

Feeding regime, adult age and sexual size dimorphism as determinants of pre-copulatory sexual cannibalism in virgin wolf spiders

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Abstract

Pre-copulatory sexual cannibalism (Pre-SC), where females attack and consume courting males, is considered an extreme case of sexual conflict. Different ultimate causes underlying this phenomenon have been proposed for more than a century and still remain unclear. The main objective of this research was to test the ‘adaptive foraging hypothesis’ in females of the non-burrowing wolf spider *Lycosa fasciiventris*. We exposed virgin adult females to either rich or poor diet treatments (differing in prey quality and availability) and offered them males at different adult female ages (a proxy of mate availability during adulthood). The analysis of correlative data served to evaluate other hypotheses/predictions. In order to test the ‘mate choice hypothesis’ and the ‘sexual size dimorphism hypothesis’, we tested how male absolute and relative (to the female) body size, respectively, affected the probability of Pre-SC. Finally, we asked if the female body size predicts the rate of Pre-SC as proposed by the ‘aggressive spillover hypothesis’. Females from the poor diet treatment and females that were offered a mate shortly after reaching maturity more likely engaged in Pre-SC than females from the rich diet

treatment and females that were deprived of males for longer periods. While females more likely cannibalized courting males of relatively or absolutely smaller body size, female absolute body size did not predict Pre-SC. Thus, our results support the ‘adaptive foraging hypothesis’, the ‘mate choice hypothesis’ and the ‘sexual size dimorphism hypothesis’ and failed to find support for one of the predictions of the ‘aggressive spillover hypothesis’.

Significance statement

Pre-copulatory sexual cannibalism, where females kill and devour courting males, may be costly for both sexes raising questions about its adaptive aspects and evolution. We used wolf spider *Lycosa fasciiventris* to test several existing hypotheses aiming to explain the phenomenon. Pre-copulatory cannibalism in virgin females was predicted by their food availability during adulthood, age at first male encounter and the relative body size of the courting male. Females more likely engaged in pre-copulatory cannibalism if they were poorly fed, young at first male encounter and relatively larger than their courting males. Thus, our data may simultaneously support the ‘adaptive foraging’, the ‘sexual size dimorphism’ and the ‘mate choice’ hypotheses as the explanations for pre-copulatory cannibalism occurrences in *L. fasciiventris*.

Keywords Adaptive foraging hypothesis · Aggressive spillover · *Lycosidae* · Mate choice · Sexual size dimorphism

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Introduction

Sexual conflict arises from asymmetric costs of reproduction for males and females, which differ in their optimal fitness strategies (Rowe et al. 1994; Schneider and Lubin 1998; Chapman et al. 2003). Sexual cannibalism (SC), a behaviour

in which one of the sexes (usually a female) kills and devours a conspecific of other sex (usually a male), is considered one of the most extreme forms of sexual conflict (Elgar 1992; Wilder et al. 2009; Schneider 2014). This behaviour, known mainly in arachnids and insects, but rare in other animal taxa, has puzzled researchers since Darwin (Darwin 1871; Polis 1981; Elgar 1992).

Females may cannibalize males during courtship or during or after mating; and the costs and benefits of SC for each sex strongly depend on this timing (Elgar 1992; Elgar and Schneider 2004). When females cannibalize males during courtship, in a phenomenon termed pre-copulatory sexual cannibalism (Pre-SC), they may obtain nutritional benefits from consumed males enhancing their survival and fecundity (e.g. Rabaneda-Bueno et al. 2008; Welke and Schneider 2012; Pruitt et al. 2014). Pre-copulatory cannibalism however may be also costly for females; virgin females may remain unmated when attacking and killing courting males, whereas females that have already mated may risk lower genetic variability in their offspring compared to females mating multiple times (Rabaneda-Bueno 2014). While females incur both costs and benefits from Pre-SC, there are no apparent advantages for males when they are cannibalized before mating. Despite the potentially high costs for both sexes, the evolution and prevalence of Pre-SC is still not entirely understood (Newman and Elgar 1991; Arnqvist and Henriksson 1997; Johnson 2001; Johnson and Sih 2005; Kralj-Fišer et al. 2013, 2016).

Various hypotheses have been proposed to explain the evolution of Pre-SC. The ‘adaptive foraging hypothesis’ (AFH; Newman and Elgar 1991) posits that females benefit from consuming males, obtaining energy and nutrients which enhance their body condition (Moya-Laraño et al. 2003a, 2003b; Barry et al. 2008) and subsequently their fecundity (Birkhead et al. 1988; Johnson 2001, 2005; Rabaneda-Bueno et al. 2008; Berning et al. 2012; Welke and Schneider 2012; Pruitt et al. 2014). An economic model associated to the AFH (Newman and Elgar 1991) explains the probability of SC depending on the trade-off between the female chances of mating and foraging. According to the model, the probability of a female attack would depend on her expected reproductive success, which in turn depends on two factors: her body condition (hunger status/feeding history) and the potential to fertilize her eggs (male availability) (Newman and Elgar 1991). Therefore, one should expect that virgin females could adaptively assess the value of a male as a meal when prey is rare, or the value of a male as a mate when male availability is low (Newman and Elgar 1991), and when both are scarce to face with a strong compromise between the two. The ‘sexual size dimorphism hypothesis’ (SSDH) proposes that the occurrence of Pre-SC depends on the male size relative to the female size, as relative size would be a proxy of the male ability to defend or escape from the female attack (Wilder and Rypstra 2008a, 2008b; e.g. Roggenbuck et al. 2011; Kralj-Fišer et al. 2016).

Pre-copulatory cannibalism has also been explained as a form of female mate choice, and it would occur when females select for favourable male phenotypic traits and reject unwanted mates through cannibalism (MCH; ‘mate choice hypothesis’; Elgar and Nash 1988; e.g. Persons and Uetz 2005; Prenter et al. 2006; Kralj-Fišer et al. 2012). Alternatively, to the above adaptive hypotheses, non-adaptive hypotheses propose that either Pre-SC may be the result of females failing to recognize a putative mate and attacking, killing and consuming males as if they were any other prey (‘mistaken identity hypothesis’; Goul 1984) or the result of an ‘aggressive spillover’. The ‘aggressive spillover hypothesis’ (ASH; Arnqvist and Henriksson 1997) proposes that Pre-SC depends on a female’s inherited level of aggressiveness; namely, females that are genetically very aggressive cannot plastically adjust their aggression levels according to the context (mating vs. foraging) due to genetic constraints. Consequently, the aggressive females that benefit from their voraciousness in the foraging context incur high costs of remaining unmated since they kill courting males with a high probability (Arnqvist and Henriksson 1997; e.g. Johnson and Sih 2005).

Some of the above hypotheses are not mutually exclusive. For example, females might more likely mate with than cannibalize larger males, and vice versa for smaller males (e.g. Kralj-Fišer et al. 2016), either because large males are high-quality mates (MCH; Prenter et al. 2006) or because they defended themselves better (SSDH; Wilder and Rypstra 2008b). In contrast, the ASH (Arnqvist and Henriksson 1997) predicts that the more voracious females attack males regardless of the male size or any other male trait (e.g. Rabaneda-Bueno et al. 2014).

Furthermore, these different hypotheses explaining SC have received mixed experimental support. For example, several studies have found a positive relationship between female hunger and the likelihood of SC (Andrade 1998; Schneider and Elgar 2001; Herberstein et al. 2002; Persons and Uetz 2005; Wilder and Rypstra 2008b), whereas others failed to support this prediction of the AFH (Jackson 1980; Breene and Sweet 1985; Elgar and Nash 1988; Arnqvist and Henriksson 1997; Fromhage et al. 2003; Johnson 2005; reviewed in Wilder et al. 2009). Similarly, some studies found evidence in the line of the ASH (e.g. Johnson and Sih 2005, Foellmer and Khadka 2013, Rabaneda-Bueno et al. 2014), while others failed to support the latter hypothesis (see Kralj-Fišer et al. 2012, 2013, 2016).

We explored different hypotheses aiming to explain Pre-SC in the spider species *Lycosa fasciiventris* Dufour 1835, a sub-desert non-burrowing wolf spider of moderate sexual size dimorphism (SSD). Using virgin females, we experimentally tested the main prediction of the ‘adaptive foraging hypothesis’; i.e. under low food availability, females will be more prone to attack the males, and we took advantage of the experimental protocols to simultaneously evaluate other

hypotheses and predictions of the ‘adaptive foraging hypothesis’, the ‘sexual size dimorphism hypothesis’, the ‘mate choice hypothesis’ and the non-adaptive ‘aggressive spillover hypothesis’. We compared the rates of Pre-SC of females that were exposed to rich and poor diet treatments. We asked whether the timing of male–female encounters, i.e. if the time from female maturation (namely adult female age), affected the propensity of females to cannibalize courting males, assuming that the longer an adult female had not been encountering a male the lower was her assessment of male availability. Simultaneously, we tested how male absolute and relative (to the female) size affected the female probability of mating and/or killing a male. Finally, we asked if female body size alone could predict the rate of Pre-SC, as predicted by the ASH (Arnqvist and Henriksson 1997). According to the ‘adaptive foraging hypothesis’ (Newman and Elgar 1991), prey and male availability influence the rate of SC; thus, we expected that ‘better fed females’ would exhibit lower rates of Pre-SC than ‘poorly fed females’. We also predicted that older virgin adult females (i.e. deprived for longer time from access to males) would be less prone to be involved in Pre-SC. Furthermore, in accordance to the ‘sexual size dimorphism hypothesis’ (Wilder and Rypstra 2008b), we predicted that relative male body size would affect the probability of a female attack. Similarly, if male absolute body size (i.e. regardless of the female body size) predicted the rate of Pre-SC, then the ‘mate choice hypothesis’ would be supported (Elgar and Nash 1988). In contrast, if our data supported the ‘aggressive spillover hypothesis’ (Arnqvist and Henriksson 1997), we predicted that female body size would explain the probability of Pre-SC regardless of the male phenotype or the female nutritional status.

Methods

Spider collection

The wolf spider *L. fasciiventris* Dufour 1835 is a moderately sexually size dimorphic species (carapace width \pm SE: males, 5.34 ± 0.06 , $N = 78$; females, 5.75 ± 0.06 , $N = 127$) inhabiting semiarid Iberian lands. This species, already known for many years, including published accounts of its biology (Parellada 1998), was described as *L. ambigua* only recently (Barrientos 2004) and later acknowledged as a synonymy and renamed as *L. fasciiventris* (Planas et al. 2013). Unlike the other Iberian species in the genus—*L. hispanica* Walckenaer, 1837 (formerly adscribed to *L. tarantula* Linnaeus 1758), *L. fasciiventris* has an annual life cycle and does not build burrows (Parellada 1998), making *L. fasciiventris* spiders more easy to maintain and handle in the laboratory.

From June 23th to August 20th, 2015, we collected 78 subadult and adult males and 127 subadult females in dry

temporal washes (‘ramblas’) in four locations within the Almeria province (South-East Spain; $36^{\circ} 48' 7''$ N, $2^{\circ} 8' 36''$ O; $36^{\circ} 57' 60''$ N, $2^{\circ} 6' 7''$ O; $37^{\circ} 20' 39''$ N, $2^{\circ} 0' 28''$ O; $36^{\circ} 47' 35''$ N, $2^{\circ} 41' 36''$ O), which were kept in individual containers (74 cm height \times 56 cm \varnothing) until arrival to the laboratory.

Laboratory rearing

Upon arrival to the laboratory, each specimen was transferred to an individual tank (22 \times 18 \times 18 cm) filled with 2–3 cm of soil at the bottom. We used the soil brought from one of the collection localities. We added two wooden blocks (10 \times 8 \times 1 and 3 \times 5 \times 1 cm) into each tank providing shelter. We fed subadult females and all males with a size-matched cricket once a week and also checked for moulting events during feeding. Spiders had ad libitum access to water, which was provided through a 40-ml vial filled with water and covered with cotton. Vials were checked for water every 2–3 days and filled when empty. The tanks were placed in a climate chamber with simulated outdoor climatic conditions (day and night temperature cycles, photoperiod with light bulbs of 54 W—mimicking natural sunshine—and a relative humidity of 50–65%). Every week, temperature and photoperiod parameters were adjusted according to the preceding weekly average conditions in the Almeria province, with temperatures changing twice per day to conform to night vs. day oscillations (ranges: temperature, 18.7–34.3 $^{\circ}$ C; photoperiod, 7:21–8:20). This unusual setting was necessary for reasons beyond the present study (i.e. using the offspring later on in an experiment in the wild, so we needed similar conditions).

Experiments

To experimentally test the hypothesis that feeding conditions after reaching maturity affect the probability of Pre-SC in virgin females, we randomly assigned females to either a rich ($N = 101$) or a poor ($N = 26$) diet treatment. Our study was part of a more extensive research protocol aiming at estimating quantitative genetic parameters in a half-sib design (i.e. using the offspring of these matings). Therefore, we had to be sure that most males survived the first mating and remated with a second female. The unbalanced sample sizes of the two treatments, namely, much larger sample size in the rich diet treatment, aimed at ensuring a sufficient number of half-sib families if feeding condition would indeed affect the rate of SC.

Spiders from the poor and rich diet treatments were provided with different quantity (number and/or size of cricket preys) and quality (congeneric spider provided or not) of food. All animals were fed once a week, and once females reached maturation, they were submitted to the diet treatments during the weekly feeding and also 48 h before the mating trials (when acclimating within the mating arenas—see below).

An adult female was not included in a mating trial until she had been at least for 2 weeks under the feeding treatment (range in days 17–65). In the rich diet treatment, females were fed with 1–3 crickets (*Acheta domesticus*) weekly, summing up a total mass similar to that of the experimental female. Two days prior to the mating trial, we additionally fed these females with 1–3 crickets (summing up approximately twice as much mass as that of the female) and also with one congeneric spider weighing approximately half the female mass. Depending on the availability of spiders collected in the field, the spider that was offered as prey could be either a conspecific or a *L. hispanica* juvenile captured in the same localities. When a *L. fasciiventris* male was used as the spider prey, the male was exposed to -20°C for 3 min prior to offering it to the female in order to prevent courtship and mating (e.g. Rabaneda-Bueno et al. 2008). Neither species nor gender of the spider prey significantly affected the probability of SC in the rich diet treatment (GLM, estimate = -0.43 ; $Z = -0.8$; $P = 0.382$).

The females from the poor diet treatment were fed weekly with one cricket that weighed approximately 25% of the female mass. Females within the poor diet received no food 48 h prior to the mating trials. However, we introduced an inanimate object 2–3 times into the mating arenas in order to subject ‘poor diet females’ to comparable disturbance levels as we did in those in the rich diet treatment.

Blinded methods were not possible to apply as females in the high feeding treatment were very apparent to the observer due to their conspicuously better body conditions (swollen abdomens). In addition, blind methods were not necessary as our main output variable was whether the female attacked and killed the male, a behaviour that is assigned without any source of uncertainty by the observer and is therefore not subjected to potential observer bias.

Mating trials

We staged mating trials between July 29th and September 18th of 2015. Mating arenas were built with plastic boxes ($26 \times 14 \times 10$ cm) cut and split in two halves (top and bottom) of equal height, which were attached to each other with transparent sticky tape. The bottom half of the arena was filled with soil, and the top half had a circular (3 cm \varnothing) opening in each of its opposite sides according to its length, which served for introducing the male into the container. The cover was made of transparent plastic and had a trapdoor in the centre, from which prey could be introduced in the container with minimum disturbance to the female. Adult virgin females were introduced into the mating arenas 48 h prior the mating trials. This allowed virgin females to acclimate to the container and to release silk threads and pheromones (Papke et al. 2001; Roberts and Uetz 2005).

Before spiders were introduced into the mating arenas, we measured their carapace and abdomen widths (nearest 0.1 mm) using a dissection microscope (Zeiss Stemi DV4) and their body masses using a scale (nearest 0.01 g). Spider carapace width is considered a good indicator of its structural body size (Hagstrum 1971; Jakob et al. 1996) measured at its maximum distance. In order to measure sexual size dimorphism (SSD), we used the RII index (Armas et al. 2004), a modification of the SDI index (Lovich and Gibbons 1992) which has been shown by simulation to be robust and to incur in minimum statistical bias (Armas et al. 2004): $\text{SDI} = (\text{male carapace width} - \text{female carapace width}) / (\text{male carapace width} + \text{female carapace width})$.

In each mating trial, the male was gently introduced into the mating arena, as far as possible from the female, i.e. through the side hole located opposite to the female. The male was placed in an open 15-mm-diameter opaque tube, and a plunger was used to slowly force the spider to exit the tube and enter the mating arena (Moya-Laraño 2002). We measured latency until mating (courtship duration), copulation duration and occurrence of Pre-SC. Not all pre-copulatory attacks ended in cannibalism, and even after some unsuccessful female attacks, the spiders later mated (13.39%). In a few cases (5.5%), neither mating nor cannibalism occurred. Once copulation started, we removed the sticky tape that attached the two halves of the mating arena (top and bottom) and carefully removed the top part in order to prevent successful post-copulatory attacks (i.e. by opening the arena, we allowed the males to jump out of the mating arena and escape after copulation ended).

Statistical analyses

We used generalized estimating equations (GEE) to test the probability of Pre-SC. The dependent variable was cannibalism occurrence (no vs. yes); therefore, we used a binomial distribution in the GEE. Due to the constraints of the half-sib design, we used males twice (with two different females). Thus, we included male identity as the within-subject repeated measure in the GEE. To control for the potential correlation among trials, we included the ‘exchangeable’ correlation structure. The GEE analysis was performed in R (R Core Team 2015) by applying the function ‘geeglm’ within the ‘geepack’ package (Højsgaard et al. 2006). In order to test the above hypotheses, namely, AFH, ASH, SSDH and MCH, we run models with sexual cannibalism as the dependent variable and different predictors according to the these hypotheses. The predictor(s) was/were treatment and female adult age (AFH), female size (ASH), male size and body condition (MCH) and SDI and male age (SSDH). Male age was included to test if older males could be more vulnerable. Male body condition was included implicitly by including abdomen

width or the cubic root of body mass as covariates in a model including carapace width (Moya-Laraño et al. 2008).

We used the quasi-likelihood information criterion (QIC), an analogous statistic to the Akaike's Information Criterion (AIC) in GEE, to select the model with the best fit, i.e. with the lowest QIC. Models with QIC differences larger than two ($|\text{QIC model 2} - \text{QIC model 1}| > 2$) were considered to be different (see also 'Discussion'). However, when QIC differences between models were smaller than two ($|\text{QIC model 2} - \text{QIC model 1}| < 2$), we considered the models were equivalent. In order to plot the final results, we used the library 'effects' (Fox 2003). We implemented a new 'method' for generic functions in R, which allowed extracting the variance-covariance matrix of estimated coefficients from a GEE in the library 'vcov'.

Results

Females under the poor diet treatment more likely engaged in Pre-SC than the females from the rich diet treatment (GEE, odds ratio = 4.95; $\chi^2 = 7.8$; d.f. = 1; $P = 0.005$).

A comparison of models representing different hypotheses by means of QIC (Table 1) revealed that the AFH model including 'female adult age' and the 'feeding treatment' as predictors had lower QIC values than the model with just the 'treatment' predictor (80.5 vs. 85.1, respectively). Indeed, female adult age negatively and significantly affected the probability of Pre-SC (estimate = -0.08 ; $\chi^2 = 13.5$; d.f. = 1; $P = 0.0002$). Namely, older females attacked courting males less often. The ASH model including only female carapace width as a predictor had higher QIC than that of the AFH model (90.0); and the effect of female carapace width was not significant (estimate = 0.44 ; $\chi^2 = 2.5$; d.f. = 1; $P = 0.115$). The SSDH model including only the relative male and female sizes (SDI) had a similar QIC value to the above models (86.0), although SDI significantly affected the probability of SC (estimate = -8.45 ; $\chi^2 = 8.7$; d.f. = 1; $P = 0.0032$). When comparing MCH models (male size and body condition as predictors), we found that all models had higher QIC values than that of the AFH model (all QIC > 87.3). In all the MCH models, male size measures were found to significantly influence probability of Pre-SC when introduced as a single predictor (male carapace width: estimate = -0.88 ; $\chi^2 = 5.2$; d.f. = 1; $P = 0.022$; male abdomen width: estimate = -0.80 ; $\chi^2 = 5.5$; d.f. = 1; $P = 0.019$; male mass: estimate = -6.92 ; $\chi^2 = 5.1$; d.f. = 1; $P = 0.024$). However, male body condition (e.g. male abdomen width or body mass in models including carapace width) was not a significant predictor of sexual cannibalism (Table 1). Male adult age as a predictor did not explain the frequency of Pre-SC either. Since we could not choose a model based on QIC to distinguish whether absolute or relative male sizes were more relevant to trigger female

attacks, we then compared two final models, which integrated the relevant variables explaining the AFH with either male carapace width or the SDI index. We found that a model including treatment, female adult age and male carapace width had slightly higher QIC values (78.9) than a similar model including the SDI index instead of male carapace width (76.4). Therefore, we consider this last model as our final one, in which all predictors were significant (Table 2; Fig. 1).

Discussion

Here, we used *L. fasciiventris* spiders to test various hypotheses that aim to explain the occurrence of pre-copulatory sexual cannibalism. Our results suggest that the incidence of Pre-SC in virgin females is related to their feeding history, i.e. food availability during adulthood, an experimental result, their adult age and the relative body size of the male; these being correlational results. Females more likely engaged in Pre-SC if they were hungry (poorly fed), young and relatively larger than their courting males. Thus, our results support the 'adaptive foraging hypothesis' (AFH; Newman and Elgar 1991) and the 'sexual size dimorphism hypothesis' (SSDH; Wilder and Rypstra 2008b). However, since the final models were of similar QIC magnitude, we cannot reject the 'mate choice hypothesis' (MCH; Elgar and Nash 1988), as absolute male body size was also relevant and meaningful in the models. In addition, adult female body size did not affect Pre-SC; thus, we failed to find support for the 'aggressive spillover hypothesis' (ASH; Arnqvist and Henriksson 1997).

We found that the virgin wolf spider females that were experimentally induced to poor diet were more likely engaged in Pre-SC when compared to the females in the rich diet treatment. While this finding supports the 'adaptive foraging hypothesis' proposing that the probability of SC depends on female hunger and body condition (Newman and Elgar 1991), to our knowledge, very few studies have previously tested the AFH in virgin females to explain Pre-SC and have contradictory results. While Schneider and Elgar (2001) found a negative relationship between food intake and the probability of sexual cannibalism in virgin, but not in mated *Nephila plumipes* females, Herberstein et al. (2002) found the opposite in *Argiope keyserlingi*. In accordance to our results, Moya-Laraño et al. (2003a) found indirect evidence that experimentally manipulated food availability determined the rate of sexual cannibalism in *L. hispanica* spiders, a sympatric congener.

Due to our experimental design, we could not discern the effect of female hunger (prey quantity) from the effect of female nutritional status (prey quality) on female decisions to attack and kill courting males. In our research, most of cannibalistic females almost completely devoured their mate, leaving only a small residual pellet afterwards. However, since the females in the high food treatment received a larger amount of

Table 1 Comparison of the statistical models proposed to explain the different hypothesis on Pre-SC

Hypothesis	Number of predictors	Model predictors	Estimate	QIC
AFH	1	Feeding treatment	1.596	85.1
	2	Feeding treatment	1.627	80.5
		Female age	-0.078	
ASH	1	Female carapace	0.441	90.0
SSDH	1	SSD index	-8.448	86.0
	1	Male age	0.013	91.8
	2	SSD index	-8.616	88.0
		Male age	-0.007	
MCH	1	Male carapace	-0.879	87.3
	1	$\sqrt[3]{\text{Male mass}}$	-6.920	87.8
	2	Male carapace	-0.842	89.5
		$\sqrt[3]{\text{Male mass}}$	-0.328	
	2	Male carapace	-0.615	88.9
		Male abdomen	-0.350	

AFH adaptive foraging hypothesis, ASH aggressive spillover hypothesis, SSDH sexual size dimorphism hypothesis, MCH mate choice hypothesis, SSD sexual size dimorphism

nutritional resources than those provided by just one male and females in the poor treatment were provided food ad libitum after resuming the trials (as we needed them to produce equally high amounts of offspring for the half-sib quantitative genetics study), we could not compare whether males add substantial nutritional value for offspring production. However, this benefit was experimentally demonstrated for a congeneric and syntopic species (*L. hispanica*) in the same area, which were fed with natural prey ad libitum (Rabaleda-Bueno et al. 2008). Therefore, the consumption of males may provide an extra nutritional value for cannibalistic females, and beyond the amount of energy summed up by natural prey, cannibalistic females may increase their fecundity (Rabaleda-Bueno et al. 2008). Several studies have reported that females who engaged in SC obtained benefits by producing more eggs per sac (*L. hispanica* and *Agelenopsis pennsylvanica*; Rabaleda-Bueno et al. 2008; Pruitt et al. 2014), increasing hatching success (*A. pennsylvanica*; Berning et al. 2012; Pruitt et al. 2014), advancing female breeding and producing spiderlings with better body condition and earlier dispersal leading to early growth and a cannibalistic advantage (*L. hispanica*; Rabaleda-Bueno et al. 2008), which in natural conditions

can lead to an increase in the survival of the offspring by allowing access to more prey and escaping from more predators (268 spider species; Verdeny-Vilalta et al. 2015), raising the survival of the offspring (*Argiope bruennichi* and *Pardosa pseudoannulata*; Welke and Schneider 2012; Wu et al. 2013), or even increasing female attractiveness to males (*Latrodectus hesperus* and *A. pennsylvanica*; Johnson et al. 2011; Pruitt et al. 2014). Hence, given an identical feeding status in females (i.e. amount of heterospecific prey and mass consumed), the nutritional contribution of a male may provide huge benefits to the female, either in the form of energy reserves to lay eggs or to restore nutritional imbalance (*Pardosa prativaga*; Mayntz and Toft 2006). Nevertheless, whether or not males constitute high-quality prey for females remains unclear. The ratio of lipid to protein that female spiders extract from their mates appears less suitable regarding oogenesis than the ratio of lipid to protein extracted from crickets (*Tigrosa helluo*; Wilder and Rypstra 2010). However, praying mantis females fed on a high-protein diet produced more offspring than females on a high-lipid diet despite the fact that they consumed the same biomass of prey (Barry and Wilder 2013), supporting the previous hypothesis that in congeneric

Table 2 Coefficients of the Pre-SC final GEE model with the feeding treatment (rich or poor diet), female adult age (time since maturation) and the SSD index

Model	Estimate	Std. error	Wald (χ^2)	Deg. free.	P value
Intercept	-2.0582	0.5657	13.24		0.00027
Feeding treatment	1.6818	0.6503	6.69	1	0.00970
Female age	-0.0851	0.0243	12.23	1	0.00047
SSD	10.0004	3.5225	8.06	1	0.00453

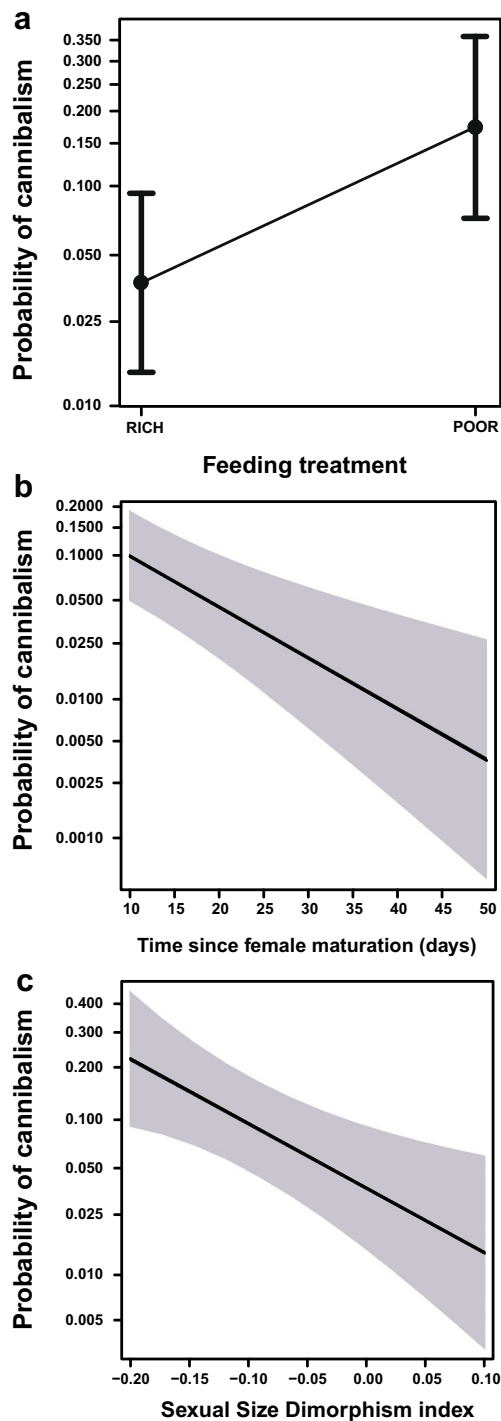


Fig. 1 Predictors of the probability of pre-copulatory sexual cannibalism according to the different hypotheses supported by the final model. **a** Feeding treatments ('rich diet females' vs. 'poor diet female' spiders). **b** Time (days) since female maturation. **c** An index of sexual size dimorphism. Effects are estimates (least-squares means for categorical variables and partial regression coefficients for continuous variables) from the GEE models, which were built with the aid of the library effects (see text for details). Errors are 95% confidence intervals, taken at the different X point estimates for continuous variables (*shaded area*)

Lycosa species, males could be a source of nitrogen to females (Rabaneda-Bueno et al. 2008).

The 'adaptive foraging hypothesis' further proposes that the probability of SC depends on the availability of males during the breeding season, which influences the probability to fertilize the female eggs (e.g. *Dolomedes triton* and *L. hispanica*; Johnson 2004; Rabaneda-Bueno et al. 2008). In our study, we manipulated the female perception of male availability indirectly and used adult female age as a proxy. Namely, due to logistic constraints during the experiment, the length of time that virgin females were deprived from access to males (female adult age) simulated the period that females experienced male shortages. In accordance to the AFH, we found that those females that had not interacted with a male for a longer period of time (older adult females) were less prone to attack males. The relationship between female age and the Pre-SC occurrence was significant and negative regardless of the feeding treatment or the mate size dimorphism.

The effect of age and feeding status could be confounded in our analyses because the older females had been also in their feeding treatment for longer time, and thus, age could be simply reflecting differences in feeding status. If that were the case, we would expect to find that the effect of feeding treatment would be stronger in older relative to younger females (i.e. a feeding treatment per age interaction) and not a simple (additive) effect of age. However, we found that such interaction did not improve the QIC of our final model, indicating that the effect on age was independent on the effect of feeding status. This implies that the negative effect of age was not spurious and free of confounding factors.

However, due to the constraints of our experimental design, we cannot confidently separate the effect of female perception of male availability from the effect of female age alone, which can be an indication of other effects as well. For instance, female age has often been shown to affect female mate choice (e.g. *Schizocosa ocreata* and *Rabidosia rabida*; Uetz and Norton 2007; Wilgers and Hebets 2012). In line with our expectations, younger virgin females exerted Pre-SC more often than older virgin females. Nevertheless, this finding could be an indication that younger females were choosier than older ones. Wilgers and Hebets (2012) found that older *R. rabida* females exhibited higher mating rates and were less choosy than that of younger ones, but they found no influence of female age on SC. Furthermore, in our study, if the response cannibalism vs. mating would be an indication of female choosiness depending on age, then we would expect to see an interaction between male body size (the trait that responded in the MCH model) and female age. However, adding such interaction to the model did not improve the QIC (results not shown), suggesting that rather the pattern found is an indication that females that were deprived from access to males for longer were assessing their environment as short of males and behave consistently with the predictions of the economic model of the AFH (Newman and Elgar 1991). This result is consistent with previous results from other studies in which

male availability was experimentally manipulated either in the laboratory (*D. triton*; Johnson 2004) or in the field (*L. hispanica*; Rabaneda-Bueno et al. 2008).

L. fasciiventris females more often cannibalized males of either absolute or relative smaller body size, which supports both the SSDH and the MCH. We cannot therefore reject the MCH which states that Pre-SC likely serves as a mate choice strategy by which females can select particular male traits, such as male body size (*Araneus diadematus*; Elgar and Nash 1988), nor the SSDH, or male vulnerability hypothesis, in which the larger the male relatively to the female (size dimorphism), the lower the vulnerability of males, and the lower the probability that females attack and kill them (*T. helluo*; Wilder and Rypstra 2008b). Thus, when females select larger male body sizes, SSDH and MCH are not mutually exclusive (*Dolomedes fimbriatus*; Kralj-Fišer et al. 2016). In fact, we observed that males that evaded female attacks later copulated with the same female, implying that the female attack may also serve as a mate check strategy (*Nephilengys livida*; Kralj-Fišer et al. 2012). Females could benefit from mating with larger males because their offspring could inherit larger adult body sizes which could be advantageous in both foraging and mating contexts (Elgar 1992; Prenter et al. 2006).

We failed to find support for one of the predictions of the ‘aggressive spillover hypothesis’ (Arnqvist and Henriksson 1997); i.e. that larger females more often engage in Pre-SC. The results of testing the ASH in other spider taxa have given mixed support. This prediction has only been supported in very few studies (*D. fimbriatus*, *D. triton*, *Argiope aurantia* and *L. hispanica*; Arnqvist and Henriksson 1997; Johnson and Sih 2005; Foellmer and Khadka 2013; Rabaneda-Bueno et al. 2014) and remains as highly controversial due to unrealistic mating trials (laboratory artefacts) or neglected male behaviour and traits (see in Kralj-Fišer et al. 2013; Pruitt and Keiser 2013). A potential drawback of our design could be that we manipulated the amount of food that juvenile females received in the laboratory, thereby possibly masking the effect of female voracity levels influencing their adult body sizes. However, this is likely not the case as we collected subadult females shortly before reaching maturity in the field, and we can assume that voracity—if it has an effect—had already influenced adult body size. Also, when these spiders mature, they stop growing (in their fixed body parts; i.e. carapace and the legs), and thus, feeding treatment during adulthood could not affect their body size.

Even though multiple mechanisms could affect the prevalence of sexual cannibalism, only a few recent studies have found support for multiple factors acting simultaneously (e.g. Roggenbuck et al. 2011; Berning et al. 2012; Kralj-Fišer et al. 2013, 2016; Rabaneda-Bueno et al. 2014). According to the final model, Pre-

SC in *L. fasciiventris* can be explained by female hunger, female age and sexual size dimorphism. Data suggests that Pre-SC strongly depends on ecological conditions such as prey and male availability. Pre-SC may enable female survival and fecundity in periods or areas of high male density and low prey availability. Yet females deprived of males for longer time rarely devoured males even if the female had a relatively poor body condition. Thus, females in the field would not devour courting males when both prey and males are scarce. However, in periods or areas of high male availability and low prey availability, Pre-SC may serve as both a mate choice and a foraging strategy to increase the female fecundity (Rabaneda-Bueno et al. 2008).

In principle, all of the above results would need to be interpreted with caution since the models do not differ substantially in QIC; e.g. our final model only differs in six QIC units from a model including only the feeding treatment. However, given the strong theoretical background behind each of the models and hypotheses, and the fact that all predictors were significant in our final model, we believe that these QIC differences provide sufficiently strong support for our final model.

In our design, we simulated natural conditions by giving males the chance to escape away from the female after copulation (Kralj-Fišer et al. 2013; Pruitt and Keiser 2013). Remarkably, we have not observed any case of cannibalism during or after mating, implying low rates of post-copulatory cannibalism in the field. Pre-copulatory sexual cannibalism may play particularly important role in the ecology of lycosids. For example, in a congeneric, *L. hispanica*, female’s diet in the field largely consists of conspecific males in situations in which heterospecific prey is limiting (Moya-Laraño 2002; Moya-Laraño et al. 2003a; Rabaneda-Bueno et al. 2008). Thus, in this gender, Pre-SC may be an adaptive and flexible behaviour strongly dependent on the females’ feeding history and the ecological conditions that they face during the mating season.

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Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All specimens were in sufficiently large containers and received water and food at libitum with the exception of the ‘low food’ individuals, which were nevertheless not starved beyond what it is often found in the wild (e.g. animals with even lower body conditions can be found).

References

- Andrade MCB (1998) Female hunger can explain variation in cannibalistic behavior despite male sacrifice in redback spiders. *Behav Ecol* 9:33–42
- Armas C, Ordiales R, Pugnaire FI (2004) Measuring plant interactions: a new comparative index. *Ecology* 85:2682–2686
- Arnqvist G, Henriksson S (1997) Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. *Evol Ecol* 11:225–273
- Barrientos JA (2004) *Lycosa ambigua* sp. nov. (*Araneae*, *Lycosidae*), una nueva “tarántula” para la fauna ibérica. *Revista Ibérica de Aracnología* 9:23–29
- Barry KL, Wilder SM (2013) Macronutrient intake affects reproduction of a predatory insect. *Oikos* 122:1058–1064
- Barry KL, Holwell GI, Herberstein ME (2008) Female praying mantids use sexual cannibalism as a foraging strategy to increase fecundity. *Behav Ecol* 19:710–715
- Berning AW, Gadd RD, Sweeney K, MacDonald L, Eng RY, Hess ZL, Pruitt JN (2012) Sexual cannibalism is associated with female behavioural type, hunger state and increased hatching success. *Anim Behav* 84:715–721
- Birkhead TR, Lee KE, Young P (1988) Sexual cannibalism in the praying mantis *Hierodula membranacea*. *Behav Ecol* 106:112–118
- Breene RG, Sweet MH (1985) Evidence of insemination of multiple females by the male black widow spider, *Latrodectus mactans* (*Araneae*, *Theridiidae*). *J Arachnol* 13:331–335
- Chapman T, Arnqvist G, Bangham J, Rowe L (2003) Sexual conflict. *Trends Ecol Evol* 18:41–47
- Darwin C (1871) *The descent of man*, 2 vols. London 81:130–131
- Elgar MA (1992) Sexual cannibalism in spiders and other invertebrates. In: Elgar MA, Crespi BJ (eds) *Cannibalism: ecology and evolution among diverse taxa*. Oxford University Press, Oxford, pp. 129–156
- Elgar MA, Nash DR (1988) Sexual cannibalism in the garden spider *Araneus diadematus*. *Anim Behav* 36:1511–1517
- Elgar MA, Schneider JM (2004) The evolutionary significance of sexual cannibalism. *Ad Stud Behav* 34:135–164
- Foellmer MW, Khadka KK (2013) Does personality explain variation in the probability of sexual cannibalism in the orb-web spider *Argiope aurantia*? *Behaviour* 150:1731–1746
- Fox J (2003) Effect displays in R for generalised linear models. *J Stat Softw* 8:1–27
- Fromhage L, Uhl G, Schneider JM (2003) Fitness consequences of sexual cannibalism in female *Argiope bruennichi*. *Behav Ecol Sociobiol*: 5560e64
- Goul SJ (1984) Only his wings remained. *Nat Hist* 93:10–18
- Hagstrum DW (1971) Carapace width as a tool for evaluating rate of development of spiders in laboratory and field. *Ann Entomol Soc Am* 64:757–760
- Herberstein ME, Schneider JM, Elgar MA (2002) Costs of courtship and mating in a sexually cannibalistic orb-web spider: female mating strategies and their consequences for males. *Behav Ecol Soc* 51: 440–446
- Højsgaard S, Halekoh U, Yan J (2006) The R package geepack for generalized estimating equations. *J Stat Soft* 15:1–11
- Jackson RR (1980) Cannibalism as a factor in the mating strategy of the jumping spider *Phidippus johnsoni* (*Araneae*: *Salticidae*). *Bull Br Arachnol Soc* 5:129–133
- Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices. *Oikos* 77:61–67
- Johnson JC (2001) Sexual cannibalism in fishing spiders (*Dolomedes triton*): an evaluation of two explanations for female aggression towards potential mates. *Anim Behav* 61:905–914
- Johnson JC (2004) Cohabitation of juvenile females with mature males promotes sexual cannibalism in fishing spiders. *Behav Ecol* 16:269–273
- Johnson JC (2005) The role of body size in mating interactions of the sexually cannibalistic fishing spider *Dolomedes triton*. *Ethology* 111:51–61
- Johnson JC, Sih A (2005) Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. *Behav Ecol Sociobiol* 58:390–396
- Johnson JC, Trubl P, Blackmore V, Miles L (2011) Male black widows court well-fed females more than starved females: silken cues indicate sexual cannibalism risk. *Anim Behav* 82:383–390
- Kralj-Fišer S, Schneider JM, Justinek Z, Kalin S, Gregori M, Pekár S, Kuntner M (2012) Mate quality, not aggressive spillover, explains sexual cannibalism in a size-dimorphic spider. *Behav Ecol Sociobiol* 66:145–151
- Kralj-Fišer S, Schneider JM, Kuntner M (2013) Challenging the aggressive spillover hypothesis: is pre-copulatory sexual cannibalism a part of a Behavioural syndrome? *Ethology* 119:615–623
- Kralj-Fišer S, Čandek K, Lokovšek T, Čelik T, Cheng RC, Elgar MA, Kuntner M (2016) Mate choice and sexual size dimorphism, not personality, explain female aggression and sexual cannibalism in raft spiders. *Anim Behav* 111:49–55
- Lovich JE, Gibbons JW (1992) A review of techniques for quantifying sexual size dimorphism. *Growth Develop Aging* 56:269–281
- Mayntz D, Toft S (2006) Nutritional value of cannibalism and the role of starvation and nutrient imbalance for cannibalistic tendencies in a generalist predator. *J Anim Ecol* 75:288–297
- Moya-Laraño J (2002) Senescence and food limitation in a slowly ageing spider. *Funct Ecol* 16:734–741
- Moya-Laraño J, Macías-Ordóñez R, Blanckenhorn WU, Fernández-Montraveta C (2008) Analysing body condition: mass, volume or density? *J Anim Ecol* 77:1099–1108
- Moya-Laraño J, Orta-Ocaña JM, Barrientos JA, Bach C, Wise DH (2003a) Intriguing compensation by adult female spiders for food limitation experienced as juveniles. *Oikos* 101:539–548
- Moya-Laraño J, Pascual J, Wise DH (2003b) Mating patterns in late-maturing female Mediterranean tarantulas may reflect the costs and benefits of sexual cannibalism. *Anim Behav* 66:469–476
- Newman JA, Elgar M (1991) Sexual cannibalism in orb-weaving spiders: an economic model. *Am Nat* 138:1372–1395
- Papke MD, Riechert SE, Schulz S (2001) An airborne female pheromone associated with male attraction and courtship in a desert spider. *Anim Behav* 61:877–886
- Parellada X (1998) Identificació i dades biològiques de tres espècies de taràntules (*Araneae*: *Lycosidae*) al Garraf. II Trobada d'Estudiosos Del Garraf 26:15–25
- Persons MH, Uetz GW (2005) Sexual cannibalism and mate choice decisions in wolf spiders: influence of male size and secondary sexual characters. *Anim Behav* 69:83–94
- Planas E, Fernández-Montraveta C, Ribera C (2013) Molecular systematics of the wolf spider genus *Lycosa* (*Araneae*: *Lycosidae*) in the western Mediterranean Basin. *Mol Phylogenet Evol* 67:414–428
- Polis GA (1981) The evolution and dynamics of intraspecific predation. *Ann Rev Ecol Syst* 12:225–251
- Prenter J, MacNeil C, Elwood RW (2006) Sexual cannibalism and mate choice. *Anim Behav* 71:481–490
- Pruitt JN, Berning AW, Cusack B, Shearer TA, McGuirk M, Coleman A, Eng RYY, Armagost F, Sweeney K, Singh N (2014) Precopulatory sexual cannibalism causes increase egg case production, hatching success, and female attractiveness to males. *Ethology* 120:453–462
- Pruitt JN, Keiser CN (2013) Debates: the aggressive spillover hypothesis: existing ailments and putative remedies. *Ethology* 119:807–810
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. Accessed 15 February 2016

- Rabaleda-Bueno R (2014) El canibalismo sexual en la tarántula ibérica (*Lycosa hispánica*): ecología y evolución de estrategias conductuales. Dissertation, Universidad Autónoma de Madrid
- Rabaleda-Bueno R, Aguado S, Fernández-Montraveta C, Moya-Laraño J (2014) Does female personality determine mate choice through sexual cannibalism? *Ethology* 120:238–248
- Rabaleda-Bueno R, Rodríguez-Gironés MA, Aguado de la Paz S, Fernández-Montraveta C, De Mas E, Wise DH, Moya-Laraño J (2008) Sexual cannibalism: high incidence in a natural population with benefits to females. *PLoS One* 3:e3484
- Roberts JA, Uetz GW (2005) Information content of female chemical signals in the wolf spider, *Schizocosa ocreata*: male discrimination of reproductive state and receptivity. *Anim Behav* 70:217–223
- Roggenbuck H, Pekar S, Schneider JM (2011) Sexual cannibalism in the European garden spider *Araneus diadematus*: the roles of female hunger and mate size dimorphism. *Anim Behav* 81:749–755
- Rowe L, Arnqvist G, Sih A, Krupa JJ (1994) Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends Ecol Evol* 9:289–293
- Schneider JM (2014) Sexual cannibalism as a manifestation of sexual conflict. *Cold Spring Harb Perspect Biol* 6:a017731
- Schneider JM, Elgar MA (2001) Sexual cannibalism and sperm competition in the golden orb-web spider *Nephila plumipes* (Araneidae): female and male perspectives. *Behav Ecol* 12:547–552
- Schneider JM, Lubin Y (1998) Intersexual conflict in spiders. *Oikos*:496–506
- Uetz GW, Norton S (2007) Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. *Behav Ecol Sociobiol* 61:631–641
- Verdeny-Vilalta O, Fox CW, Wise DH, Moya-Laraño J (2015) Foraging mode affects the evolution of egg size in generalist predators embedded in complex food webs. *J Evolution Biol* 28:1225–1233
- Welke KW, Schneider JM (2012) Sexual cannibalism benefits offspring survival. *Anim Behav* 83:201–207
- Wilder SM, Rypstra AL (2008a) Sexual size dimorphism predicts the frequency of sexual cannibalism within and among species of spiders. *Am Nat* 172:431–440
- Wilder SM, Rypstra AL (2008b) Sexual size dimorphism mediates the occurrence of state-dependent sexual cannibalism in a wolf spider. *Anim Behav* 76:447–454
- Wilder SM, Rypstra AL (2010) Males make poor meals: a comparison of nutrient extraction during sexual cannibalism and predation. *Oecologia* 162:617–662
- Wilder SM, Rypstra AL, Elgar MA (2009) The importance of ecological and phylogenetic conditions for the occurrence and frequency of sexual cannibalism. *Ann Rev Ecol Evol S* 40:21–39
- Wu L, Zhang H, He T, Liu Z, Peng Y (2013) Factors influencing sexual cannibalism and its benefit to fecundity and offspring survival in the wolf spider *Pardosa pseudoannulata* (Araneae: Lycosidae). *Behav Ecol Sociobiol* 67:205–212
- Wilgers DJ, Hebets EA (2012) Age-related female mating decisions are condition dependent in wolf spiders. *Behav Ecol Sociobiol* 66:29–38