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Sexual cannibalism in the spider *Alpaida veniliae* (Keyserling 1865) (Araneae: Araneidae)

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Abstract. Postmating cannibalism where a female attacks, kills and consumes a male after a sexual encounter is frequently influenced by certain male morphological and behavioral characteristics. We conducted behavioral assays in the laboratory to test the predictions that male *Alpaida veniliae* (Keyserling 1865) with larger absolute and relative size in relation to their mate and those having longer courtship and copulation duration would have lower probability of being cannibalized by females after a sexual encounter. We performed a set of mating trials exposing males of different sizes to virgin females. We observed copulation in 88.8% of mating trails; its duration was very brief compared to courtship. Only a few attempts (16.7%) of recopulations with the same female were recorded, and in all these cases the first copulation was significantly shorter than the mean copulation duration of those who had only one copulation. The percentage of postcopulatory cannibalism was 47.6%. There was no correlation between the relative and absolute male size and duration of courtship and copulation. Postcopulatory cannibalism was independent of courtship and mating durations but was affected by absolute and relative male size. Smaller males were more frequently cannibalized than large ones. However, it remains unclear whether sexual cannibalism in *A. veniliae* may be explained by female mate choice or whether smaller males are less able to escape or defend themselves. More studies are needed to understand the underlying factors of postcopulatory cannibalism of *A. veniliae*, as well as to elucidate their possible ecological and evolutionary implications.

Keywords: Sexual behavior, courtship, copulation, postcopulatory cannibalism

Sexual cannibalism is a common behavior in some spider families, including Araneidae, Theridiidae and Pisauridae. In this process, the female attacks, kills and consumes a male before, during or after a sexual encounter (Elgar 1992; Elgar & Schneider 2004; Schneider & Andrade 2011). Many ecological factors such as food availability, population density, sex ratio, and body mass of individuals affect the selective benefits of cannibalism (Wilder et al. 2009). The adaptive significance of sexual cannibalism is often sex-specific because the costs and benefits for males and females can differ significantly, and depends on the time of occurrence during sexual interaction and if the mating system is monogamous or polygamous (Newman & Elgar 1991; Elgar 1992; Andrade 1996, 1998; Elgar & Schneider 2004).

Differential sexual cannibalism takes place when females respond differently to certain conspecific males according to some measurable morphological or behavioural trait. However, to date, studies where cannibalism occurs during or after copulation do not provide strong evidence that differential postcopulatory cannibalism occurs (Prenter et al. 2006). Male size, both absolute and relative to female size, often predicts the likelihood of cannibalism. Wilder & Rypstra (2008) tested for a relationship between sexual size dimorphism and sexual cannibalism within and among species of spiders and found that cannibalism was more likely when males were much smaller than females. Elgar & Nash (1988) observed in *Araneus diadematus* Clerck 1757 that sexual cannibalism was determined by male body size. While females attacked males of different sizes at approximately the same rate, relatively larger males were better than smaller males at resisting attack. Roggenbuck et al. (2011) also reported that smaller males of *A. diadematus* were more likely cannibalized than the larger ones. Similarly, in the wolf spider *Pardosa pseudoannulata* (Bösenberg & Strand 1906), Lingbing et al. (2013) found that there was a strong positive relationship between mate size dimorphism and the occurrence of sexual cannibalism. In contrast, mating advantages for smaller males are reported in *Argiope keyserlingi* Karsch 1878 (Elgar et al. 2000), and in *Nephila edulis* Labillardière 1799 (Schneider et al. 2000).

Courtship behavior may function to reduce cannibalism by the female. During courtship, both sexes can transfer information on species identity, mating status and quality (Wignall & Herberstein 2013). Andrade & Banta (2001) experimentally manipulated courtship duration in the sexually cannibalistic redback spider (*Latrodectus hasselti* Thorell 1870) and did not find any difference in size, mass or condition between cannibalized and noncannibalized males, but noted that males exhibiting short courtship had lower mating success and experienced more female rejection behavior than did males with long courtship.

Another factor that seems to be related to postcopulatory cannibalism is copulation duration which has long been thought to be one of the most important measures of male reproductive success (Simmons 2001). While it is presently recognized that postcopulatory cannibalism can extend or shorten the duration of copulation for cannibalized males compared to survivors, specific details of female discriminatory behavior remain unclear (Prenter et al. 2006). Although postcopulatory cannibalism was not significantly related to any male morphological trait in the orb-weaving spiders *Argiope aurantia* Lucas 1833 and *Argiope bruennichi* (Scopoli 1772) (Fromhage et al. 2003; Foellmer & Fairbairn 2004; Schneider et al. 2006), it was correlated with prolonged copulation duration. Moreover, Andrade (1996) showed that males of *Latrodectus hasselti* were cannibalized during or after copulation and those that were cannibalized had longer

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copulations and fertilized a larger proportion of the eggs than males that were not eaten.

Alpaida veniliae (Keyserling 1865) (Araneae, Araneidae) is one of the most abundant orb weaving spiders in transgenic soybean crops in Buenos Aires province, Argentina. Our previous studies have focused primarily on biological and ecological attributes that indicate *A. veniliae*'s importance as a predator of soybean crop pests (Minervino 1996; Benamú et al. 2011). *Alpaida veniliae* is moderately size–dimorphic and most females are monogamous. Laboratory observations (Benamú et al. 2012) described the process of courtship, mating and postmating behavior of this species. Courtship and copulation occurred in the capture web of the females, and courtship represented 75% of the total duration of a sexual encounter with a male. These observations suggested that male courtship is critical for recognition by the female and for avoiding female aggression. Precopulatory cannibalism was not observed in *A. veniliae* and 50% of males were cannibalized post-copulation. There were no genital plugs and during copulation the male inserted only one of the two pedipalps into only one of the paired copulatory ducts of the female. The males did not try to insert the second palp.

Although several studies have investigated many potential causes of cannibalism separately, there is a paucity of studies that tested multiple causes simultaneously. Here we conducted behavioral assays in the laboratory to test the predictions that males with larger absolute and relative size (male/female ratio) and those having longer courtship and copulation durations would have lower probability of being cannibalized by females after a sexual encounter.

METHODS

Study system.—We collected adult gravid females and adult males of *A. veniliae* in transgenic soybean crops located in Chivilcoy (35°01′ S, 60°06′ W), Buenos Aires, Argentina, and reared them at 25 ± 2 °C, 75 ± 5% RH and a photoperiod of 16:8 (L:D). Egg-sacs were incubated and spiderlings were reared until adulthood. Juveniles and adults were transferred to 500 ml transparent glass jars. Once a week, juveniles were fed *ad libitum* with *Drosophila melanogaster* and subadults and adults were fed with *Musca domestica*.

Experimental protocol.—Adult virgin females collected at random from the colony were placed individually in glass frames (15 × 10 × 5 cm) to allow web building. The female was placed in the cage three hours before introducing the male, and fed with one *Musca domestica* immediately before the experiment to avoid cannibalism elicited by hunger. We selected virgin and non-virgin males from the colony, choosing individuals that differed conspicuously in size (total body length). Males were assigned at random to each female and each was set on the bottom of a frame so it could detect the female's pheromones on the threads of the web (n = 116 pairs). At the end of the experiment individuals were preserved in 75% ethanol. We measured body length of each individual using an optical stereo microscope (NIKON SMZ-10) with an ocular micrometer and calculated the relative size (male/female ratio).

During mating trials, we defined a male as being cannibalized if the female wrapped it in silk after copulation. In these cases, the mating pair was immediately removed and killed so that we could measure the male before the female could consume it. If, after copulation, the female and male unhooked and the male jumped away from the female, we interpreted this as being no cannibalism. The mating pair was removed, killed, and measured.

Durations of courtship, first and second copulations, and palp insertion and the occurrence of post-mating cannibalism were video-recorded using a SONY HDR-XR160 High-Definition Handycam with 30X optical zoom. Recorded data were analyzed by Pinnacle Studio 9 (version 3.8).

Voucher specimens.—All specimens of *A. veniliae* used in this study were deposited in the insect collection at the Museum of Natural Sciences of La Plata (UNLP), Argentina.

Statistical analysis.—To assess for differences in body size between male and female, as well as in duration of courtship and copulation between the first and second copulation, paired Student t tests were used. The frequency of insertion of the right or left palp during copulation was analyzed by chi-square contingency test. To examine the dependence between the absolute and relative male size (male/female ratio) and duration of courtship and copulation, the Pearson correlation coefficient (r) was used. To test the effect of absolute and relative male size (male/female ratio) and duration of courtship and copulation on postmating cannibalism, we used generalized linear models (GLM). A multiple regression model that explicitly assumes binomially distributed errors and a logit link function was used (McCullagh & Nelder 1989). Before fitting the model, explanatory variables were checked for multicollinearity. Cannibalism was considered a categorical (binary) response variable, and courtship duration, copulation duration, male body size, and male/female size ratio as explanatory variables. The model was fitted with maximum likelihood, and the statistical significance of each variable was tested in turn by a stepwise procedure, and the Wald statistic was used to test the significance of each regression coefficient. Statistical analyses were performed using Statistica v. 7 (StatSoft 2007), and in all tests, $P < 0.05$ was considered significant.

RESULTS

Alpaida veniliae females were significantly larger than males (t = 22.46; df = 204; $P < 0.001$); body length (mean ± SE) was 6.08 ± 0.03 mm (n = 103) for females and 5.07 ± 0.03 (n = 103) mm for males. The ratio male/female body length (mean ± SE) was 0.84 ± 0.009 (n = 103).

Spider pairs copulated in 103 (88.8%) out of 116 observed trials. Males started courtship with alternating constant and intense vibratory movements of the third pair of legs on the threads of the female's web. If the female did not react aggressively, the male constructed a mating thread at the edge of the orb web. Then, males engaged in a series of vibratory movements with their legs and abdominal shudders on the mating thread with no direct female contact. The female then advanced over the mating thread, slipped down, apparently being held only by the third and fourth pairs of legs, and adopted the receptive posture. Male and female touched each other repeatedly with their first and second pair of legs, while the male performed abdominal shaking and rubbed the female epigyne with his palps until finally assuming the copulation position. Duration of copulation was very brief compared to

Table 1.—Correlations between absolute and relative male size and duration of courtship and copulation in *Alpaida veniliae*.

Variable	*r*	*n*	*P*
Male size			
courtship	0.079	105	0.419
copulation	−0.109	105	0.265
Male/female ratio			
courtship	0.033	103	0.734
copulation	0.085	103	0.390

courtship; mean duration was 10.99 ± 0.11 (n = 103) seconds for copulation and 278.05 ± 6.54 (n = 103) seconds for courtship, and they were not correlated with either the absolute male size or the relative male size (male/female ratio) (Table 1). Males inserted a single palp during copulation and the right one was used more often than the left one (χ^2 = 25.81; $P < 0.001$).

When the male was not cannibalized after mating, both sexes unhooked from each other quickly and the male jumped out of the female's reach, cut the mating thread and left. The female stopped being receptive and kept the male away with aggressive attacks or by eating its own web, preventing a new courtship by that male or others.

Premating cannibalism was never observed in 103 mating trials. The percentage of postcopulatory sexual cannibalism, i.e., trials in which females devoured males after copulation, was 47.6% (n = 49). We recorded nine attempts of a second copulation. In all nine pairs, the first copulation lasted 6.22 ± 0.27 seconds, which was significantly shorter than the mean duration of copulation of those who had only one copulation (t = 11.35; df = 110; $P < 0.001$). When the male attempted to copulate again with the same female, it used the other palp and repeated the courtship, which was significantly longer (603.22 ± 22.5 seconds) than the first (t = 3.92; df = 112; $P < 0.001$). Duration of the second copulation was very similar (11.67 ± 0.3) to the duration of the first copulation of males that did not remate (t = 1.604; df = 112; P = 0.111). Percentage of postcopulatory sexual cannibalism after remating was 55.6% (n = 5).

Postcopulatory cannibalism was not related to the duration of either courtship or copulation (Table 2). By contrast, absolute and relative male size significantly affected postcopulatory cannibalism. Postcopulatory cannibalism was inversely related to male size and to male/female size ratio. Thus, the larger the absolute size of the male and the higher the ratio of male/female size of the interacting pair, the lower the likelihood that the male would be cannibalized.

DISCUSSION

We found that mating behavior of *Alpaida veniliae* strongly resembles those of related araneids (Robinson 1982). We conclude that absolute and relative size of a male in relation to the female affected the occurrence of postcopulatory cannibalism. Smaller males were more frequently cannibalized than large ones. Furthermore, postcopulatory cannibalism was not related to the length of courtship or mating. Sexual cannibalism was common in this species, with ≈ 50% of males cannibalized after their first insemination.

Table 2.—Logistic regression analysis of the probability of cannibalism of the male by the female of *Alpaida veniliae* in the laboratory.

Predictor	Coefficient	S.E.	Wald	*P*
Intercept	12.955	5.435	5.682	0.017
Courtship duration	−0.002	0.003	0.490	0.483
Copulation duration	0.200	0.172	1.344	0.246
Male size	−1.840	0.709	6.739	0.009
Male/female ratio	−6.489	2.629	6.089	0.013

In accordance with our results, there is some evidence that spider females in other species react differently to some conspecific males and that discrimination relies on measurable morphological or behavioral traits such as size and aggressiveness (Prenter et al. 2006; Kralj-Fišer et al. 2012). Prenter et al. (2006), analyzing original data from Rubenstein (1987) in *Metellina segmentata* (Clerck 1757), confirmed that smaller males were cannibalized at a significantly greater frequency than larger males.

The frequency of postcopulatory cannibalism in *A. veniliae* is in agreement with that of other sexually size monomorphic species like *Araneus diadematus* (Roggenbuck et al. 2011). We showed here that post-insemination sexual cannibalism is driven by size differences between the mating partners, a finding that is in accord with the sexual size dimorphism (SSD) hypothesis proposed by Wilder & Rypstra (2008).

As in other orb-web spiders (Maklakov et al. 2003), courtship of *A. veniliae* involved males generating vibrations on the web. Some studies suggest that courtship and signalling during courtship functions to alter the likelihood of cannibalism (pre- or postcopulatory) (Eberhard & Huber 1998; Maklakov et al. 2003; Wignall & Herberstein 2013). Stoltz et al. (2008, 2009) reported that, when males of the redback spider *Latrodectus hasselti* compete with rivals in the presence of females, those that exhibited shorter courtship were cannibalized before mating was completed, while longer courting males were able to inseminate both sperm storage organs. Females employ premature cannibalism to reduce the paternity of males that had reduced investment in courtship if they were clearly distinct from their rivals. Although in our experiments courtship was the most prolonged part of the sexual activity, its duration was not related to postcopulatory cannibalism. Given that our trials were carried out using individual pairs, we are unable to speculate about whether the presence of other males within a competitive context might affect the cannibalistic response of *A. veniliae* female to the duration of male courtship.

The brief copulation observed in *A. veniliae*, possibly due to the insertion of a single male palp, is similar to that of *Argiope bruennichi* (Schneider et al. 2005, 2006). Given the duration of copulation and that females mate only once raises the question of whether this time is enough for a complete fertilization. According to these authors, males of *A. bruennichi* can transfer 50% of their sperm in the 10 seconds, which is the duration of the whole mating, and even during an average mating of 8 seconds they can transfer enough sperm to ensure a full fertilization. As the duration of mating in *A. veniliae* is similar to that of *A. bruennichi*, it is also possible that a complete fertilization takes place during mating. Additionally, when Snow & Andrade (2004) tested duration-dependent sperm

transfer in the redback spider, *L. hasselti*, the found that although copulations ranged between 5 and 20 min, the redback males transferred the majority of their sperm within the first 5 min of copulation.

Interestingly, second copulations took place only when the duration of the first mating was less than 10 seconds, suggesting that this length of time was too short for a complete insemination. In these cases, and probably to overcome the resistance of the female, the male had to perform a significantly longer courtship for a successful second copulation.

We demonstrated that absolute and relative male's size affected postmating cannibalism, although from our data we cannot conclude whether sexual cannibalism in *A. veniliae* is a mechanism of mate choice or whether smaller males are less competent to escape or defend themselves (Elgar & Nash 1988; Elgar 1992; Persons & Uetz 2005; Prenter et al. 2006). Sexual conflict in this species should be lower compared with those that have premating cannibalism. However, further studies are needed to understand the underlying factors of postcopulatory cannibalism in *A. veniliae*, as well as to elucidate their possible ecological and evolutionary implications.

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