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Polyandry in the absence of fitness benefits in a species with female-biased sexual size dimorphism

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Abstract

Most studies exploring the evolution of female mating systems focus on species in which females are either monandric (mate with a single male) or highly polyandric (mate with multiple males), but less is understood about variation in mating decisions within a species. How and why do females of a single species decide whether or not to copulate with additional mates? In this study we attempt to answer this question in the highly dimorphic crab spider, *Mecaphesa celer*, whose females may be either monandric or polyandric. We tested three hypotheses: (1) a female's decision to remate is based on sequential mate choice; (2) a female's decision to remate has fitness consequences, with polyandry providing increased benefits; and (3) mating order predicts male paternity, following a pattern of first-male sperm precedence. We conducted double-mating trials between females and males of varied sizes and age, quantified six putative fitness benefits obtained by monandric and polyandric females, and tested sperm precedence patterns using the sterile male technique. We found no evidence that female *M. celer* are performing sequential mate choice. Moreover, we found no difference in fitness between monandric and polyandric females. Finally, we found that paternity in polyandric females follows a pattern of sperm mixing. *Mecaphesa celer* females' decisions regarding mating with multiple males do not appear to be influenced by comparisons of male attributes or by future fitness benefits. We recommend future studies examining male ejaculate components that might influence female mating decisions.

Keywords: female mating strategy, mating system, *Mecaphesa celer*, offspring survival, protandry, Thomisidae

Female mating strategies select for traits that extend far beyond the conspicuous secondary sexual characteristics typically associated with sexual selection (Bocedi & Reid, 2016; Boulton & Shuker, 2015; Kvarnemo & Simmons, 2013; Pizzari & Wedell, 2013; Yasui & Garcia-Gonzalez, 2016). The number of mates a female accepts is expected to depend on the costs and benefits of each additional copulation (Emlen & Oring, 1977; Hubbell & Johnson, 1987). These costs and benefits are often mediated by the males' mating strategies (Parker & Birkhead, 2013), which in turn are affected by the females' decision to mate multiply, leading to complex evolutionary feedbacks between the sexes (Kvarnemo & Simmons, 2013; Wade & Arnold, 1980). To understand the evolution of female mating strategies we must thus explore not only the costs and benefits females obtain from copulations, but also how their mating decisions mediate, and are mediated by, the mating strategy of the males.

For females, the costs of copulating with multiple partners (i.e. polyandry) include reduced reproductive success, infanticide (e.g. Maklakov, Bilde, & Lubin, 2005; Schneider & Lubin, 1997), physical harm

(e.g. Blanckenhorn et al., 2002) and decreased female longevity (Arnqvist & Nilsson, 2000), among others. Given that monandrous females (i.e. females mated to a single male) receive sperm from only one male, monandrous species are often characterized by high levels of precopulatory mate choice and/or male–male competition (Emlen & Oring, 1977; Kvarnemo & Simmons, 2013). Benefits of polyandry are also varied (see reviews by Arnqvist & Nilsson, 2000; Kvarnemo & Simmons, 2013), and include increased female longevity and reproductive success (Wagner, Kelley, Tucker, & Harper, 2001; Worthington & Kelly, 2016), increased genetic variability of the brood (Zeh & Zeh, 2001), larger and faster-growing offspring (Watson, 1998) and/or sequential mate choice through acquisition of sperm from more attractive and/or genetically more compatible males (trading-up strategies: Jennions & Petrie, 2000; Schneider & Elgar, 1998; Watson, 1991). Benefits of polyandry are likely to be influenced by sperm precedence patterns (Elgar, 1998; Simmons, 2005) and may lead to cryptic female choice or strategic mating decisions in terms of order of mating partners (Kvarnemo & Simmons, 2013; Simmons & Beveridge, 2010).

Male mating strategies may evolve in response to the intensity of postcopulatory competition and to the species' sperm precedence pattern (Wade & Arnold, 1980; Simmons & Beveridge, 2010, and references therein). Males from species with first-male sperm precedence, for instance, may develop faster and compete over access to virgin females (Dodson & Beck, 1993; Huber, 2005; Kasumovic & Andrade, 2009; Singer, 1982; Zonneveld, 1996). This earlier male development (protandry) often results in a male-biased operational sex ratio and female-biased sexual size dimorphism (Danielson-François, Hou, Cole, & Tso, 2012; Vollrath, 1998; Vollrath & Parker, 1992; but see Legrand & Morse, 2000; Wiklund, Nylin, & Forsberg, 1991). In species with some degree of sperm mixing, however, polyandry may hinder the benefits obtained by early-matured males (Birkhead & Møller, 1998).

Much theoretical and empirical research has been conducted on species where females are predominantly monandric or polyandric, but less attention has been paid to variation in mating decisions within a species (but see Boulton & Shuker, 2015). How do females of a single species decide whether or not to copulate with additional mates? Why do some females in a population exhibit monandry while others exhibit polyandry?

Females of the flower-dwelling crab spider *Mecaphesa celer* (family Thomisidae) appear to be both monandrous and polyandrous: some females accept only one partner over their life, but others remate with a second male (Chelini & Hebets, 2016). The temporal window of receptivity to additional copulations is small in this species, and females become increasingly aggressive towards males following their initial copulation. While approximately 85% of females are willing to remate immediately after their first copulation (M.-C. Chelini, personal observation; $N = 40$), only 15% of the females are willing to remate after 2 days, and approximately 5% of the females still remate after 4 days (Chelini & Hebets, 2016). In contrast to what we might expect in a monandrous mating system, *M. celer* females do not exhibit any form of mate choice while virgin (Chelini & Hebets, 2016; see also Morse, 2009), and, unlike other closely related crab spiders (Dodson & Schwaab, 2001), males do not appear to enter into direct contests (M.-C. Chelini, personal observation). Additionally, *M. celer* males are known to mate with multiple females if given the opportunity (Chelini & Hebets, 2016; Muniappan & Chada, 1970).

Mecaphesa celer crab spiders are also sexually dimorphic, with females approximately twice the body size of males (Dondale & Redner, 1978) and often weighing more than 10 times the average male mass (Chelini et al., n.d.). The female-biased sexual size dimorphism observed in *M. celer* appears to be at least partly driven by early male maturation (Muniappan & Chada, 1970; see also Danielson-François et al., 2012; Maklakov, Bilde, & Lubin, 2004). It has been demonstrated that female and male body sizes do not influence the likelihood of first copulations, as all virgin females mate with the first male that they encounter (Chelini & Hebets, 2016).

In this study we ask three explicit questions. (1) What factors influence *M. celer* females' decision to copulate with a second mate? (2) Do females receive fitness benefits from copulating with a second male? (3) What is the pattern of sperm precedence in this species? Given the lack of female choice in first copulations (Chelini & Hebets, 2016), we hypothesize that a female's decision to remate is based on sequential mate choice (i.e. trading-up hypothesis: Jennions & Petrie, 2000; Schneider & Elgar, 1998), and as such depends on the comparison between the first and second male they encounter (Hypothesis 1). We also hypothesize that polyandry provides fitness benefits to females (Hypothesis 2). Finally, given the observed early male maturation (Muniappan & Chada, 1970), we hypothesize that male paternity is dependent on mating order (Hypothesis 3) and predict a pattern of first-male sperm precedence.

Methods

Animal Collection and Maintenance

We collected male and female *Mecaphesa celer* as juveniles and subadults (fifth–seventh instar) at Holmes Lake Park, Lincoln, Nebraska, U.S.A. in June 2014. In the laboratory, we housed spiders in individual $4 \times 4 \times 6$ cm acrylic cages with the internal walls covered in plastic netting, allowing spiders to climb and perch. All spiders were in a room held at 26°C and 60% relative humidity, under a 14:10 h light:dark cycle, with *ad libitum* water. We fed all individuals two juvenile crickets (*Acheta domesticus*, 1 mm, Ghann's Cricket Farms, Augusta, GA, U.S.A.) twice a week and recorded the date of all molts, including the date of maturity. No ethical approval was required for the study.

Hypothesis 1: Female Decision to Remate Is Based on Sequential Mate Choice

Predictions

We tested the following predictions of hypothesis 1.

Prediction 1a: A female's decision to remate is based on the first and second males' body size. If female *M. celer* remate only if the second male is somehow superior to the first male, we predicted that females mated with males of similar size would not remate, while females paired with males of very different sizes would. In the closely related *Misumena vatia* and *Misumenoides formosipes*, larger males tend to win more male–male contests (Dodson & Schwaab, 2001; Legrand & Morse, 2000) and are faster and more agile than small males (Morse, 2014), an advantage likely to be important in a system with sedentary females and intense male mate search. As such, we predicted that the highest remating rates would occur when the second male had a larger cephalothorax width (our chosen measure of body size) than the first male.

Prediction 1b: A female's decision to remate is based on the first and second males' leg length. Despite being extremely sexually dimorphic in body size, female and male *M. celer* have similarly long legs. Prior to copulation, *M. celer* males typically touch the female's abdomen from afar with their first and second pairs of legs. Females respond to this first contact either passively, folding their legs close to their body, or with an attack (Chelini & Hebets, 2016). Longer legs may therefore confer an advantage to males by allowing them to touch the female from further away, minimizing the exposure of their vital body parts to a potentially aggressive female. As such, while both cephalothorax width and femur length are measures of size, they may potentially convey different information to the female.

Prediction 1c: A female's decision to remate is based on the first and second males' age (in days postmaturation). Age is known to affect female receptivity in many species (e.g. Mack, Priest, & Promislow, 2003; Moore & Moore, 2001; Wilgers & Hebets, 2012). In addition, male age may be inversely related to the amount and quality of their sperm (Radwan, 2003; Jones & Elgar, 2004). In the closely related *M. vatia*, older males are more often rejected and cannibalized than younger males (Morse & Hu, 2004). As such, we expected older *M. celer* females to be less receptive to second males than young females, and we expected females to be polyandric when the first male presented to them was older than the second one.

Mating trials and data collection

We conducted a total of 77 double mating trials during 8–29 July 2014, from 1000 to 1700 hours. We measured all spiders with a caliper immediately postmaturation (at least 3 days prior to a trial). We then paired our spiders in a manner such that females over the entire size range were paired with all possible combinations of first and second males' sizes. Given the small size of these animals and the difficulty of accurately measuring them while alive, we conducted our statistical analyses using measurements taken under the microscope after all spiders were sacrificed (see Female and male size quantification, below). There was no difference in the average size of first and second males (t test: $t_{127.564} = -0.6136$, $P = 0.5406$).

Trial arenas were cylindrical 12×7 cm (diameter \times height) acrylic tubes. We placed an artificial plant composed of a ramified plastic stem with two flowers set 3 cm apart in the center of the arenas prior to each trial, fixing them in a 1 cm^3 piece of plasticine. To observe the spiders' behavior on the underside of the flowers without disturbing the focal individuals, we placed each arena on top of a small square mirror set on top of a rotating platform (20 cm diameter).

We transferred females gently from their cages to the top of the artificial flower using a soft paintbrush, and allowed them to acclimate for 20 min before introducing males. All females were virgin and had matured in the 3–25 days prior to being tested. We transferred males in the same manner and placed them at the base of the artificial flower, at least 5 cm away from the females. Males typically climbed on the flower stem immediately, quickly reaching the female.

As with the females, all males matured in the laboratory and were virgin. *Mecaphesa celer* males mature much earlier than females (Muniappan & Chada, 1970), so mating trials were run 15–50 days after male maturation. Our trials ran for 30 min, or until copulation ended for trials in which mating occurred. We cleaned arenas and mirrors with 70% ethanol and used new pieces of plasticine for each trial to eliminate potential chemical cues.

Immediately following the end of each initial trial (i.e. after 30 min when no copulation occurred, or after the female and the male had stopped copulating and distanced themselves), we left females alone in the arenas for 2 h and then introduced a second virgin male using a soft paintbrush. The pair was then allowed to interact for 30 min or until copulation ended. For each initial and second mating trial, we recorded the occurrence of copulation and aggressiveness of females towards males (e.g. attacking, biting, killing).

Female and male size quantification. Female mass in spiders may vary rapidly according to their feeding schedule, but measurements of structural size, such as cephalothorax width or leg length, are fixed at maturity. As such, cephalothorax width is the most commonly used proxy for size in studies with sexually size dimorphic spiders (Foellmer & Moya-Laraño, 2007). After sacrificing all adult individuals by freezing (the most ethical method of sacrificing spiders) and transferring them to 75% ethanol, we photographed them using a Spot Flex digital camera (Model 15.2 64 MP, Diagnostic Instruments, Inc., Sterling Heights, MI, U.S.A.) mounted on a Leica DM 4000 B microscope. Cephalothorax width and femur length of all adults were then measured on the photographs using the software ImageJ (National Institutes of Health, Bethesda, MD, U.S.A., <http://rsbweb.nih.gov/ij/>).

Statistical analyses

Prediction 1a: A female's decision to remate is based on the first and second males' body size. To test this prediction we used a binomial generalized linear model (GLM) with logit link function to account for the binomial dependent variable (remated/nonremated). We used

female, first and second male cephalothorax width and the interaction between these three variables as independent variables and conducted a stepwise regression to select the simplest and best-fit model possible. To minimize the number of parameters involved in the model, we repeated this analysis using multiple indexes of similarity between first and second males' sizes (first male/second male; first male – second male) and indexes adapted from two indexes of sexual size dimorphism (Lovich & Gibbons, 1992; Smith, 1999). All analyses yielded results similar to the GLM described above (data not shown), and thus we report only the GLM including female, first male and second male cephalothorax width.

Prediction 1b: A female's decision to remate is based on the first and second males' leg length. We tested for the effect of male leg length with an approach identical to that of Prediction 1a, but replacing female, first male and second male cephalothorax width with femur length.

Prediction 1c: A female's decision to remate is based on the first and second males' age (in days post maturation). We tested for the effect of female, first and second male age using the same approach described in Prediction 1a, replacing female, first male and second male cephalothorax width with days postmaturation.

As our trials occurred over 3 weeks, we had a strong correlation between female and male age and the days elapsed along *M. celer*'s reproductive season (which in Nebraska falls between early June and early August; M.-C. Chelini, personal observation). To test for an effect of the timing of trials within the reproductive season, we ran another binomial GLM using trial delay (i.e. number of days elapsed between 14 June, when the first mature female was found, and the trial date) as the only independent variable.

Because of the large window of time during which females were tested, we repeated all analyses included in this manuscript using age as a covariate, but our results remained unchanged (data not shown). We ran the analyses of Predictions 1a–1c using the software R (R Development Core Team, 2009) and the function 'glm'.

Hypothesis 2: Polyandry Provides Fitness Benefits to Females

Predictions and fitness proxies

To test the hypothesis that females accrue reproductive benefits from being polyandric, we collected data on six different proxies of fitness benefits that females could obtain from the time period immediately following copulation until after offspring dispersal.

Prediction 2a: Polyandric females lay eggs faster than monandric females.

Polyandry may benefit females through the transference of nutritious seminal products and/or hormones that accelerate oviposition rate (Perry, Sirot, & Wigby, 2013; see review by Arnqvist & Nilsson, 2000). As such, our first fitness proxy was the number of days between copulation and eggsac laying.

Prediction 2b: Polyandric females' eggs hatch faster than monandric females' eggs.

Following the argument presented above, double-mated females may also benefit from faster-developing embryos, minimizing the time spent caring for each eggsac and optimizing the female's chances of laying multiple eggsacs in a single season. The number of days between eggsac laying and eggsac hatching was therefore our second fitness proxy.

Prediction 2c: Polyandric females have higher lifelong reproductive success than monandric females. Increased reproductive success is one of the most common benefits of polyandry (e.g. Arnqvist & Nilsson,

2000; Fedorka & Mousseau, 2002; McNamara, Brown, Elgar, & Jones, 2007; Newcomer, Zeh, & Zeh, 1999; Wagner et al., 2001). The total number of spiderlings produced by a female over her lifetime and across all of her eggsacs was our third fitness proxy.

Prediction 2d: *Polyandric females have more spiderlings in their first eggsac than monandric females.* Not all females lay multiple eggsacs and food availability is known to influence the number of eggsacs laid by *M. celer* (see Chelini & Hebets, 2016). In the field, food availability is likely to decrease abruptly towards the end of the season (i.e. late summer/early autumn), so females may invest more heavily in their first clutch than in subsequent ones. As such, the putative benefits of polyandry may be quantifiable only in *M. celer* females' first eggsac, making this our fourth fitness proxy.

Prediction 2e: *Polyandric females have higher fertilization success than monandric females.* If mating with more than one male allows females to minimize risks of genetic incompatibility (e.g. Yasui & Garcia-Gonzalez, 2016), we would expect double-mated females to have a higher clutch success (i.e. a greater percentage of eggs fertilized in each clutch) than single-mated females.

Prediction 2f: *Polyandric females' spiderlings are more resistant to starvation than monandric females' spiderlings.* Mating with multiple males may allow females to have larger and longer-lived offspring (e.g. Watson, 1998). As *M. celer* spiderlings are extremely small, we were unable to obtain accurate measurements of their mass at birth. Spiderling mass at birth is strongly correlated with spiderling survival and resistance to starvation (Walker, Rypstra, & Marshall, 2003), being highly dependent on the amount of yolk allocated to each egg by the female. Thus, the number of days that spiderlings survived starvation conditions was our last fitness proxy.

Data collection

To obtain data regarding Prediction 2a, the time to lay eggsacs, and Prediction 2b, the egg-hatching time, we fed and monitored all females three times per week following copulations to record eggsac deposition and hatching dates. To determine Prediction 2c, the number of spiderlings that each female had over her lifetime, and Prediction 2d, the number of spiderlings that each female had in her first eggsac, we separated and counted the spiderlings once they had hatched and dispersed from the eggsac (approximately 3–5 days after eclosion, Chelini & Hebets, 2016; Muniappan & Chada, 1970). To acquire data on Prediction 2e, fertilization success, we preserved the remainder of each eggsac in 70% ethanol and later counted all undeveloped eggs under a Leica DM 4000 B microscope in order to calculate the percentage of eggs fertilized in each clutch. Finally, to determine Prediction 2f, the number of days spiderlings could survive starvation conditions, we separated out 10 spiderlings from each female's first clutch and housed them individually in 3 cm tall \times 1 cm diameter cages, with *ad libitum* water. We monitored these spiderlings approximately every 2 days, recording the date of all deaths. We sacrificed all remaining spiderlings by freezing them, and later preserved them in 70% ethanol.

Statistical analyses

Prediction 2a: *Polyandric females lay eggs faster than monandric females.* We ran a quasi-Poisson GLM with the number of successful copulations as the independent variable and the number of days between copulation and eggsac laying as the dependent variable.

Prediction 2b: *Polyandric females' eggs hatch faster than monandric females' eggs.* We tested this prediction by repeating the analysis described in Prediction 2a, but replacing the number of days between

copulation and eggsac laying with the number of days between copulation and eggsac hatching as the dependent variable.

Prediction 2c: *Polyandric females have higher lifelong reproductive success than monandric females.* We tested this prediction with a linear model (LM), using the total number of spiderlings per female as the dependent variable and the number of successful copulations (single-mated/remated) as the independent variable.

Prediction 2d: *Polyandric females have more spiderlings in their first eggsac than monandric females.* We repeated the analysis described in Prediction 2c, but focusing only on the number of spiderlings hatching from the first eggsac laid by each female.

Prediction 2e: *Polyandric females have higher fertilization success than monandric females.* We ran a quasi-binomial GLM with the ratio of the total number of spiderlings/the total number of eggs laid by each female (hereafter referred to as 'clutch success') as the dependent variable and the number of successful copulations as the independent variable. We tested Predictions 2a–2e using the software R (R Development Core Team, 2009) and the function 'glm'.

Prediction 2f: *Polyandric females' spiderlings are more resistant to starvation than monandric females' spiderlings.* We ran a mixed-effects Cox model with the functions 'Surv', 'survfit' and 'coxme' of the R software library package 'survival'. We used each clutch (i.e. each female) as a random variable and the female's mating status (single versus double-mated) as the independent variable.

Hypothesis 3: Male Paternity Is Dependent on Mating Order

Male sterilization and mating trials

We used the sterile male technique to determine sperm priority patterns (Boorman & Parker, 1976; Magris, Wignall, & Herberstein, 2015; Parker, 1970; Schneider & Lesmono, 2009). We collected penultimate males and juvenile females in late June 2015 and housed them in conditions identical to those described above (see Animal collection and maintenance). Once all females had matured, we sterilized 60 males through exposure to 1500 rads of X-ray irradiation, using a RADSOURCE RS2000 irradiator® (12.5 min at 120 rads/min). Two days after irradiating these males, we conducted a new round of double-mating trials following the methods described above (see Mating trials), but reducing the interval between males from 2 h to 20 min, in order to maximize our chances of obtaining double-mated females. Females were sorted into four treatments: irradiated male followed by normal male ($N = 25$); normal male followed by irradiated male ($N = 25$); two irradiated males ($N = 10$); and two normal males ($N = 10$). After all mating trials had been conducted we killed all males and allowed the females to lay eggsacs until their natural death. We estimated male paternity share through egg development and differences in spiderling numbers (Boorman & Parker, 1976; Schneider & Lesmono, 2009). We obtained spiderling numbers following the same methods described above (Hypothesis 2, Data collection).

Statistical analyses

To test our prediction of first-male sperm precedence (i.e. that first males would fertilize the majority of the eggs), we ran two analyses. First, we ran a GLM with a quasi-Poisson distribution using the total number of spiderlings as the dependent variable and male order as the independent variable, followed by a Tukey contrasts test for multiple comparisons of means. Second, to test for differences in the percentage of fertilized eggs laid in the female's first eggsac only, we repeated the same analysis using the number of spiderlings from the first eggsac only as the dependent variable.

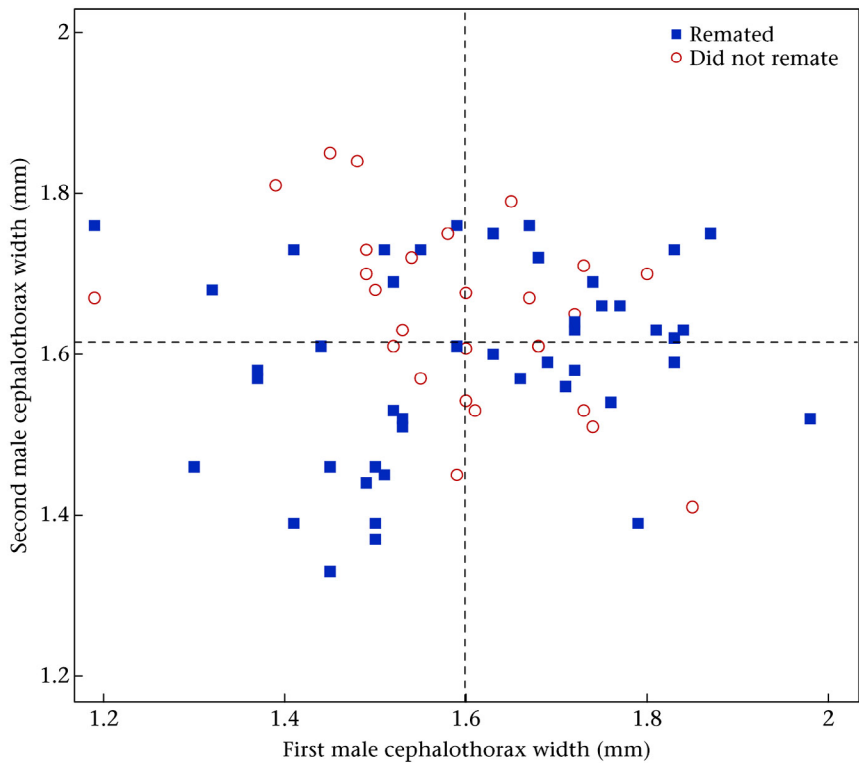


Figure 1. Female *M. celer* that did or did not accept a second copulation, according to the size of the first and second male presented to them. All females mated with the first male. The vertical dashed line represents the average cephalothorax width of first males and the horizontal line represents the average cephalothorax width of second males.

Results

Hypothesis 1: Female Decision to Remate

Males always attempted to copulate, regardless of the females' mating status (virgin versus previously mated), showing no evidence of male mate choice. Similarly, all females mated with the first male presented to them, indicating a lack of virgin female mate choice. One female attacked the first male, subsequently accepted his approach, but killed him postcopulation. Out of the 74 trials included here, 46 females accepted a second copulation (65%; Figure1). Although mated females showed more aggressive behaviors than virgins, few females attempted to cannibalize their mates: 10 females attacked the second male prior to copulation, and two females remated and later killed the second male postcopulation. The model best fit to explain the effect of cephalothorax width on the females' decision to mate multiply was the full model containing the triple interaction between female, first and second male cephalothorax width. However, even in this best-fit model we found no effect of female cephalothorax width, first male cephalothorax width, second male cephalothorax width or their interaction on the females' decision to remate (Table 1). None of the indexes of similarity between males had an effect on the probability of remating (data not shown). Female and male femur length also had no effect on the probability of females mating multiply (Table 2), and neither did female and male age (Table 3). Finally, the days elapsed along the season did not influence the female's decision to mate multiply ($Z^2 = 0.018$, $P = 0.985$, deviance = 0.00032).

Hypothesis 2: Female Fitness Consequences of Polyandry

Prediction 2a: Time to lay eggs — Females took between 9 and 75 days to lay their first eggsac (mean \pm SD = 20.32 \pm 10.33). Polyandry did not affect the number of days elapsed between copulation and eggsac laying ($t_1 = 1.27$, $P = 0.21$; estimate = 0.17, SE = 0.13, deviance = 8.13).

Table 1. Binomial GLM on females' decision to remate based on female, first male and second male cephalothorax width (chi-square goodness-of-fit test: deviance = 14.92, $df = 7$, $N = 72$, $P = 0.04$)

Parameter	Estimate	SE	Z	P
Intercept	1046.20	685.10	1.53	0.13
Female CW	-451.80	315.20	-1.43	0.15
First male CW	-729.40	452.10	-1.61	0.11
Second male CW	-625.40	422.10	-1.48	0.14
Female*first male CW	320.00	208.60	1.53	0.12
Female*second male CW	269.60	194.00	1.39	0.16
First male*second male CW	438.20	278.20	1.57	0.11
Female*first male*second male CW	-191.90	128.20	-1.50	0.13

CW: cephalothorax width

Table 2 Binomial GLM on females' decision to remate based on female, first male and second male femur lengths (deviance = 6.69, $df = 7$, $N = 72$, $P = 0.46$)

Parameter	Estimate	SE	Z	P
Intercept	-93.43	400.81	-0.23	0.82
Female FL	52.22	137.70	0.38	0.70
First male FL	29.27	122.44	0.24	0.81
Second male FL	28.16	126.04	0.22	0.82
Female*first male FL	-16.02	42.10	-0.38	0.70
Female*second male FL	-15.99	43.26	-0.37	0.71
First male*second male FL	-8.72	38.51	-0.23	0.82
Female*first male*second male FL	4.88	13.2	0.37	0.71

FL: femur length

Prediction 2b: Time for eggsacs to hatch — Once laid, eggsacs took between 4 and 21 days to hatch (mean \pm SD = 14.34 \pm 2.98). Polyandry also had no influence on the number of days necessary for the first eggsac to hatch ($t_1 = -0.191$, $P = 0.849$; estimate = -0.01, SE = 0.054, deviance = 0.33).

Table 3. Binomial GLM on females' decision to remate based on female, first male and second male ages (deviance = 4.85, $df = 7$, $N = 69$, $P = 0.74$)

Parameter	Estimate	SE	Z	P
Intercept	16.26	16.44	0.99	0.32
Female age	-1.30	0.92	-1.49	0.16
First male age	-0.46	0.49	-0.94	0.35
Second male age	-0.42	0.41	-1.00	0.32
Female*first male ages	0.04	0.03	1.42	0.16
Female*second male ages	0.03	0.02	1.43	0.15
1st Male*second male ages	0.01	0.01	1.02	0.31
Female*first male*second male ages	-0.01	0.00	-1.45	0.15

Prediction 2c: Lifelong reproductive success — Females laid between 25 and 154 eggs over their lifetime (mean \pm SD = 80 ± 33.71), spread across an average of 1.9 eggsacs (minimum = 1; maximum = 3). Seven females never laid a successful clutch. Polyandry did not affect the lifelong reproductive success of *M. celer* females ($F_{1,63} = 1.77$, $P = 0.19$; estimate = -11.578; Figure 2).

Prediction 2d: Spiderlings in first clutch — An average of 51.38 spiderlings hatched from *M. celer* females' first eggsac (minimum = 18; maximum = 95; SD = 20.07). Polyandry did not influence the number of spiderlings hatching from *M. celer* females' first eggsac ($F_{1,63} = 1.047$, $P = 0.31$; estimate = -5.33).

Prediction 2e: Lifelong clutch success — The overall clutch success varied from 64% to 100% (mean \pm SD = $97 \pm 0.06\%$). This percentage was not affected by the number of mates each female had ($t_{63} = 0.41$, $P = 0.68$; estimate = 0.20, SE = 0.49, deviance = -2.25).

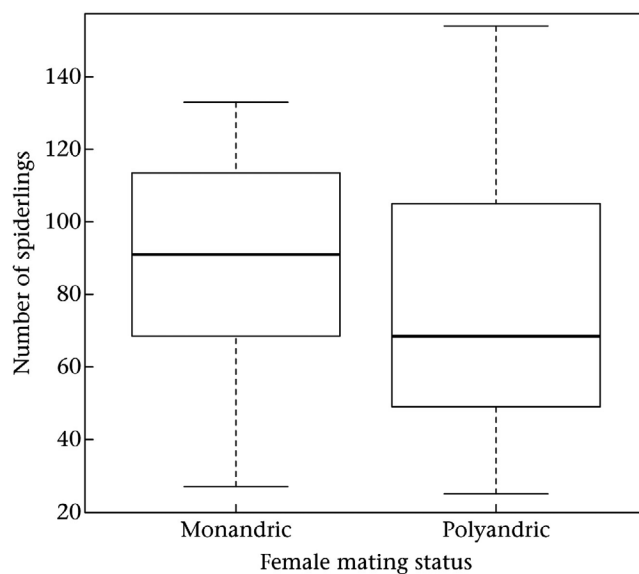
Prediction 2f: Spiderling survival — Polyandry did not significantly influence the survival of *M. celer* spiderlings (Cox survival model: $Z = 1.51$, $P = 0.13$; coefficient = 0.43, hazard (exp(coef)) = 1.54, SE = 1.28; Figure 3).

Hypothesis 3: Male Paternity and Sperm Precedence Patterns

Remating rates in all treatments ranged from 75% to 85%. None of the females that mated only with irradiated males produced live spiderlings, indicating that our sterilization treatment was successful. Females in the normal-irradiated treatment produced significantly fewer spiderlings than females in the normal-normal control, but showed only a nonsignificant tendency to produce fewer spiderlings than females in the irradiated-normal treatment (Figure 4, Table 4). We found no difference in the number of spiderlings produced by normal-normal control females and irradiated-normal females (Table 4). Contrary to the females' lifetime reproductive success, the number of spiderlings hatching from the first eggsac was not influenced by the males' irradiation treatment (quasi-Poisson GLM: $F_{2,44} = 1.94$, deviance = 116.1, $P = 0.15$; Figure 5).

Discussion

Although some female *M. celer* crab spiders copulate with a second male (approximately 65% are polyandric within a 2 h window), we did not find any evidence that females' remating decisions are based on male physical attributes or female fitness outcomes. We were unable to identify any factors that influence *M. celer* females' decision to copulate with a second mate, as female remating was independent of female and/or male size, age and seasonal progression. Our results also show that male *M. celer* do not discriminate between virgin and previously mated females. We were also unable to identify any fitness benefits females might receive from copulating with a second male. In terms of the pattern of sperm precedence in *M. celer*, our irradiation

**Figure 2.** Lifelong reproductive success of monandric and polyandric female *M. celer*. Boxes correspond to first quartile, median and third quartile, whiskers correspond to the range.

results suggest a pattern of sperm mixing in first eggsacs and a potential for last-male sperm priority in subsequent eggsacs. This last finding reinforces the growing body of evidence indicating that the relationships between morphology, behavior and sperm precedence patterns are complex and require direct testing (Elgar, 1998; Herberstein, Schneider, Uhl, & Michalik, 2011; Huber, 2005).

Polyandry in *M. celer* does not fit a 'trading-up' strategy, with females remating only if the second male encountered is somehow superior to the first (e.g. Schneider & Elgar, 1998). The decision to remate also seems independent of the female's physical attributes or age. Similarly, virgin *M. celer* exhibit no obvious mate choice, passively accepting copulation from their first mate (Chelini & Hebets, 2016). Together, results from virgin female matings (Chelini & Hebets, 2016) and second matings (present study) suggest that neither female nor male size (either cephalothorax width or leg length), or their interaction, are good predictors of copulation success. As such, the sexual size dimorphism observed in *M. celer* is unlikely to have been driven by mate choice.

The apparent lack of benefits of polyandry could explain why we were unable to identify any decision criteria that females might be using to accept or reject a second mating. Our previous study focusing only on virgin females and their first mating similarly found no evidence of fitness benefits associated with male body size, leg length or the degree of sexual size dimorphism between a female and her mate (Chelini & Hebets, 2016). Variation in the benefits provided by males is often tied to the evolution of female mate choice (Hubbell & Johnson, 1987; Kokko, Brooks, Jennions, & Morley, 2003). As such, if fitness benefits were associated with *M. celer*'s male phenotype, we would expect it to be reflected in female mate choice decisions. The absence of mate choice could also indicate that polyandrous female *M. celer* are not trading up, but rather bet hedging (i.e. maximizing the genetic diversity of their offspring and minimizing the risk of infertility or low fitness; Fox & Rauter, 2003; Jennions & Petrie, 2000; Watson, 1991). Benefits due to bet hedging could be difficult or impossible to identify in a short-term experiment (Holman, 2015), which could explain the apparent lack of benefits of *M. celer*'s polyandry. Long-term field studies would be required to test the hypothesis that *M. celer* is bet hedging and that the benefits of polyandry are not tied to the males' characteristics.

Table 4. Quasi-Poisson GLM followed by Tukey post hoc comparison test on the number of spiderlings hatching from the first eggsac of females mated to males sterilized by irradiation and normal males in all possible combinations ($F_{2,44} = 3.42$, $N = 45$, $P = 0.04$, deviance = 281.51)

Parameter	Estimate	SE	Z	P
Normal-Irradiated-Irradiated-Normal	-0.86	0.41	-2.11	0.08
Normal-Normal-Irradiated-Normal	0.19	0.36	0.54	0.85
Normal-Normal-Normal-Irradiated	1.05	0.46	2.28	0.05

Protandric species such as *M. celer* are typically expected to have first-male sperm priority, with the first male to mate with a virgin female fertilizing most of her eggs (Kvarnemo & Simmons, 2013; Wiklund et al., 1991; Zonneveld, 1996). In spiders, the morphology of the female reproductive tract is also hypothesized to influence the pattern of fertilization (Austad, 1982). Specifically, entelegyne spiders such as *M. celer* have a “conduit” reproductive tract, where the first sperm entering the spermatheca is considered the most likely to be used in egg fertilizations (Elgar, 1998). Nevertheless, many exceptions to this

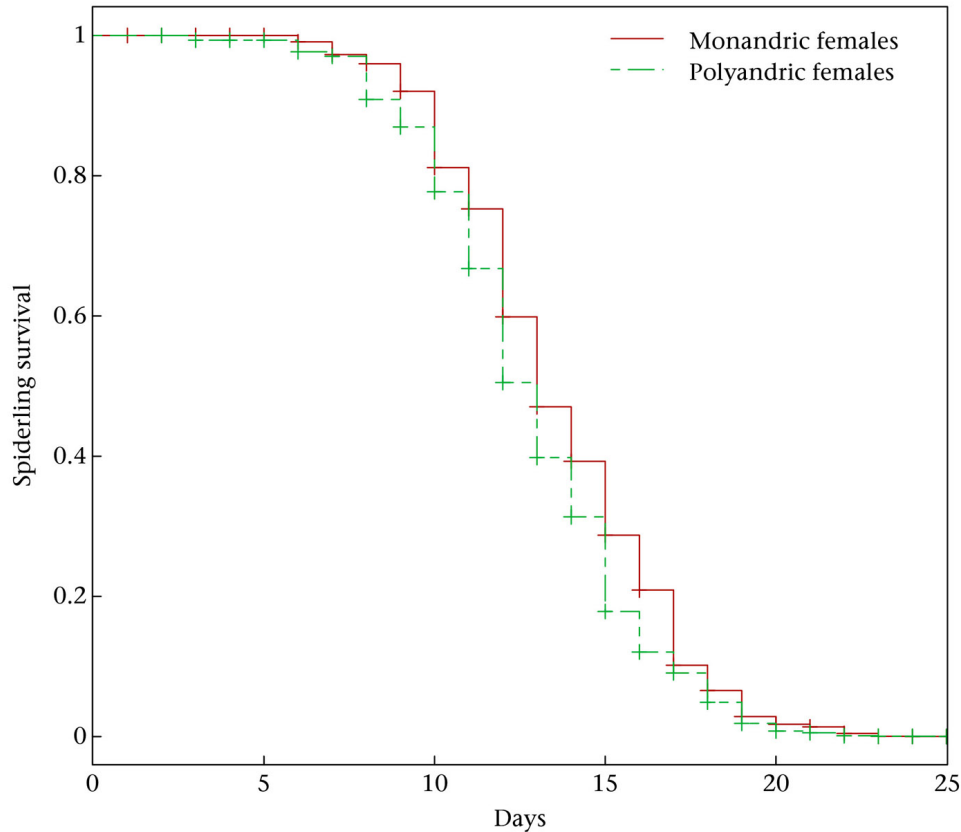


Figure 3. Average survival curves of spiderlings hatched from clutches produced by monandric and polyandric *M. celer* females.

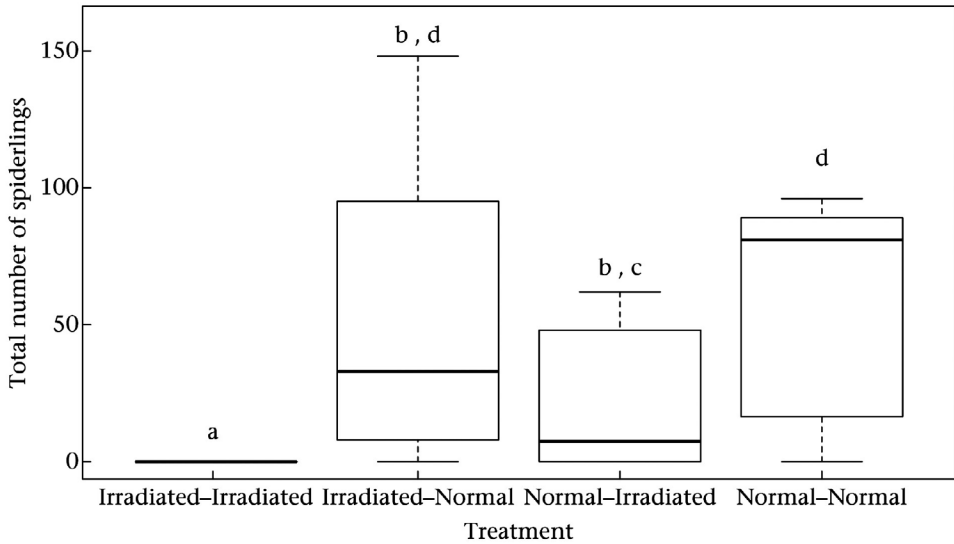


Figure 4. Total number of spiderlings resulting from matings between *M. celer* females and two males, some sterilized through X-ray irradiation, and some normal, in all possible combinations. Different letters represent statistically significant differences.

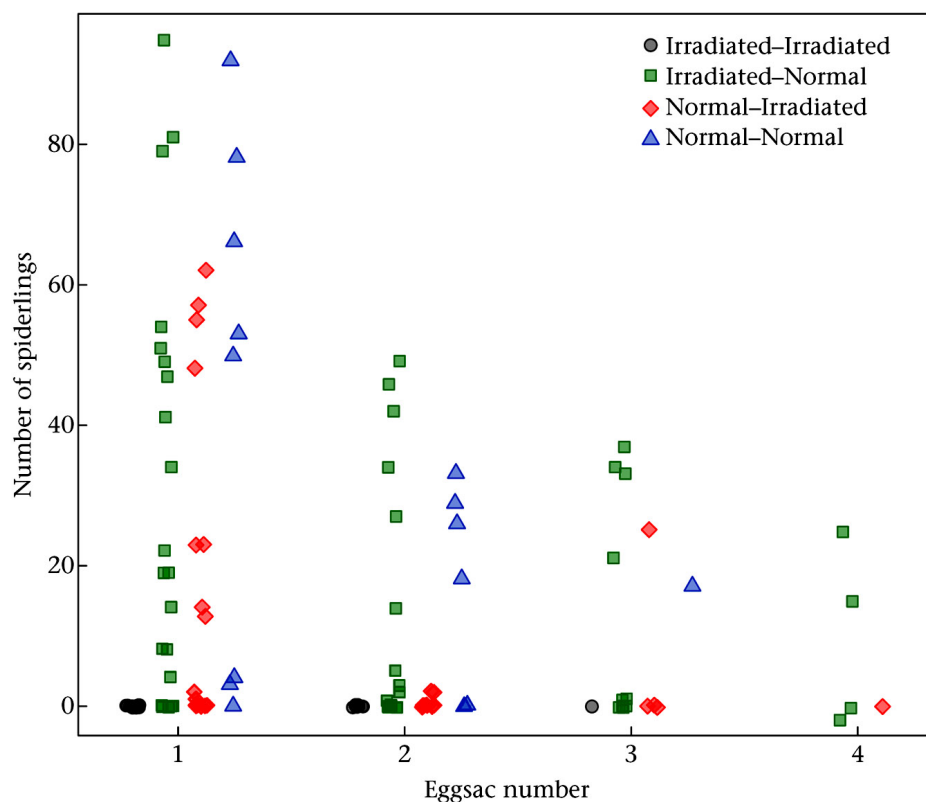


Figure 5. Number of spiderlings hatching from each *M. celer* eggsac, by male irradiation treatment.

pattern have been described, and the most common sperm precedence pattern in spiders seems to be sperm mixing (see Elgar, 1998; Herberstein et al., 2011). Given that *M. celer* shows both early male maturation and a “conduit” reproductive tract, we predicted a priori that this species presented first-male sperm priority. Contradicting our prediction, our results suggest a strong degree of sperm mixing in first eggsacs of *M. celer* females.

Surprisingly, second males tended to fertilize most of the subsequent eggsacs, showing a putative advantage for males to mate with a previously mated female. The likelihood of a female *M. celer* laying more than one eggsac in the field is probably highly dependent on the length of the season and on the date of the female’s first copulation, making it difficult to estimate the magnitude of this advantage under natural conditions. We do know, however, that females are more aggressive following their first copulation, increasing the risk of sexual cannibalism (see results from: Chelini & Hebets, 2016; present study). Thus, from a male’s standpoint, the most advantageous strategy might be to benefit from the ready acceptance of virgin females to copulate and guard these females after copulation until their short window of receptivity is closed. Although a few males have been observed guarding their mates in the laboratory and in the wild (Chelini & Hebets, 2016; M.-C. Chelini, personal observation), postcopulatory mate guarding does not seem to be a widespread strategy in this species.

With or without postcopulatory mate guarding, the decision of *M. celer* females to remate may still be partly under male control. Some male spiders, and many insects, may influence a female’s receptivity to subsequent matings through their seminal fluids (Aisenberg, Estramil, Toscano-Gadea, & Gonzalez, 2009; Ringo, 1996; Sirot, Wolfner, & Wigby, 2011; Wigby et al., 2009). Substances incorporated into the seminal fluid may allow males to benefit from access to virgin females

through early maturation while minimizing the risks of decreased paternity by reducing females’ likelihood of remating (Elgar, 1998; Elgar & Bathgate, 1996; Rice, 1996). As the number of mating partners does not seem to influence *M. celer* females’ reproductive success (present study), a strategy of male-driven female monandry would not impose costs on females but would potentially confer great benefits to males under scenarios of male-biased sex ratio and high male–male competition. The seminal fluid composition of *M. celer* and the relationship between seminal fluid transmission and female mating decisions are yet to be explored in depth.

Although mating strategies of females and males are hypothesized to be driven by their respective costs and benefits, these are often tested (perhaps simply for logistical reasons) in species where these costs and benefits are fairly evident (Boulton & Shuker, 2015; Huber, 2005; Kvarnemo & Simmons, 2013). In this study, we present a species that defies many predictions regarding traditional mating systems. Species such as *M. celer* provide an important challenge to our understanding of the coevolutionary dynamics of male and female mating strategies and the often assumed role played by pre- and postcopulatory sexual selection on the evolution of sexual size dimorphism (Vollrath & Parker, 1992).

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