Male mate choice in a sexually cannibalistic widow spider

Shevy Waner [a, *], Uzi Motro [a], Yael Lubin [b], Ally R. Harari [c]

[a] Department of Ecology, Evolution and Behavior and Federmann Center for the Study of Rationality, The Hebrew University of Jerusalem, Jerusalem, Israel
[b] Department of Desert Ecology, Ben Gurion University of the Negev, Midreshet Ben Gurion, Israel
[c] Department of Entomology, The Volcani Center, Rishon Lezion, Israel

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Males of the brown widow spider, *Latrodectus geometricus* (Theridiidae), invest energy in courtship displays and are often cannibalized after mating; accordingly, partial sex role reversal is expected. In this species, subadult females are able to mate and produce viable offspring. In contrast to mature females, these subadult females do not cannibalize their mates after copulation. Nevertheless, when given a choice, males preferred mature over subadult females and older over young mature females. We found no benefit for males in mating with the females of their choice. Older females were significantly less fecund than young mature females, and were not more fecund than subadult females. We tested possible advantages in mating with cannibalistic (mature) females, such as an increased probability of plugging the female's genital duct or longer copulations, or disadvantages in mating with subadult females, such as higher remating risk. None of these explanations was supported. Thus, we lack an adaptive explanation for male preference for mature older females. We suggest that older females produce more pheromone to attract males and that males are thus misled into mating with older, more aggressive and less fecund females.

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Both females and males are known to be choosy to different degrees in selecting mates, depending on the species and factors such as the parental investment of each partner (Trivers, 1972), the sex ratio of the available mating partners (Emlen & Oring, 1977) and the variation in quality among available mates (Owens & Thompson, 1994; Parker, 1983).

Even though sexual selection is considered to act more strongly on males, in a variety of taxa there may be a reversed sex role, in which males are choosy and females compete for males (Clutton-Brock, 2009; Eens & Pinxten, 2000), or only partially reversed if males, or both sexes, are selective, but females do not compete for males directly (Edward & Chapman, 2011). For example, Gwynne and Simmons (1990) showed that, when food is scarce, katydid females competed for males and the nutritious spermatophores that they produce. Other notable examples are two wolf spider species, *Allocosa alticeps* and *Allocosa brasiliensis*, in which females actively search for males waiting in their burrows. The females initiate a courtship display at the entrance of the males' burrows and males either accept or reject them (Aisenberg, Costa, González, Postiglioni, & Pérez-Miles, 2010).

Choosy females may gain direct benefits such as paternal care and nutritional nuptial gifts (Lehmann & Lehmann, 2016) and indirectly from 'good genes' (Bertram et al., 2016). Males are expected to benefit from mating selectively if they invest heavily in parental care (Trivers, 1972) or in courtship and mating (Edward & Chapman, 2011; Petrie, 1983; Scharf, Peter, & Martin, 2013), or if female quality varies, such that males are able to distinguish better quality or more fecund females. The benefits for a male of being choosy should outweigh the costs of searching (Edward & Chapman, 2011) and mate assessment (Dewsbury, 1982; Petrie, 1983).

While female preference for males is often based on the male's secondary traits (display and ornaments, Darwin, 1871), in most cases the key factor determining the male's mate choice is the female's potential fecundity (Bonduriansky, 2001; Fitzpatrick, Berglund, & Rosenqvist, 1995). Phenotypic indicators of potential female fecundity (e.g. age, body size, mating status) are often subtle and are assessed by males either directly or by means of pheromones or other cues produced by the females.

Maternal age has been found to influence growth and survival of different fish species, for example. Larvae from older black rockfish, *Sebastes melanops*, grow faster and survive starvation longer than the larvae of younger fish (Berkeley, Chapman, & Sogard, 2004). Larval survival, some disease resistance and larval growth in

* Correspondence: S. Waner, Department of Ecology, Evolution and Behavior, The Hebrew University of Jerusalem, Jerusalem 9190401, Israel.
E-mail address: shevyww@gmail.com (S. Waner).

Atlantic cod, *Gadus morhua,* increase with maternal age (Hansen, Puvanendran, & Bangera, 2015).

Female age is particularly important for relatively short-lived organisms such as most arthropods. In many arthropods, older females are selected against by males, owing to the negative effect on various traits of the offspring. Maternal age has been found to have a negative effect on egg size in Lepidoptera (Wasserman & Asami, 1985) and in the cockroach *Nauphoeta cinerea* (Moore & Harris, 2003). It also has a negative effect on offspring viability, as in *Drosophila serrata* (Hercus & Hoffmann, 2000), and in the oleander aphid, *Aphis nerii* (Zehnder, Parris, & Hunter, 2007).

In spiders, as in many other taxa, female mating status and pattern of male sperm precedence strongly affect male choice (Bonduriansky, 2001). Studies of male mate choice in spiders have found that males favour virgin females, in an attempt to avoid sperm competition. A few of the many examples are St Andrew's cross spider, *Argiope keyserlingi* (Herberstein, Schneider, & Elgar, 2002), the wasp spider, *Argiope bruennichi* (Schulte, Uhl, & Schneider, 2010) and the widow spider, *Latrodectus hesperus* (MacLeod & Andrade, 2014). The first male to mate may avoid sperm competition by plugging the female's genital duct and preventing access by additional males. Plugs are often formed by males adding secretory substances into or over the female's genital opening after sperm transfer (Parker, 1998), but can also be devised by males leaving copulatory organs that break off partly or completely during copulation in the genital duct of the female. Plugging this duct with part of the copulatory organ is common among widow spiders, for example *Latrodectus curacaviensis* (Bhatnagar & Rempel, 1962), *Latrodectus mactans* (Abalos & Baez, 1963) and *Latrodectus revivensis* (Berendonck & Greven, 2002), and occurs also in other spider genera, for example *A. bruennichi* (Nessler, Uhl, & Schneider, 2006) and the nephilid spider *Herennia multipuncta* (Kuntner, Kralj-Fišer, Schneider, & Li, 2008).

Mate choice critically depends on the ability to discriminate between qualities of potential mates. Chemical signalling is a common mode of intersexual communication in many arthropod taxa, which can be used as a means of assessing a mate, as well as being a common form of long-distance sex attractant (Roelofs, 1995; Wyatt, 2003). In many spider species, the female remains in her web while adult males travel in search of mates, while females may communicate with potential mates over long distances. These chemical signals (pheromones) pass through a noisy chemical background, and are often complex and species specific. Males discriminate between virgin and mated females by means of pheromones deposited on the web, on dragline silk or on the female's body surface (Anava & Lubin, 1993; Herberstein et al., 2002; Papke, Riechert, & Schulz, 2001; Perampaladas, Stoltz, & Andrade, 2008; Riechert & Singer, 1995; Roberts & Uetz, 2005; Stoltz, McNeil, & Andrade, 2007). Discrimination may result from quantitative or qualitative differences in pheromones produced by females based on their mating status (Papke et al., 2001; Riechert & Singer, 1995; Stoltz et al., 2007) or postmating inhibition of pheromone production by females (Perampaladas et al., 2008; Riechert & Singer, 1995; Stoltz et al., 2007). For example, male redback spiders, *Latrodectus hasseltii*, were more active on extracts from webs of virgin females than on extracts from webs of just-mated females. Moreover, males were less active on extracts from webs of just-mated females than on extracts from webs of the same females 3 months after they had mated (Perampaladas et al., 2008). Male *L. hesperus* respond differently to silk-borne pheromones extracted from webs of females depending on whether they were well or poorly fed. This may indicate quantitative or qualitative differences in pheromones produced by females, based on their nutrient status, maybe affecting their fecundity (Baruffaldi & Andrade, 2015).

Mate search can be risky and energetically costly. For example, after their final moult vagabond males of many spiders do not catch prey, but rely on nutrients stored as juveniles (Foelix, 2011), increasing the cost of energy depletion and mortality due to exhaustion (Kasumovic, Bruce, Herberstein, & Andrade, 2006). In addition, mortality rates, due to predation of actively searching males, can be relatively high (Kasumovic et al., 2006). Therefore, even though male choosiness decreases the probability of mating with inappropriate females, it is also likely to increase searching time, and thereby male mortality rate (Kasumovic et al., 2006). Accordingly, males are expected to discriminate a female's quality from a distance (Stoltz et al., 2007).

Another reason to be choosy is the risk of sexual cannibalism. This is a widely documented phenomenon in spiders (Prenter, MacNeil, & Elwood, 2006), occurring in many genera, including widow spiders (*Latrodectus*, Theridiidae; Andrade, 1996; Biaggio, Sandomirsky, Lubin, Harari, & Andrade, 2016; Harari, Ziv, & Lubin, 2009; Segoli, Arieli, Sierwald, Harari, & Lubin, 2008). In some species, cannibalized males contribute to female reproductive success (e.g. in the fishing spider, *Dolomedes triton*; Johnson, 2001), but this may not be the case generally (Fromhage, Uhl, & Schneider, 2003). The small size of *Latrodectus* males relative to females suggests that they do not provide nutritious benefit to the female; therefore, the parental investment by which the male provides himself as nutrition for the female seems unlikely (Andrade, 1996; Segoli et al., 2008). Male *L. hasseltii* show a somersault behaviour, in which the male places the dorsal surface of his abdomen onto the female's mouthparts during copulation; sexual cannibalism in this species prolongs copulation duration, thereby enabling the male to fertilize more eggs (Andrade, 1996). Similar self-sacrifice behaviour occurs in the brown widow spider, *Latrodectus geometricus* (Biaggio et al., 2016; Segoli et al., 2008). Male *L. geometricus* invest time and energy when courting adult females (Harari et al., 2009; Segoli et al., 2008). Courting activities include vibrating, and removing portions of, the female's web, followed by contact courtship that involves adding threads around the female and vibrating on her (Biaggio et al., 2016; Harari et al., 2009; Knoflach & Van Harten, 2002; Segoli et al., 2008). However, males of both species can also mate with subadult females (females in their final juvenile instar), resulting in the production of viable eggsacs following the females' final moult to adulthood. Courting display towards subadult females is significantly shorter than with adult females; the males do not somersault and are rarely (if ever) cannibalized (Biaggio et al., 2016), and consequently may survive to mate again with another female.

Here we hypothesized that males of *L. geometricus*, when given the choice, would prefer to court and mate with subadult females based on the notable benefits they can obtain. To test this hypothesis, we assessed the courting effort and survival outcome of males introduced to females that varied in their age and reproductive status and tested male mate choice accordingly.

METHODS

Natural History and Mating Behaviour

Latrodectus geometricus (Theridiidae) has a worldwide distribution, found mainly in drier and warmer regions (Garb, Gonzalez, & Gillespie, 2004; Knoflach & Van Harten, 2002; Levy & Amitai, 1983), and is associated with human habitats. Like other species of the genus, it is characterized by an extreme female-biased sexual size dimorphism (Knoflach & Van Harten, 2002; Segoli et al., 2008). Mated females can produce an eggsac as early as 1 week after mating, and multiple eggsacs over their lifetime; spiderlings emerge several weeks after oviposition.

During mating, *L. geometricus* males insert one of their two copulatory organs (pedipalps) into one of the two female genital openings. When mating with a mature female, a male often copulates more than twice, inserting each pedipalp in turn, alternating with bouts of courtship. When mating with subadult females, males usually copulate only once with each pedipalp. During copulation, the tip of the male's embolus (terminal part of the copulatory organ, serving to transfer the sperm into the female's genital opening) may break off and become lodged in the spermatheca (paired female sperm storage organ), potentially acting as a mating plug (Berendonck & Greven, 2002). After mating with subadult females, males were able to successfully mate again (S. Waner, U. Motro, Y. Lubin & A. R. Harari, personal observations). When copulating with a mature female, an *L. geometricus* male may perform a somersault after the first pedipalp insertion, placing his abdomen in front of the female's chelicerae. The female wraps the male and either cannibalizes or discards him. The male sometimes releases himself from the female and resumes courting, and may copulate with the second pedipalp, repeating the somersault after the second copulation.

Rearing Spiders in the Laboratory

Latrodectus geometricus spiders were collected from several playgrounds in central Israel, and a few from the northern Negev (Sde Boker area). All spiders were brought to the laboratory to obtain virgin males and females for the behavioural experiments. Spiders were kept under constant conditions (25 ± 1 °C, 60% relative humidity, 14:10 h light:dark). Those that participated in the experiments were kept individually in plastic containers to avoid their mating. The date of the final moult of each spider was recorded. Individual spiderlings and males were fed with one to two Mediterranean fruit flies, *Ceratitis capitata*, twice a week. Subadult and mature females were fed with five to seven flies twice a week, alternating every other week with one cricket meal per week. Females with eggsacs and their spiderlings were fed with more flies (12–15).

Males that participated in the mating trials were released at the end of the trials, whereas females remained in their containers with their multiple eggsacs, and were fed as described above, until their natural death.

Male Courting Duration and Efforts

We compared the courting behaviour of mature virgin males that were introduced to virgin females belonging to one of three age groups: (1) subadult females, no longer than 1 week before their final moult ($N = 55$), (2) young adult females, no longer than 2 weeks after their final moult ($N = 31$) and (3) older adult females, 2–3 months after their final moult ($N = 27$). Trials were done throughout the year at different times of day. Not all males courted and mated with the female they were introduced to, especially when introduced to subadult females, and we were left with 26, 25 and 27 matings with subadult, young and older adult females, respectively, for the analysis.

Most of the 78 females that participated in our trials (81%) were descendants of the central Israel founders, and only 19% were descendants of the northern Negev spiders. The percentages for the males were 88% and 12%, respectively.

In each trial, a male was introduced onto the female's web at about 3 cm from the female. We measured the male's total courting duration, which is the time from the moment that the male was placed on the female's web until the male's first pedipalp insertion.

To analyse whether the time of day, as well as the female's age group, affected the courting length of the males, we divided the starting time of a courtship into three categories: morning (0800–1200), afternoon (1200–1700) and evening (1700–2200). We tested the effect of both factors using a two-way ANOVA, with Bonferroni post hoc pairwise comparisons. We also tested the effect of the geographical origin of the spiders (females and males) on the courtship length using a two-way ANOVA.

Courting observations were recorded ca. every 3 min. At each observation we recorded the male's distance from the female and his courting activities: web cutting and bundling of web silk, walking along the threads, walking on the female and adding threads around her, mouthing of the female's epigynum, vibrating or standing still. We compared the percentage of time between the different age groups in which the male was engaged in web cutting and adding threads (which are the main courtship activities), using the Kruskal–Wallis *H* test.

Cannibalism and Somersaulting

For each mating, we documented the length of the male's first copulation (the time between the male's first insertion and first extraction of his pedipalp), whether the male somersaulted and whether he was cannibalized. For females of different age groups, we compared the percentage of matings in which males were cannibalized and in which the males somersaulted. We also tested the association of somersaulting and cannibalism, all using Fisher's exact test. Percentages of matings with cannibalism within each age group were also compared between the two geographical origins (using Fisher's exact tests).

For 57 mature females (including several females for which complete courtship information was lacking but copulation data were available), the length of the first copulation was compared depending on whether the male somersaulted or not, using a two-way ANOVA. Since males mating with subadult females generally do not somersault (we only observed one such case), matings with subadult females were not included in this analysis.

Embolus Tips as a Possible Genital Plug

Spermathecae were dissected after the female's natural death and immersed in 5% KOH solution for 8 days, until the cuticular spermathecae were transparent and broken tips of the male pedipalp, if present, could be observed. We then counted the tips (two, one or zero) that were left in the spermathecae. The association between the number of tips and the occurrence of cannibalism was tested, using a chi-square test. Since the test involved some rather small expected frequencies, the chi-square distribution cannot be a reliable approximation, and thus the *P* values were estimated by randomization: 1000 iterations were randomly simulated under the null hypothesis of independence between the number of tips and the occurrence of cannibalism, and the proportion of iterations having a chi-square statistic larger than (or equal to) the observed chi-square was taken as an estimate of the real *P* value.

Mate Choice

We looked for evidence of mate consent when a male was presented to a single female (subadult, young or older adult). The percentage of these presentations in which the pair consented to mate was compared between the three female age groups using Fisher's exact test. Pairs were defined as not mated if the male did not court the female or the male stopped courting a few minutes after introduction.

A choice experiment, in which males could choose between females from the three age groups (subadult, young and older adults) was done outdoors in a closed greenhouse. In eight trials,

each done on a different day, we placed nine females (three of each age group), each in her own plastic container, in a rectangle (ca. 1×1.5 m) with approximately equal distances between adjacent females. Females were placed at random, provided that two females of the same age group were never adjacent. Each container was placed 15 cm above the ground, allowing the female to extend her threads downwards. The females were given 2 h to acclimatize before releasing the males. At dusk, 11 virgin males were released in the middle of the rectangle, at a similar distance from each female. We followed the dispersing males and noted the number of males in each female's web within the first minute after their release, and then after 45, 90 and 135 min from release.

The attractiveness of each female was established in two ways: (1) based on the number of males found within her web in the four observations, giving priority to earlier observations ('males' quantity'), and (2) based on the presence of males in her web within the first 45 min after the release of the males ('male promptness'). Thus the 'male quantity' measure was obtained by first discounting the numbers of males in each female's web by multiplying by 1.0, 0.9, 0.8 or 0.7 for times 1, 45, 90 and 135 min (respectively), and then summing these discounted numbers. The 'male promptness' was a binary measure: 1 if any male was found in the female's web during time 1 or 45 and 0 otherwise.

To compare the attractiveness of each of the three female age groups, we ranked the nine females in each of the eight trials according to the 'male quantity' of each female (i.e. the female having the most male visits is the most attractive, while the least visited female has the lowest rank). For each of the eight trials we summed the females' ranks of each of the three age groups. The eight ranks of each age group were summed again, to obtain the three test statistics (i.e. the total ranks of the subadult, the young and the older adults). The significance of deviations from random distributions was determined by a Monte Carlo randomization (10 000 repetitions). We first compared the subadult to the adult (both young and older) females, and then we compared within the adults, between young and older females. A similar procedure was applied for the 'male promptness' measure.

Reproductive Outcomes of the Male's Mate Choice

To test whether differences in female fecundity can explain the male's choice, we counted the eggsacs, the eggs in each sac and the viable eggs in each sac (determined by the empty eggshells) produced by a female during her lifetime. We collected a full data set for 10 subadult, 18 young adult and 15 older adult females that participated in the male courting time and effort experiment. Comparison was done using a one-way ANOVA, with Tukey's post hoc test.

RESULTS

Male Courting Duration and Effort

Courting duration differed significantly between the three starting time categories (mean ± SE: morning: 2.91 ± 0.56 h, $N = 9$; afternoon: 2.76 ± 0.20 h, $N = 45$; evening: 1.69 ± 0.28 h, $N = 24$; two-way ANOVA: $F_{2,69} = 5.31$, $P = 0.007$; post hoc pairwise comparisons indicated a significant difference in courting duration between all starting times of courtship). More interestingly, courting duration differed significantly between the three female age groups. The mean duration of courting older adult females (3.98 ± 0.34 h, $N = 27$) was significantly longer than courting young adults (2.90 ± 0.38 h, $N = 25$), and courting young adults was significantly longer than courting subadult females (0.63 ± 0.13 h, $N = 26$; two-way ANOVA: $F_{2,69} = 23.46$, $P < 0.001$; post hoc

pairwise comparisons indicated significant differences between all pairs; Fig. 1). There was no effect of geographical origin on courting duration for either females (two-way ANOVA: $F_{1,72} = 0.697$, $P = 0.407$) or males ($F_{1,72} = 0.116$, $P = 0.735$).

The percentage of courting time a male spent cutting and/or bundling the female's web during his courtship display was largest when courting an older adult female ($18.40 \pm 1.66\%$, $N = 27$), intermediate when courting a young adult female ($7.34 \pm 1.73\%$, $N = 25$) and smallest when courting a subadult female ($0.48 \pm 1.70\%$, $N = 26$; Kruskal–Wallis H test: $\chi^2_2 = 41.84$, $P < 0.001$; all three comparisons were significant after a Bonferroni correction; Fig. 2).

The percentage of courting time a male spent walking on the female and adding threads on her was not significantly different between courting an older adult ($19.50 \pm 2.18\%$, $N = 27$) and courting a young adult female ($18.18 \pm 2.27\%$, $N = 25$). Both were significantly larger than when courting a subadult female ($4.85 \pm 2.23\%$, $N = 26$; Kruskal–Wallis H test: $\chi^2_2 = 29.71$, $P < 0.001$; pairwise comparisons with a Bonferroni correction; Fig. 2).

Cannibalism and Somersaulting

Percentages of males cannibalized were 56.7, 47.6 and 0.0% when mating with older, young and subadult females, respectively (Fisher's exact tests: older adults versus young adults: $P = 0.725$; older adults versus subadults: $P < 0.001$; young adults versus subadults: $P < 0.001$; Fig. 3). There were no significant differences in the percentage of males cannibalized between the two geographical origins (central Israel versus northern Negev) for any of the age groups (Fisher's exact tests: $P = 0.509$, $P = 1.000$ and $P = 1.000$ for older, young and subadults, respectively).

Percentages of males that somersaulted were 96.7, 90.5 and 4.0% when mating with older, young and subadult females, respectively (Fisher's exact tests: older adults versus young adults: $P = 0.733$; older adults versus subadults: $P < 0.001$; young adults versus subadults: $P < 0.001$; Fig. 3).

Somersaulting by males almost exclusively occurred when mating with adult females; thus, the following analysis refers only to matings with adult females (young and older combined). None of the nonsomersaulting males were cannibalized, whereas 57.1% of the somersaulting males were cannibalized (Fisher's exact test: $P < 0.001$).

There was no significant effect of female's age group on copulation length (mean ± SE: older adults: 5.77 ± 0.98 min, $N = 31$; young adults: 7.46 ± 0.88 min, $N = 26$; two-way ANOVA: $F_{1,53} = 1.67$, $P = 0.202$). There was a significant effect of somersaulting on copulation length (somersaulting males: 4.97 ± 0.60 min, $N = 45$;
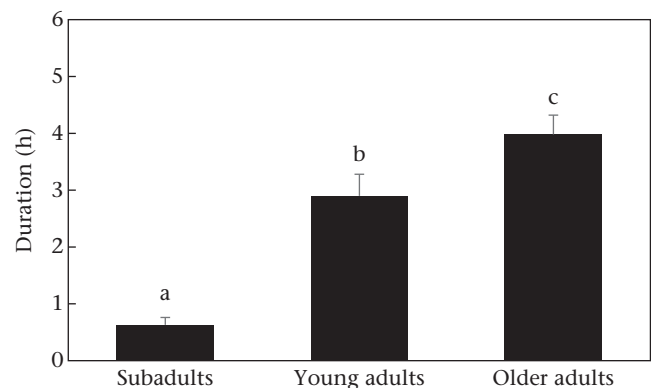
Figure 1. Mean length of courting duration (+SE) as a function of the female's age group. Different letters indicate significant differences.
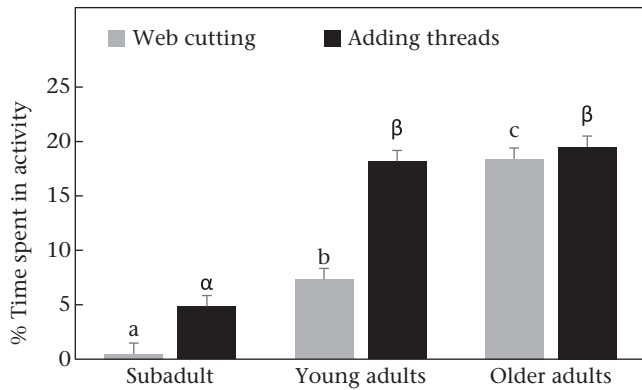
Figure 2. Mean percentage of courtship time (+SE) the male spent cutting the female's web and walking on the female/adding threads around her, as a function of the female's age group. Different letters indicate significant differences within each activity.
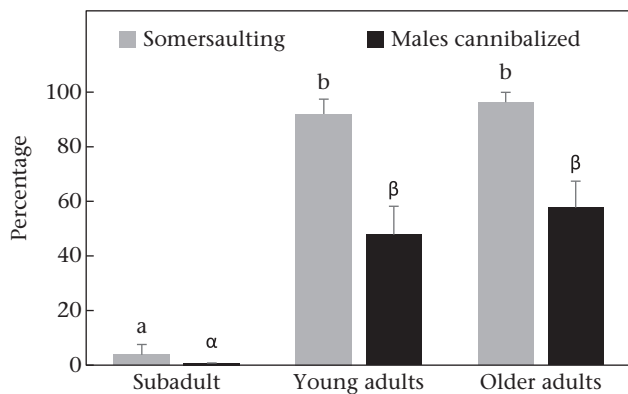
Figure 3. Somersaulting and cannibalism of males. Percentage of matings (+SE) in which the male somersaulted and was cannibalized, both as a function of the female's age group. Different letters indicate significant differences within each factor.

nonsomersaulting males: 8.26 ± 1.17 min, $N = 12$; two-way ANOVA: $F_{1,53} = 6.25$, $P = 0.016$), indicating a significantly shorter copulation length for the somersaulting males. Note that only young and older adults were considered in this analysis as males mating with subadult females did not somersault.

Embolus Tips as a Possible Genital Plug

The distribution of the number of tips remaining in female spermathecae after mating with cannibalized and noncannibalized males is given in Fig. 4. There was no difference in the distribution of number of pedipalp tips left in the females' spermathecae between cannibalized and noncannibalized males ($\chi^2_2 = 0.713$, $P = 0.756$, based on 1000 random iterations).

Mate Choice

No-choice experiment
The percentages of presentations of a male to a single female that resulted in the pair mating were 100, 80.6 and 47.3% for older, young and subadults, respectively (Fisher's exact test: older versus young adults: $P = 0.036$; older adults versus subadults: $P < 0.001$; young adults versus subadults: $P = 0.004$; only the last two comparisons are significant when considering the Bonferroni correction). Thus, adult females were significantly more likely to mate than subadults.
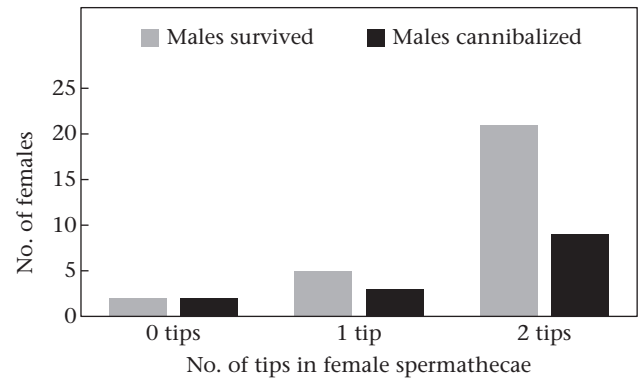
Figure 4. Number of pedipalp tips remaining in the spermathecae of females after mating, in relation to whether the males survived or were cannibalized.

Male choice experiment
Both measures indicated that subadult females were significantly less attractive than adults (male quantity measure: $P = 0.003$; male promptness measure: $P = 0.004$). Among adult females, young adults were significantly less attractive than older adults (male quantity measure: $P = 0.012$; male promptness measure: $P = 0.003$; Fig. 5).

*Reproductive Outcomes of the Male's Mate Choice*

There were significant differences in fertility between the age groups (Table 1). Post hoc comparisons revealed a significant difference between young and older adults in the number of eggsacs, the total number of eggs and the number of fertile eggs. There was no significant difference between young adults and subadults in these three parameters. Between the older adults and subadults there was a significant difference only in the number of eggsacs (Table 1).

DISCUSSION

In this study we found a consistent preference of *L. geometricus* males to mate with mature females versus subadult females. Males particularly preferred older mature females, even though there was a higher energetic cost involved in courting these females, and males were more likely to be cannibalized.

Males can have an advantage in mating with a cannibalistic female if sexual cannibalism leads to an increase in their paternity. This may be achieved through (1) prolonging copulation duration while being cannibalized, thus transferring more sperm to the female (e.g. *L. hasseltii*: Andrade, 1996; *A. bruennichi*: Fromhage et al., 2003), or (2) increasing the plugging probability when cannibalized, by leaving parts of their emboli tips in the female's duct/spermathecae, thus preventing sperm competition (Berendonck & Greven, 2002; e.g. the theridiid *Tidarren argo*: ; Knoflach & Van Harten, 2001; *A. bruennichi*: Nessler et al., 2006; *H. multipuncta*: Kuntner et al., 2008).

In this study, however, *L. geometricus* males that somersaulted while in copula (thereby increasing the risk of cannibalism) in fact copulated for shorter durations than males that did not somersault and were not cannibalized. Thus, the hypothesis that cannibalism increases mating duration is not supported. Furthermore, cannibalized males were not more likely to leave their emboli tips in the female's spermathecae than males that were not cannibalized. Therefore, if tips function as plugs against subsequent inseminations (Berendonck & Greven, 2002), cannibalized *L. geometricus* males did not gain an increased benefit.
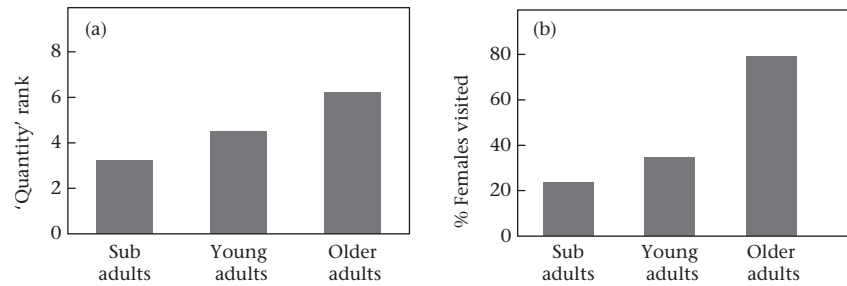
Figure 5. Female attractiveness in the greenhouse experiment. (a) Mean female attractiveness rank according to the 'male quantity' measure (see text) for females in each age group. (b) Female attractiveness according to the 'male promptness' measure (see text), quantified by the percentage of females in each age group that were visited by at least a single male during the first 45 min of the experiment.

Table 1
Fertility of the various female groups (mean ± SE)

	No. of eggsacs	Total no. of eggs	No. of fertile eggs
Subadults (N=10)	17.30 ± 2.12	1446 ± 208	1226 ± 184
Young adults (N=18)	17.67 ± 1.33	1616 ± 142	1496 ± 137
Older adults (N=15)	11.27 ± 1.31	954 ± 161	869 ± 168
ANOVA	$F_{2,40}$=5.88	$F_{2,40}$=4.81	$F_{2,40}$=4.37
	P=0.006	P=0.013	P=0.019
Post hoc			
Subadult vs Young	NS	NS	NS
Subadult vs Older	$P < 0.050$	NS	NS
Young vs Older	$P < 0.010$	$P < 0.050$	$P < 0.050$

In some species of spiders, males avoid cannibalism using different strategies (Prenter et al., 2006; Robinson, 1982) such as copulating with newly moulted females that are less dangerous (Uhl, Zimmer, Renner, & Schneider, 2015), mating during a time of day when they are less aggressive (Moya-Laraño, Pascual, & Wise, 2004) or mating with females that are engaged in eating (Austin & Anderson, 1978). In *L. geometricus* not only do males give up their future reproductive success by being cannibalized, but they also seem to choose the females that are more likely to cannibalize them, and avoid mating with fertile, noncannibalizing subadult females.

Males may benefit from mating with mature females over subadults if the former are more fecund (Bonduriansky, 2001). We found no apparent benefits for *L. geometricus* males in mating with mature females; neither did we find benefits in their preference for older adults: Older, mature females were significantly less fecund than young mature females (they produced fewer eggsacs, fewer total number of eggs and fewer fertile eggs), and were not more fecund than subadult females (they had significantly fewer eggsacs, and the total number of eggs and the number of fertile eggs were also smaller, although not significantly so). Young mature adults and subadult females did not differ in their fecundity outcome. A preference for mature females may be better understood if mated subadult females tend to remate after moulting, diluting the first male's sperm, while mature, mated females refrain from remating. We observed the remating tendency of nine subadult females 1 week after mating and before laying their first eggsac. All had access to virgin, adult males, but none of them remated. While the sample size is small, it does not support the possibility of sperm dilution. Interestingly, the preference for older mature females over young mature females is consistent with male mate choice findings in some other spiders, for example the funnel web spider, *Agelenopsis aperta* (Riechert & Singer, 1995) and *A. bruennichi* (Cory & Schneider, 2016).

Female size may have influenced the males' preference for adult over subadult females. Although spiders' size was not measured in this study, females seemed to grow larger with age. The size of female arthropods is known to affect mate choice of males (Harari, Handler, & Landolt, 1999) and is positively correlated with fecundity (Honek, 1993). The pattern in spiders in unclear. In the sexually cannibalistic orb weaver, *Cyrtophora citricola*, heavier females were preferred by males, and were also more fecund (Yip & Lubin, 2016; Yip, Berner-Aharon, Smith, & Lubin, 2016), whereas male *A. aperta* did not show a preference for larger females (Riechert & Singer, 1995). In our experiments, we did not analyse the effect of size per se on male choice. Previous data, however, suggest that *L. geometricus* males have a weak preference for heavier adult females (I. Sandomirsky, Y. Lubin & A. R. Harari, personal observations).

From the female's standpoint, she should avoid postponing mating, especially when the population density is low, males can be a limiting resource and delaying fertilization can be costly (Kokko & Wong, 2007). As females age, egg maturation progresses and by mating too late, oviposition will take place, even though eggs are unfertilized (e.g. *A. bruennichi*: Cory & Schneider, 2016). Therefore, if no males have appeared by a certain time after maturation, it may be adaptive for females to increase their signalling effort to attract them. This is despite the physiological cost of pheromone production (Cory & Schneider, 2016; Harari, Zahavi, & Thiery, 2011) and extrinsic costs of pheromone emission, such as attracting undesirable predators and parasitoids (Herberstein et al., 2002; Noldus, Potting, & Barendregt, 1991; Zegelman, Hassner, Mendel, & Dunkelblum, 1993), or additional males that cohabit in the female's web and steal her food (Erez, Schneider, & Lubin, 2005; Schneider & Lubin, 1998). Volatile and contact male-attractant pheromones are known to be deposited on the web silk of females in several widow spider species (Anava & Lubin, 1993; Jerhot, Stoltz, Andrade, & Schulz, 2010; Kasumovic & Andrade, 2004).

We suggest that older *L. geometricus* females may release larger quantities of pheromone, regardless of its cost, in the attempt to lure males for mating. Subadult females, may refrain from producing pheromones altogether.

The intensity of pheromone production may be a function of the amount of pheromone produced per unit length or mass of silk, or simply the total quantity of pheromone-bearing silk laid by the female. Neither of these could be measured in our experiments. In our no-choice experiment, nearly half of the subadult females paired with virgin males did not mate. It is unclear whether the subadult females were not 'recognized' as potential mates, possibly due to a lack of pheromone production. There is mixed evidence from other spider species regarding male attraction to silk of subadult or juvenile individuals. In the congeneric *L. revivensis*, males responded with courtship behaviour to silk from webs of adult females, but not that from webs of subadult females (Anava &

Lubin, 1993). Males of *L. hesperus*, however, reacted positively to webs of immature females, although their behaviour seemed qualitatively less vigorous than to adult females' webs (Ross & Smith, 1979). Similarly, in *A. bruennichi* no sex pheromones were detected on subadult female webs (Chinta et al., 2010), whereas in the wolf spider, *Lycosa longitarsis*, female sex pheromone was detected in subadult female nests (Lizotte & Rovner, 1989).

Finally, if older, mature females indeed produce more pheromone, it is possible that males are duped into mating with them. There are conflicting views regarding honest communication through signalling in the animal world; one view asserts that animals can manipulate others (Krebs & Dawkins, 1984), while the other approach considers signals as honest indicators (Zahavi, 1975; 1977). In recent years, theoretical models (Grafen, 1990) as well as behavioural experiments (Harari et al., 2011; Kotiaho, 2002) support the latter view. However, we cannot preclude manipulation as a possible strategy. In some species, males may gain access to females by adopting a parasitic satellite strategy that exploits the reproductive investment made by other males. A few such examples are the synchronizing firefly, *Pteroptyx malacca* (Buck & Buck, 1978), the field cricket, *Gryllus integer* (Rowell & Cade, 1993), or the tree frog, *Hyla arborea* (Brepson, Troïanowski, Voituron, & Lengagne, 2012). There are also examples of females using deceiving strategies. In the long-tailed dance fly, *Rhamphomyia longicauda*, males show a preference for females with larger abdomens. Females in this species swallow air, inflating expandable pouches on their abdomen before entering a lek to compete for males bringing nuptial gifts (Funk & Tallamy, 2000). Here we demonstrated that despite the higher energetic cost, lower reproductive success and, above all, the much higher risk of sexual cannibalism, the male *L. geometricus* prefer to mate with older females over subadults. Although we have suggested some possible solutions for this peculiar behaviour, the enigma has not yet been resolved.

Acknowledgments

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