$ ; Fig. 2a). Silk cutting occurred in 88% of trials with adult females and never with subadult females (proportion test:  $\chi^2 = 20.1$ ,  $P < 0.005$ ; Fig. 2a). Males that chose adult females vibrated in only 40% of trials, whereas vibration occurred in 80% of trials with subadult females (proportion test:  $\chi^2 = 3.1$ ,  $P = 0.07$ ; Fig. 2a). The latencies to contact the female's web and body and to vibrate did not differ significantly between adult ( $N = 25$ ) and subadult females ( $N = 10$ ; Mann–Whitney  $U$  test: web contact:  $U = 160.5$ ,  $P = 0.2$ ; body contact:  $U = 127.5$ ,  $P = 0.79$ ; vibrating:  $U = 54$ ,  $P = 0.24$ ; Fig. 2d).

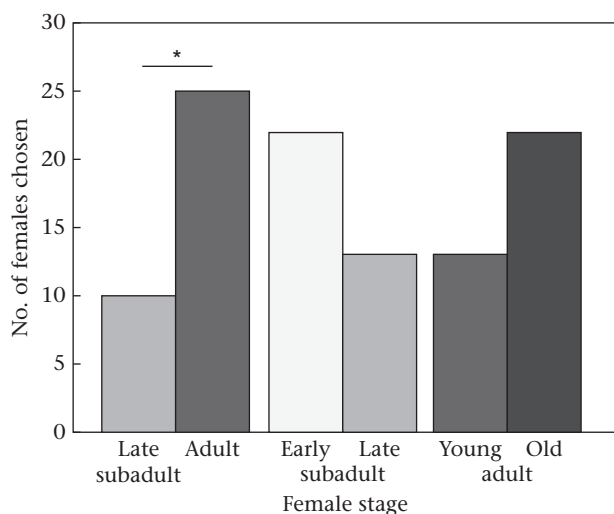
#### Early versus Late Subadult Females

In two of 37 trials (5.4%), the unchosen female (both late subadults) did not mate with a control male and so these trials were excluded from the analyses. Males showed no preference for either of the subadult female stages (binomial test:  $N = 35$ ,  $P = 0.17$ ; Fig. 1).

Neither the probability of adding silk nor the latency to silk adding differed between groups (proportion test:  $\chi^2 < 0.01$ ,  $P = 1.00$ ; Fig. 2b; Mann–Whitney  $U$  test:  $U = 7$ ,  $P = 0.57$ ; Fig. 2e). Males never cut silk from webs of early or late subadult females (Fig. 2b). Males vibrated less in the webs of early subadult than late subadult females (50% versus 92%; proportion test:  $\chi^2 = 4.75$ ,  $P = 0.03$ ; Fig. 2b) but the latency to vibrate did not differ (Mann–Whitney  $U$  test:  $U = 49$ ,  $P = 0.97$ ; Fig. 2e). Males that entered webs of the early subadult females vibrated for only a few seconds, touched the females and then stayed in the webs and did not attempt to mate. Males did not differ in the latency to contact the web (Mann–Whitney  $U$  test:  $U = 111$ ,  $P = 0.54$ ; Fig. 2e) or the body (Mann–Whitney  $U$  test:  $U = 86$ ,  $P = 0.69$ ; Fig. 2e) of early ( $N = 22$ ) versus late ( $N = 13$ ) subadult females.

#### Young versus Old Adult Females

In three of 38 trials (7.9%), the unchosen female (three old adults) did not mate with a control male and these trials were not



**Figure 1.** Number of females chosen by males according to their developmental stage: adult versus subadult females, early versus late subadult females and young versus old adult females. \* $P < 0.05$ .

included in the analyses. None of the males showed a preference for adult females based on their age (binomial test:  $N = 35$ ,  $P = 0.17$ ; Fig. 1). None of the other male behaviours differed in relation to female age (proportion tests on occurrences: adding silk:  $\chi^2 = 0.07$ ,  $P = 0.79$ ; cutting silk:  $\chi^2 = 1.3$ ,  $P = 0.25$ ; vibrating:  $\chi^2 = 0.09$ ,  $P = 0.76$ ; Fig. 2c; Wilcoxon tests on latencies: adding silk:  $W = 119.5$ ,  $P = 0.66$ ; cutting silk:  $W = 86$ ,  $P = 0.25$ ; vibrating:  $W = 46$ ,  $P = 0.90$ ; Fig. 2f). The latency to contact the female's web did not differ significantly between groups (Wilcoxon test:  $W = 136$ ,  $P = 0.48$ ; Fig. 2c), nor did the latency to body contact (Wilcoxon test:  $W = 97$ ,  $P = 0.48$ ; Fig. 2f).

#### Single Female Tests

Within 30 min, all but five of 41 males (12.2%) entered a box with a female. After contacting the web, all males ( $N = 36$ ) began to court and remained with the female during the observation time. There were no significant differences for males' latencies to contact the female's web (Mann–Whitney  $U$  test:  $U = 140.5$ ,  $P = 0.29$ ) or the female's body (Mann–Whitney  $U$  test:  $U = 118$ ,  $P = 0.71$ ) when presented with either an adult ( $N = 18$ ) or a late subadult ( $N = 18$ ). As in the two-female trials described above, when presented with late subadult females, males performed significantly less silk adding (16.7% versus 100%; proportion test:  $\chi^2 = 22.4$ ,  $P < 0.005$ ) and silk cutting (5.6% versus 83.3%; proportion test:  $\chi^2 = 19.00$ ,  $P < 0.005$ ) than when presented with adult females, but vibrated more (94.4% versus 33.3%; proportion test:  $\chi^2 = 12$ ,  $P < 0.005$ ). Latencies to vibrate did not differ between the groups (Mann–Whitney  $U$  test:  $U = 22$ ,  $P = 0.51$ ).

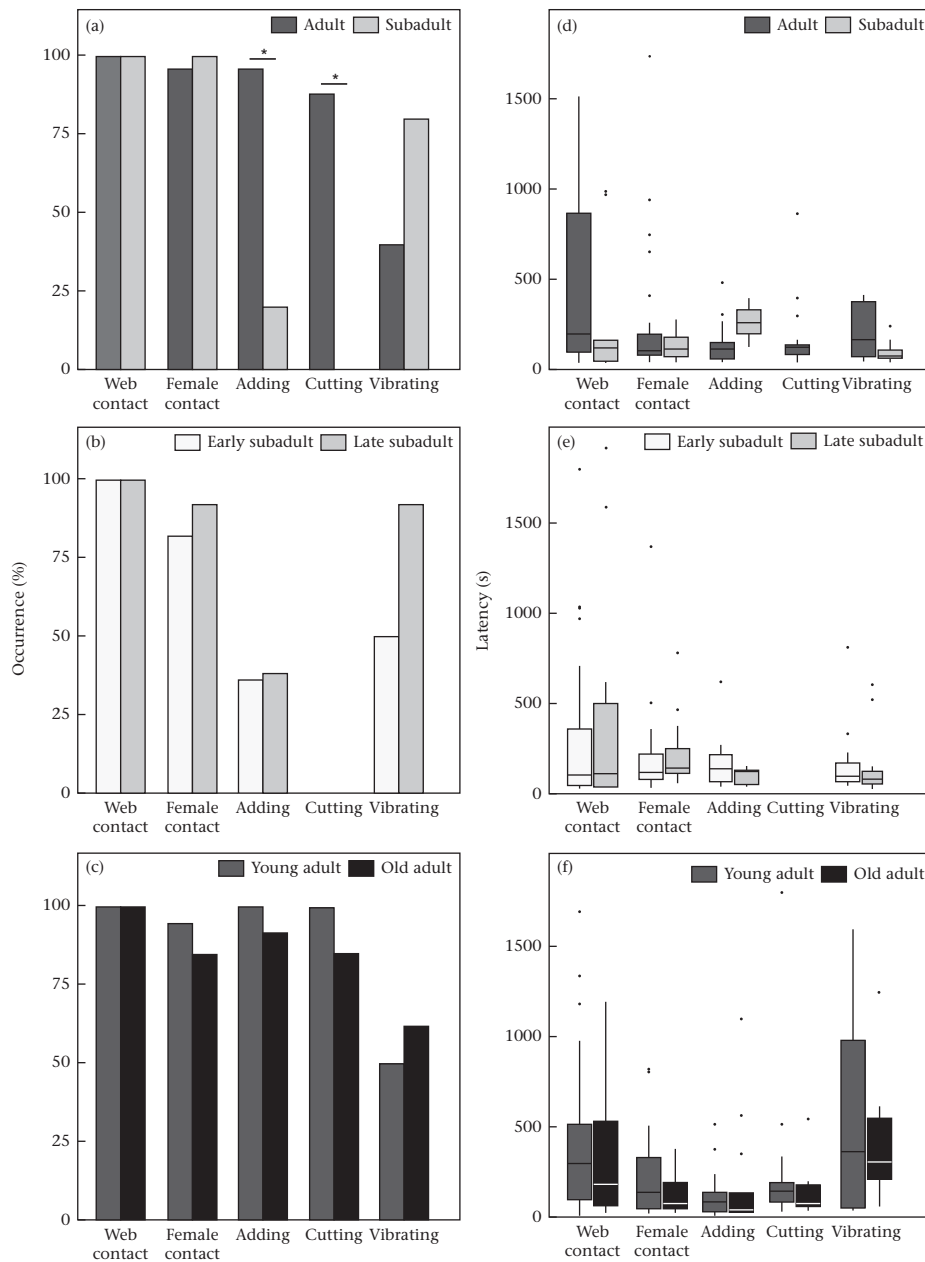
## DISCUSSION

Male *L. geometricus* mated with late subadult females, which would seem to be highly advantageous for the male due to the reduced courtship combined with consistently successful copulation with both palps and lack of sexual cannibalism (Biaggio et al., 2016; Waner et al., 2018; this study). However, when we presented males with late subadult and adult females simultaneously, they typically chose adult, cannibalistic females over the noncannibalistic subadult females. Males showed this preference even when we controlled for web age, a confounding factor that might have affected their choice for adult females observed in a previous study (Waner et al., 2018).

#### Sexual Attractants

Male *L. geometricus* might not recognize subadult females as potential mates due to a lack of sex pheromone (Waner et al., 2018). For example, Fisher et al. (2018) suggested that subadult females of the false black widow spider, *Steatoda grossa*, do not produce a sexual-attractant pheromone. However, male widow spiders are often found on webs of subadult females in nature (Y. Lubin, personal observation). Furthermore, males readily approached the subadult females in their webs, when we exposed the males to late subadult females against an empty control and they did so as rapidly as when they approached adult females. These observations suggest that even before the final moult females produce cues that act as sexual attractants by which males recognize subadult females as potential mates.

In our experiments, we found no evidence that the age of adult females influenced the male's response in choice tests, contrary to previous findings (Waner et al., 2018) where males preferred older adult females to young adults and late stage subadults. The result of Waner et al. (2018) was probably due to the presence of a stronger pheromone cue that had accumulated in the older web. We also



**Figure 2.** (a–c) Occurrences and (d–f) latencies of male contact of webs and females and of different components of courtship (silk adding, silk cutting and vibrating) towards different developmental stages of females. \* $P < 0.05$ . In (d–f) the box represents the first and third quartile, the horizontal line the median and whiskers the 95% confidence interval; circles represent outliers.

observed no difference in the male's approach towards late subadult females, which are ready to mate, and early subadult females, which are not. This suggests that males cannot identify from a distance whether the subadult females are ready to mate. However, after contacting their webs, males courted the early subadult females only very briefly and then remained in their webs without any further courting, but males immediately courted and then mated with late subadult females. Thus, our observations agree with other studies on spider chemical communication, which suggest that airborne chemicals provide less specific information than chemicals detected by contact with the web (reviewed in Gaskett, 2007; Uhl, 2013). Similarly, when presented with adult and late subadult females, males seemed to recognize the female stage only upon contacting the web because only then did they begin to

add silk when with an adult female or vibrate when with a late subadult female.

The observed preference of males for adult rather than subadult females could be due to quantitative differences in male-attracting signals or to different cues emitted by subadult and adult females. Virgin adult females of many spider species attract males by producing pheromones that signal readiness to mate (Gaskett, 2007; Kasumovic & Andrade, 2004; Riechert & Singer, 1995; Roberts & Uetz, 2005; Stoltz et al., 2007; Uhl, 2013). Subadult mating in *L. geometricus* occurs during a 4-day period before the final moult (late subadult stage). In general, adults seem to produce more pheromone and thus provide a stronger signal than immature females and often only adults produce pheromones (Gaskett, 2007; Uhl & Elias, 2011; Uhl, 2013; Fischer, et al., 2018). Then, the

observed preference for adult females represents rather an attraction to a stronger signal than a preference for a certain stage per se. A few pheromones have been chemically characterized for virgin adult females (e.g. in the genus *Latrodectus*; Jerhot, Stoltz, Andrade, & Schulz, 2010) but none for subadults. Although males often cohabit with subadult females (Jackson, 1986), these females might not produce sex pheromones, and males may identify them by unintentionally produced chemical cues (Fischer, 2019). Therefore, olfactory cues produced by subadult females may be qualitatively different from pheromones of adult females, potentially allowing males to differentiate between the two stages. If these chemicals are distinguishable by males, the choice of adult females over late subadults might indicate that the former are perceived as higher-quality mates, even though mating with them limits males to a single copulation and sometimes even to a single insertion (Segoli, Arieli, et al., 2008).

#### Costs to Males of Mating with Subadult Females

There may be fitness costs to males adopting the subadult mating tactic. After mating, subadult-mated females still have to undergo a final moult to adulthood and may have a lower probability of surviving to oviposition than adult-mated females. The moulting process itself is a sensitive period due to the risk of predation on moulting or freshly moulted spiders, the risk of desiccation or an inability to release the old cuticle (e.g. Horner & Starks, 1972; Jones, 1941; Tanaka, 1984). Thus, males may prefer to mate with adult females due to the overall greater probability of successful reproduction.

Costs of mating with late subadult females could also arise from the specific mating behaviour and the mechanisms of copulation, sperm transfer and sperm storage. When mating with subadult females, *L. geometricus* males do not somersault and are not cannibalized. In the congener *L. hasselti*, cannibalism reduces the likelihood of a female remating (Andrade, 1996). Thus, the lack of cannibalism might lead to a greater probability of remating in subadult-mated females and consequently to paternity loss for the first male. Furthermore, during courtship with adult females, the male removes a large part of the female's web and adds his own silk. Webs of adult female *L. hasselti* that were thus altered by males attracted fewer suitors (Scott, Kirk, McCann, & Gries, 2015), a phenomenon observed also in other web-building species (reviewed in Scott, Anderson, & Andrade, 2018). By contrast, we showed here that webs of subadult-mated females were not altered by the male; male courtship was brief, the web remained intact and the male added little silk. A subsequent male might thus have no indication of a previous visitor. It is unclear whether subadult-mated females remain attractive to males and whether these females will remate after maturing to adults (Biaggio et al., 2016; Waner et al., 2019). Finally, although mating with a subadult female enables the male to seek an additional female, high mortality during mate search (more than 80% of *L. hasselti* males die without finding a mate; Andrade, 2003) may reduce the benefit of such matings.

A male mating with subadult females may have lower paternity than expected for the first male in adult matings due to unfavourable sperm storage conditions or incorrect placement of sperm in the subadult female spermathecae, or to lower competitive ability of his sperm against a second male's sperm. If the internal genitalia of late subadult females are not fully developed, sperm storage conditions may differ from those in adult females and might result in a lower paternity share for a male's sperm when competing with ejaculates of other males. It is possible that the mating plugs cannot be placed correctly or can shift when subadult females moult and thus may be a less effective barrier to remating.

Additionally, the lack of somersaulting while mating with late subadult females may mechanically alter the insertion mechanism and affect where sperm is deposited within the female's reproductive tract. The location of deposited sperm and the storage conditions, together with potentially insufficient plugging in subadult matings, may yield lower reproductive success. In the congener, *L. hasselti*, the first of two males mating with an adult female achieves approximately 80% paternity (Snow & Andrade, 2005). However, the paternity share may differ if a subsequent male inseminates a female mated as subadult after she has moulted. These potential costs of mating with a subadult female can be revealed through paternity assessments in double-mating trials.

Our observation showed that *L. geometricus* males mate with late subadult females, but do not attempt to do so with younger subadults. Despite this, they did not show any preference for late over early subadult females. Cohabiting with subadult females and then mating with them when they moult is known for many spider species (Jackson, 1986) including the widow spiders (Biaggio et al., 2016; Segoli et al., 2006). Although in *L. hasselti* and *L. geometricus* the encounter with late subadult females often leads to immediate mating, cohabiting with early subadult females and waiting for them to mature may be another mating tactic in a male's repertoire. Additionally, it is likely that males cannot determine from a distance subadult females' readiness to mate.

#### Conclusions

When given a choice, male *L. geometricus* preferred to mate with adult over late subadult females. Although males that mate with subadult females survive to mate again, the absence of some courtship components (web reduction) and of cannibalism (triggered by the male somersault) may negatively affect their reproductive success. These behaviours were shown to reduce the remating probability of adult *Latrodectus* females. We hypothesize that in matings with subadult females there are costs arising from unfavourable sperm storage conditions and/or lower survival of females until egg laying compared to matings with adult females. Thus, the lack of male behaviours might represent reduced male investment in the subadult female.

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