**Hogna radiata** males do not deplete their sperm in a single mating

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Abstract. To the extent that sperm production or mating opportunities are limited, males are expected to allocate their sperm optimally, so as to increase their overall fitness. Among spiders, sperm depletion and monogyny are known to be optimal male mating decisions either under strong sperm competition or as terminal investment strategies, when future mating opportunities are limited. In a medium-sized wolf spider, *Hogna radiata* (Latreille 1817), we investigated sperm depletion, terminal sperm investment and the potential for sperm competition in laboratory mating trials in which we allowed males and females to pair sequentially with two mates. Males mated with as many females as they encountered. We found no evidence of sperm depletion or terminal sperm investment, as mating duration and female fitness were unaffected by male mating history or age. Polyandry was rare and did not involve any clear fitness benefit to females, whereas pre-mating sexual cannibalism was a rather common outcome of mating interactions involving inseminated females. Our results indicate that *H. radiata* males are not sperm limited and support the hypothesis that the potential for sperm competition shapes the evolution of sperm allocation in this species. Monandrous females do not incur any fitness cost and could potentially benefit from cannibalizing their prospective mates.

Keywords: Lycosidae, mating systems, monandry, polygyny, sperm competition

Given males’ reduced parental investment, sexual selection theory postulates that males will generally maximize their fitness by mating with as many females as possible (i.e., polygyny: Trivers 1972). Since sperm allocation to a single female would likely reduce male fitness, theory predicts that monogyny will only evolve when males cannot monopolize multiple mates and/or paternal care is necessary (Emlen & Oring 1977). Among spiders, a taxon in which paternal care has never been observed, monogyny is a surprising common mating strategy (Knoflach & Van Harten 2000; Foellmer & Fairbairn 2003; Fromhage et al. 2007). For example, when mating with virgin females, *Nephila clavipes* males deplete their sperm in a single mating and thus cannot mate with a second partner (Christenson 1989; Rittschof 2011). Spider sperm depletion is usually accompanied by other male strategies like genital mutilation or sexual cannibalism (Michalik & Rittschof 2011). For example, *Nephila fenestrata* and *Argiope aurantia* males break off their genital organs during insemination (Fromhage & Schneider 2006; Foellmer 2008), whereas *Latrodectus hasselti* and *L. geometricus* males may sacrifice themselves during copulation (Andrade & Banta 2002; Andrade 2003; Segoli et al. 2008).

Spider monogyny and sperm depletion might be an optimal male mating strategy, at least under particular conditions (Rittschof et al. 2012). Firstly, ejaculate costs may not be trivial, limiting sperm production (Dewsbury 1982; Michalik & Rittschof 2011). For example, *Nephila clavipes* males do not produce sperm during adulthood (Michalik & Rittschof 2011), and female *Pardosa australis* paired with previously mated males are more likely to fail to produce a clutch (Jiao et al. 2011a). Secondly, sperm competition may also promote monogyny (Fromhage et al. 2005; Fromhage et al. 2008). For example, in the false garden mantid *Pseudomantis albomembrata*, sperm transfer during mating increases when males experience higher risks of sperm competition, as a strategy to outcompete rivals’ sperm (Allen et al. 2011). Likewise, breaking off male genital organs and sexual cannibalism may limit female insemination by future prospective mates (Fromhage et al. 2005, 2007, 2008; Fromhage & Schneider 2006). Finally, male-biased mortality may contribute to the reduction of expected value of future mating to males, favoring sperm allocation to the current mate and monogyny (i.e., terminal investment hypothesis) (Andrade & Banta 2002; Segoli et al. 2006).

In this paper we analyze sperm allocation and mating strategies in a wolf spider, *Hogna radiata* (Latreille 1817). We conducted two experiments in the laboratory, using mating duration and female fitness as indirect measures of sperm allocation. The first experiment assessed sperm depletion and sperm terminal investment. In this experiment we allowed males of variable ages to pair sequentially with two unmated females and measured the effect of male mating history and age on mating duration and female fitness. To control for mate encounter rate prior to the experiment we used previously isolated unmated males (simulating a reduced mate encounter rate). We predicted that if males deplete their sperm in a single mating (sperm depletion hypothesis), mated males would mate for less time and/or females mated only to these males would have lower fitness (1). If males show terminal sperm investment, mating duration and reproductive output (measured as female fitness) will increase with increasing male age (2).

The second experiment tested the potential for sperm competition. Because females must mate with more than one partner for sperm competition to occur, we paired females sequentially with two unmated males. If sperm competition is a normal part of the reproductive biology of this species, we predicted that females would usually accept multiple mates and polyandry would relate positively to female fitness (3). Alternative to sperm competition, females may mate multiply to avoid sperm limitation, which might involve an increased likelihood of remating by females experiencing reduced sperm supply (i.e., shorter first mating) (4).
METHODS

The species.—Hogna radiata (Latreille 1817) is a medium-sized European wandering wolf spider. It is very common and widely distributed in the Iberian Peninsula, where it inhabits a variety of terrestrial habitats, from wet to relatively arid sites, dominated by grassy vegetation. In the laboratory, they have a rather short post-embryonic development of ca. 10 months and 10–12 molts (C. Fernández-Montraveta unpubl. data). In central Spain, males and females mature early in summer and mating occurs shortly afterwards. The moderately long-lasting mating includes repeated and alternated insertions of both male emboli into the female genital tract. Females produce an egg sac within a few weeks following mating, while males disappear completely from natural populations only a few weeks following maturation. As in other wolf spiders so far studied, females carry egg sacs and then spiderlings until dispersal. The species is semelparous, but females may produce more than one egg sac during their only reproductive season (C. Fernández-Montraveta unpubl. data). Sexual size dimorphism is only moderately female-biased; females are on average 10% larger than males (C. Fernández-Montraveta unpubl. data).

General procedures.—Male and female H. radiata mating history cannot be properly assessed morphologically, as there are no external signs of fertilization. We therefore investigated male sperm depletion, terminal investment and the potential for sperm competition using laboratory-matured spiders in laboratory mating trials. For experiments, we captured subadults (immature but sexually differentiated spiders cf. Foelix 1996) late in spring near Madrid (central Spain). We used headlamps to capture spiders by hand during the night, which facilitates capture without physical damage. In the laboratory, we housed spiders individually in 1-l plastic containers, keeping the spiders visually isolated. We provided sand as substrate, on top of which we placed a small leaf as refuge. We kept laboratory conditions in a natural light regime provided by artificial lighting and 25 ± 2°C ambient temperature. To ensure humidity, we sprayed water on each spider container twice a week. We reared spiders on a monotypic diet (blowflies) provided three times a week.

We checked molting of each spider on a daily basis until maturation, recognized as complete development of female and male external genitalia. We calculated spider age as the number of days after date of maturation. For each spider, we measured the maturation size as the maximum prosoma width and male external genitalia. We also weighed spiders to the nearest 0.05 mm (SZX9 Olympus microscope with 57x magnification provided with a micrometer). Additionally, we weighed spiders to the nearest 0.1 mg (Mettler Toledo ABS54 electronic balance) before each mating trial and again before oviposition. We kept spiders at the laboratory until their sex was known. In this study, we used 21 spiders (7 males and 14 females).

In Experiment 2, designed to test the potential for sperm competition, we prepared for a mating trial, we introduced a mature female H. radiata into the experimental arena and left her there for 24 h, allowing her to lay draglines. Wolf spider female draglines are known to contain silk-bound sex-pheromones that elicit a remarkably strong courtship response by conspecific males (Tietjen 1977; Fernández Montraveta & Ruano Bellido 2000). After checking visually for the presence of draglines, we gently placed a randomly chosen male ca. 10 cm from the female. We videotaped male and female behaviors (JVC TK-C621 video camera, JVC SVHS HR-S7000 video recorder, FOR-A VTG 55 video timer) for a maximum of 30 min if courtship and/or mating did not occur, otherwise until mating was over. Following mating, we checked females daily, noting the day of egg sac production and spiderling hatching, the egg sac weight, and the number of spiderlings hatching from the egg sac.

Experimental design.—We conducted two independent mating experiments. Experiment 1 used unmated females to test for sperm depletion (1) and terminal investment (2). Specifically, we tested whether, compared to unmated males, mated males mated for less time and/or had lower fitness (Prediction 1) and whether older males mated for longer and experienced an increased reproductive output, measured as female fitness (Prediction 2). To manipulate male mating history, we tested each male twice, first as unmated and again several days following mating, paired with different females. Male age varied randomly. Because female mating history may affect the female response to courting males, we only used unmated females in this experiment. To control for the likely effect of hunger on the female response to courting males, and particularly on sexual cannibalism (Wilder & Rypstra 2008), we always provided females with food 24 h before each mating trial. Female age may also affect the female response to courting males (Wilgers & Hebets 2012), but the delay between consecutive trials prevented controlling for female age. In this experiment we used 21 spiders (7 males and 14 females).

In Experiment 2, designed to test the potential for sperm competition, we tested predictions 3 and 4. Particularly, we measured whether polyandry was a common output of mating interactions and related positively to female fitness (Prediction 3) and whether the likelihood of female remating increased following relatively short first matings (Prediction 4). We allowed each female to mate twice. In this experiment we controlled female age. We only used 16 to 18 day-old virgin females for the first mating and re-mated females 5 d afterward. We also controlled for male and female hunger by providing spiders with food 24 h before being used in a trial and presented a single unmated male to every female per trial. In this experiment we used 39 spiders (13 females and 26 males) in all.

For mating trials, we measured the interaction outcome (courtship, mating, or sexual cannibalism) and the courtship and mating durations. Also, we estimated fitness from 1) female mass gain from mating to oviposition, 2) egg sac production, 3) number of egg sacs produced, 4) egg sac weight, 5) time to egg sac production (days elapsed since mating until the production of the first egg sac) and 6) hatching success. Lastly, we calculated (7) the developmental time inside the egg sac as the number of days from egg sac production to spiderling hatching, and (8) the number of hatched spiderlings.
Table 1.—Results of Experiment 1, summarizing male and female body mass and size and female mass gain from mating to oviposition, courtship and mating duration and several estimates of female fitness (mean ± SE), depending on the male mating condition (mated, unmated). Results of statistical tests, including $P$ values. Significant differences in bold.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Male condition</th>
<th>Test statistic</th>
<th>Value</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unmated</td>
<td>Mated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female body size (mm)</td>
<td>6.7 ± 0.4</td>
<td>7.2 ± 0.03</td>
<td>$X^2_1 = 1.9$</td>
<td>0.2</td>
</tr>
<tr>
<td>Female mass gain (g)</td>
<td>0.22 ± 0.03</td>
<td>0.32 ± 0.03</td>
<td>$X^2_1 = 4.6$</td>
<td>0.03</td>
</tr>
<tr>
<td>Male body mass (g)</td>
<td>0.3 ± 0.04</td>
<td>0.3 ± 0.03</td>
<td>$F_{1,6} = 0.1$</td>
<td>0.9</td>
</tr>
<tr>
<td>Male age (days)</td>
<td>15 ± 4</td>
<td>30.7 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Courtship duration (min)</td>
<td>12.6 ± 4.2</td>
<td>8.8 ± 1.9</td>
<td>$F_{1,6} = 0.8$</td>
<td>0.4</td>
</tr>
<tr>
<td>Interaction outcome (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating</td>
<td>100</td>
<td>100</td>
<td>Fisher exact</td>
<td>0.2</td>
</tr>
<tr>
<td>Sexual cannibalism</td>
<td>0</td>
<td>28.6(2)</td>
<td>Fisher exact</td>
<td>0.6</td>
</tr>
<tr>
<td>Mating duration (min)</td>
<td>31.2 ± 2.2</td>
<td>26.6 ± 6.1</td>
<td>Wilcoxon signed</td>
<td>0.6</td>
</tr>
<tr>
<td>Number of egg sacs (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>71.4(5)</td>
<td>57.1(4)</td>
<td>Fisher exact</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>28.6(2)</td>
<td>42.9(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to egg sac production (days)</td>
<td>24.3 ± 1.1</td>
<td>16.4 ± 1.5</td>
<td>$F_{1,12} = 17.1$</td>
<td>0.01</td>
</tr>
<tr>
<td>Egg sac weight (g)</td>
<td>0.21 ± 0.03</td>
<td>0.28 ± 0.03</td>
<td>$F_{1,12} = 2.3$</td>
<td>0.1</td>
</tr>
<tr>
<td>Hatching success (%)</td>
<td>71.4(5)</td>
<td>71.4(5)</td>
<td>Fisher exact</td>
<td>0.7</td>
</tr>
<tr>
<td>Time to cocoon hatching (days)</td>
<td>29.8 ± 0.5</td>
<td>30.8 ± 1.3</td>
<td>$F_{1,6} = 0.5$</td>
<td>0.5</td>
</tr>
<tr>
<td>Spiderling number</td>
<td>115.8 ± 15</td>
<td>155.4 ± 54.6</td>
<td>$X^2_1 = 0.6$</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Statistical analyses.**—In Experiment 1, we used mating duration and female fitness as estimates of sperm allocation, and analyzed whether any of these parameters varied, depending on the male mating history and age. In Experiment 2, we measured multiple mating by females, its fitness consequences and its relationship with first mating duration as a proxy measure of sperm supply. We present quantitative data as mean ± 1 SE. We used parametric statistics (General Linear Models) whenever possible. We inspected data visually for outliers, which we excluded from final analyses. We tested for normality (Shapiro-Wilks test), homoscedasticity (Levene test) and model fitting. When data were not homoscedastic we used Generalized Linear Models (Normal Identity link function). We used the Log likelihood ratio, compared to the Chi-square test, to check for statistical significance. When data failed to fit normality we applied non-parametric statistical tests. We used the IBM SPSS 19.0 package (IBM Corp.) for statistical tests.

**RESULTS**

**Sperm depletion and terminal investment.**—In Experiment 1, unmated male age ranged from 6 to 32 d, and time between the two male mating trials was 15.7 ± 1.9 d, representing a long time span. However, males did not experience any significant body mass loss between consecutive mating trials, and females tested with unmated and mated males were also similar in size (Table 1). All experimental females laid draglines on the terrarium surface, and males always courted upon contact with female draglines. Courtship duration did not differ depending on the male mating history, and courtship interactions always led to mating (Table 1). Male mating history did not affect mating duration (Table 1). We observed a few instances of post-mating sexual cannibalism, always in mating trials involving mated males, but the relationship between male mating history and sexual cannibalism was not significant (Table 1).

As expected, females increased their body mass before oviposition, and females paired with mated males gained relatively more mass (Table 1). All females paired in mating trials succeeded in producing an egg sac, and a few females produced two egg sacs. Male mating history did not affect the number of egg sacs produced by females, but females paired with mated males took less time to produce their first egg sac (Table 1). Male sperm allocation was unaffected by male mating history in all measures of female fitness (egg sac weight, developmental time, hatching success and female fecundity; Table 1).

Male age was unrelated to mating duration, both for unmated (Spearman correlation test: $\rho = -0.2$, $P = 0.3$) and for mated males ($\rho = 0.4$, $P = 0.2$). Likewise, there was no significant relationship between male age and female mass gain [Spearman correlation tests: $\rho = 0$, $P = 0.5$ (unmated); $\rho = -0.3$, $P = 0.3$ (mated)] or fecundity [Spearman correlation tests: $\rho = -0.6$, $P = 0.3$ (unmated); $\rho = -0.6$, $P = 0.3$ (mated)], whichever the male mating history.

**Sperm competition.**—In Experiment 2, males started courtship immediately upon contacting a females’ draglines with forelegs or pedipalps. Courtship was significantly shorter in trials involving unmated females (2.9 ± 0.13 min), compared to mated females (12.4 ± 1.7 min, Wilcoxon signed test: $P = 0.02$). Likewise, female mating history affected the female response to male courtship, and polyandry was extremely rare. Courtship interactions involving unmated females always led to mating, compared to only 15.4% of the interactions involving mated females (McNemar test: $P = 0.001$, $n = 13$). Unmated females never attacked males prior to mating, compared to 84.6% ($n = 13$) of the inseminated females. Nearly half of these females cannibalized their prospective
mates (5 out of 11, or 45.4%). A few (15.4%, n = 13) unmated females attacked males following mating, but these attacks never led to sexual cannibalism.

We found no morphological difference between monandrous and polyandrous females, nor between males succeeding or failing to mate with inseminated females (Table 2). Polyandry had no effect on female reproductive output (egg sac production, number of egg sacs produced, or time to first egg sac production: Table 2). Spiderlings hatched from all first egg sacs produced by females. Because only one polyandrous female succeed in reproducing, we could not test further for effects of mating history on female fitness. However, for this female, fitness estimates were within the range of monandrous females, except for spiderling number, which was lower (Table 2). Finally, first mating duration did not differ significantly between monandrous and polyandrous females (Table 2).

**DISCUSSION**

Our experimental results indicate that *H. radiata* males do not deplete their sperm in a single mating, but are capable of mating with more than one female. We found no support for the terminal investment hypothesis, as males in our first experiment effectively inseminated both females they encountered, in spite of increasing age and prior mating experience. Males who mated more than once therefore had an advantage, and we conclude that this species shows a potentially polygynous mating system. Moreover, we kept spiders isolated except for mating trials, and males would have experienced an natural conditions. Timing of reproduction is known to negatively affect spider reproductive success (Rittschof et al. 2012), and the value of future mating is expected to decrease as the males get older. Moreover, we kept spiders isolated except for mating trials, and males would have experienced an extremely low mate encounter rate (roughly a single female every two weeks). If terminal investment shapes male mating strategy, both getting older and experiencing a reduced mate allocation of increasing age and prior mating experience. Males who mated more than once therefore had an advantage, and we conclude that this species shows a potentially polygynous mating system.

Moreover, and contrary to the sperm depletion hypothesis, females mating with mated males took less time to produce an egg sac and gained more body mass before oviposition than those mating with unmated males. We interpret the increased mass gain as indicating that mated males had supplied resources other than sperm during mating, particularly nutritional resources. In this experiment, some females mating with mated males cannibalized their partners, and sexual cannibalism is known to have positive effects on female fecundity in a closely related wolf spider (Rabaneda-Bueno et al. 2008). Nutritional resources supplied by cannibalized males would explain the average increased mass gain experienced by females paired with mated males.

In this experiment, the reduced time to oviposition experienced by females paired with mated males did not necessarily result in a fitness benefit. Second mating by males also occurred extremely late in the reproductive season, and reducing the time to oviposition actually compensated delayed mating and ensured no delayed hatching. Females mating later seem to invest their resources to produce spiderlings earlier, thus limiting any negative effect of hatching time on the likelihood of spiderling survival, which could indicate that female reproduction is time-limited.

We found no support for the terminal investment hypothesis, as there was no evidence that sperm allocation by males depended on the value of future mating. In central Spain, the *H. radiata* mating season ranges from mid-July to early August (C. Fernández-Montraveta, unpubl. data), and the age at which we tested males in laboratory mating trials (up to 43 d) greatly exceeded the time at which males survive under natural conditions. Timing of reproduction is known to negatively affect spider reproductive success (Rittschof et al. 2012), and the value of future mating is expected to decrease as the males get older. Moreover, we kept spiders isolated except for mating trials, and males would have experienced an extremely low mate encounter rate (roughly a single female every two weeks). If terminal investment shapes male mating strategy, both getting older and experiencing a reduced mate allocation of increasing age and prior mating experience. Males who mated more than once therefore had an advantage, and we conclude that this species shows a potentially polygynous mating system.

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encounter rate should have affected sperm allocation, with males increasing the sperm allocated to their current partner the older they get. No such trend appeared in our experiments.

Besides terminal investment, spider monogyny has been also related to sperm competition (Fromhage et al. 2008), with reduced potential for sperm competition in polygynous species. As predicted under this hypothesis, we found no evidence for sperm competition in *H. radiata*. The only polyandrous female reproducing in our experiment produced two egg sacs, but spiderlings did not hatch from the second egg sac. Moreover, the number of spiderlings produced by this female was actually not above, but below the range of values representing the reproductive output of monandrous females. Again, a high percentage (roughly 50%) of monandrous females in Experiment 2 cannibalized their second prospective mate, and sexual cannibalism could improve female reproductive output more than polyandry. The likely relationship between *H. radiata* sexual cannibalism and female fitness requires further testing.

If sexual cannibalism has a positive effect on female fitness, and a single mating supplies sperm enough to fertilize female eggs, then mating with a single male and cannibalizing all prospective mates would be the optimal reproductive strategy from the female point of view. This interpretation would indicate that *H. radiata* monandry is female-driven, as has been recently demonstrated in a closely related wolf spider (Jiao et al. 2011b). In our experiment, most females followed this strategy, and only a tiny portion mated twice. In species in which polyandry is female-driven, multiple mating by females has been related either to sperm competition or to sperm supply, which is demonstrated by a negative relationship between multiple mating and first mating duration (Weldingh et al. 2011). We found no relationship between number of matings and mating duration in *H. radiata*, though first mating duration by polyandrous females represented, on average, roughly half the mean mating duration by monandrous females. Again, lack of significant differences might relate to our small sample size, and we cannot completely discard the hypothesis that *H. radiata* multiple mating relates to lack of sperm supply.

In our experiments we used three indirect estimates of sperm allocation, based on male behavior (i.e., courtship duration, mating occurrence and mating duration), but did not measure sperm transfer directly. The relationship between spider mating duration and sperm transfer is complex (Schneider et al. 2005; Wilder and Rypstra 2007; Herberstein et al. 2011). For example, higher mating duration might not relate to effective insemination, but to reproductive failure (cf. Jiao et al. 2011a). However, we also included direct estimates of female fitness that pointed in the same direction. We therefore conclude that *H. radiata* shows a female-driven monandrous mating system. The risk of potential sperm competition seems to be low, which favors a polygynous mating system. Sexual cannibalism probably improves female fitness more than polyandry, which could relate to sperm supply. These possibilities need further investigation.

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LITERATURE CITED


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