

Female control of paternity in the sexually cannibalistic spider *Argiope keyserlingi*

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Sexual conflict theory predicts an antagonistic coevolution, with each sex evolving adaptations and counter-adaptations to overcome a temporary dominance of the other sex over the control of paternity. Polyandry allows sexual selection to operate after mating has commenced, with male and female interests competing for control of fertilization. There are numerous examples of male control of paternity, but few studies have unambiguously revealed female control. Attributing variance in paternity to females is often difficult since male and female influences cannot be separated unambiguously. However, we show that polyandrous female orb-web spiders *Argiope keyserlingi* (Araneidae) control the paternity of their offspring by adjusting the timing of sexual cannibalism. Our experiments reveal that females copulating with relatively smaller males delay sexual cannibalism, thereby prolonging the duration of copulation, and that these males consequently fertilize relatively more eggs.

Keywords: sperm competition; cryptic female choice; sexual cannibalism; sexual conflict

1. INTRODUCTION

Studies of sperm competition and sexual selection have, until recently, emphasized adaptations that enhance the competitive abilities of males in the struggle over fertilization success (Birkhead & Møller 1998). In theory, males are under stronger selection to control fertilization than females because males have more to lose by failing to fertilize eggs than do females mating with inferior partners (Parker 1984; Stockley 1997; Simmons & Siva-Jothy 1998). Nevertheless, females may 'set the play-ground' (*sensu* Eberhard 2000) because sperm uptake, storage and fertilization occur within the female's reproductive tract (see also Stockley 1997). While there is compelling evidence that females play an important role in controlling sperm competition (e.g. Thornhill 1976; Simmons 1986, 1991; Watson 1998; Eberhard 1996, 1998), their ability to discriminate or choose between sperm from different males is ambiguous. Indeed, some authors doubt whether cryptic female choice can overcome male adaptations to sperm competition (Birkhead 1998; Birkhead & Møller 1998; but see Eberhard 2000).

A major problem in demonstrating cryptic female choice unambiguously is that traits favoured by females are often the same traits that may give one male a direct advantage over another. It is therefore often difficult to clearly separate female from male contributions to variation in paternity. Birkhead (1998) and Birkhead & Møller (1998) suggest three criteria that should be met in order to establish unambiguously that paternity is under female control. These include demonstrating that (i) there is variation in P_2 , the proportion of offspring sired by the last male; (ii) that some of this variation is attributable to females; and (iii) that the variation in P_2 is linked to a male characteristic. The second criterion is usually the most difficult to meet because the trait under selection and the mechanism of selection are often confounded. This is clearly illustrated by the common correlation

between male size and the duration of copulation (e.g. Elgar 1995; Simmons & Siva-Jothy 1998; but see Archer & Elgar 1999).

Polyandrous females may exert choice over the paternity of their offspring by regulating the quantity of sperm transferred to their spermatheca by a particular male, assuming that the sperm of several males compete numerically in a fair raffle (see Parker 1998). Controlling the duration of copulation is one mechanism of regulating the quantity of sperm transferred; in several insects, the number of sperm transferred by a male is a linear function of time spent copulating (e.g. Thornhill 1976; Simmons & Siva-Jothy 1998). However, determining which sex controls the duration of copulation can be difficult because sexual selection may favour mechanisms that allow either sex to prolong or terminate copulation. For example, males of many insects provide females with nuptial gifts prior to copulation, and males with larger gifts mate for longer and apparently transfer more sperm to the spermatheca (see Vahed 1998). If the variation in P_2 is correlated with male body size, it remains unclear whether females actively prefer large males or whether large males have a competitive advantage over other males by providing a larger gift and transferring more sperm. Indeed, most correlations between the duration of copulation and a male trait can be difficult to interpret; are particular males better equipped to prolong copulation or do females prefer these males and thus allow copulation to persist for longer?

The sexually cannibalistic behaviour of the orb-web spider *Argiope keyserlingi* provides an excellent opportunity to demonstrate that variation in paternity is under female control. Typically, males of *A. keyserlingi* are cannibalized shortly after mating has commenced; copulation ceases as soon as the male is captured and wrapped by the female. Males of *A. keyserlingi* attempt vigorously to escape capture, even though the majority do not survive copulation. This suggests that, unlike other species (e.g. Sasaki & Iwahashi 1995; Andrade 1996), there is little male complicity and sexual cannibalism is not a male mating strategy to increase

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paternal investment (e.g. Buskirk *et al.* 1984) or fertilization success (Elgar 1992; Andrade 1996). Instead, sexual cannibalism may allow females to control the timing of sexual cannibalism and thus the duration of copulation.

Here, we investigate whether sexual cannibalism in *A. keyserlingi* is a female strategy to control the paternity of her offspring, a male or female strategy to increase female fecundity, or a male strategy to increase his fertilization success. We also examine whether the opportunity for sperm competition influences the frequency of sexual cannibalism.

2. MATERIAL AND METHODS

Subadult male and female *A. keyserlingi* were collected from the Brisbane metropolitan region during October 1997 and 1998. The spiders were maintained in separate containers on a diet of bush-flies (*Lucilia cuprina*) and water. Mature females were transferred to Perspex frames (59 cm × 58 cm × 15 cm) where they constructed typical orb-webs. The patterns of paternity were determined using standard double-mating trials (Parker 1970); mature males were randomly assigned either normal (N) or irradiated (I) treatments; the latter were subjected to 10 krad from a cobalt γ -emitter. The proportion of developed eggs was used to calculate P_2 , the number of eggs fertilized by the second male. The mean proportion of eggs that developed from matings with normal males only was 0.96, s.d. = 0.02, $n = 5$ (mean clutch size = 202.8, s.d. = 67.1, $n = 5$). No eggs developed from matings with irradiated males only (mean clutch size = 213.4, s.d. = 65.6, $n = 9$).

Adult females were weighed and then randomly assigned to one of two mating treatments that altered the likelihood of competition between males; in one, a normal (N) and an irradiated (I) male were simultaneously placed in the frame. In the other, the first male was removed after mating had taken place and replaced with the second male. The sequence of normal and irradiated males in these treatments was randomized. P_2 was not influenced by the sequence of normal and irradiated males. The NI group showed a mean P_2 of 0.51 (s.e. = 0.06, $n = 22$) and the IN group had a mean P_2 of 0.49 (s.e. = 0.07, $n = 26$). The difference was not significant ($F_{1,46} = 0.05$, $p > 0.8$) indicating that irradiation had no measurable impact on the competitiveness of the sperm. Males were weighed a few minutes before the mating trial, and then placed in the lower corner of the frame.

Typically, the male walked up the side of the frame and onto the thread. He traversed the web to the hub, and then proceeded a short distance from the hub where he cut a hole in the web across which he stretched a mating thread (see also Robinson & Robinson 1980). Courtship and mating take place on this thread; the male strums the mating thread with his legs and the female ventures onto the web and assumes a copulatory position that exposes her epigyne. The male then leaps onto the female, inserts one pedipalp and twists his body away from the female's mouthparts. Males do not change pedipalps during a copulatory bout. Copulation ends when the male either leaps off the female or when she commences wrapping and capturing him. We measured the time from when the male inserted his pedipalp to when copulation ceased; either when he removed his pedipalp and leapt off the female or when the female started to wrap him with silk.

After the mating trial, the female was removed from the frame and returned to her container, where she was maintained on a diet of bush-flies and water. All egg sacs were removed from the spider's container and placed in small sterile vials, sealed with a perforated cap and housed in an incubator at

24 °C. The eggs were inspected daily for normal development, which was usually obvious after 14 days. The developed and undeveloped eggs were counted under a microscope.

Adult female *A. keyserlingi* of unknown reproductive status were located in a single field population in Sydney, and individually marked. Over a period of several weeks, while addressing other research questions, we noticed opportunistically when males entered the web of a female and subsequently recorded whether the male mated with the female and if he was subsequently cannibalized.

3. RESULTS

Sexual cannibalism was frequently observed in both staged laboratory (80 out of 138) and natural field (seven out of ten) matings. The fecundity of females that did not cannibalize a male (mean = 265, s.e. = 38 eggs, $n = 10$) was not significantly different from females that consumed either one (mean = 263, s.e. = 12 eggs, $n = 18$) or two (mean = 254, s.e. = 28 eggs, $n = 19$) males (ANCOVA, number of males, $F_{2,28} = 0.09$, n.s.; female weight, $F_{1,28} = 4.02$, $p < 0.06$; power, $1 - \beta = 0.99$). This is not surprising because the mass of a mature male (mean = 0.015 g, s.d. = 0.004, $n = 113$) is *ca.* 7% of a recently matured female (mean = 0.212 g, s.d. = 0.060, $n = 46$), and would be even less of older, more fecund females. There was also no evidence that the frequency of cannibalism was influenced by the condition of the female when she first mated. The condition of females (body mass/tibia–patella length of first leg) did not differ significantly among females that subsequently consumed no males (mean = 0.26, s.e. = 0.02, $n = 3$), one male (mean = 0.32, s.e. = 0.02, $n = 13$) or two males (mean = 0.35, s.e. = 0.02, $n = 6$; $F_{2,19} = 2.65$, $p = 0.1$).

Double matings involving a sterile male revealed considerable variation in P_2 , which ranged from 0 to 0.98 (mean = 0.499, s.d. = 0.288, $n = 41$). P_2 was not influenced by cannibalism of either the first ($F_{1,44} = 0.12$) or second ($F_{1,44} = 2.66$, $p > 0.10$) male (interaction, $F_{1,44} = 0.82$). If sexual cannibalism were a male strategy to increase paternity, it should be more common when there was greater opportunity for sperm competition. However, the frequency of cannibalism did not differ between virgin (43 out of 70 matings) and mated (37 out of 68 matings) females ($\chi^2 = 1.16$, $p > 0.2$). Nor was the frequency of cannibalism influenced by whether there was one (33 out of 62 matings) or two (47 out of 76 matings) males on the web at the time of mating ($\chi^2 = 1.04$, $p > 0.3$).

The duration of copulation is unambiguously under female control in those cases where the female cannibalized the male, since copulation ceased only when she commenced wrapping the male in silk. In these cases, P_2 was significantly greater if the second male copulated for relatively longer than the first male (figure 1). If females were attempting to control paternity, then they should do so according to some feature of the male. Analysis of covariance revealed that the difference in copulation duration was significantly influenced by the size difference between the two males ($F_{1,56} = 5.53$, $p < 0.025$), and whether the second male was cannibalized ($F_{1,56} = 6.25$, $p < 0.02$). Thus, cannibalized males mate for longer and, in cases where the female cannibalized the second male, relatively smaller second males copulated for relatively longer (figure 2).

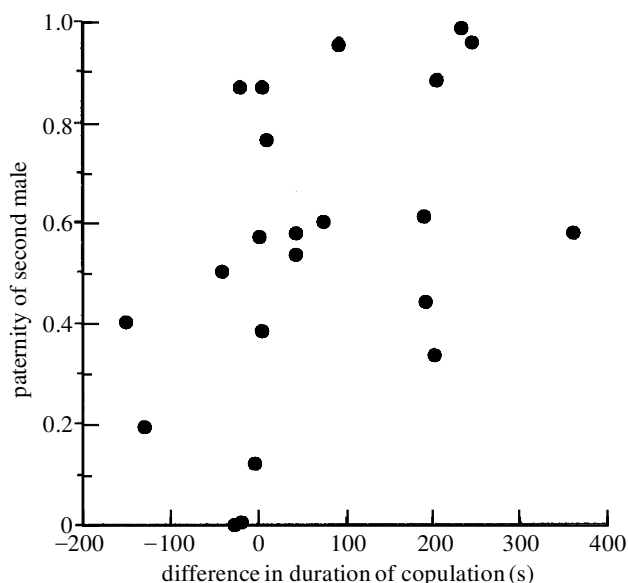


Figure 1. The proportion of eggs fertilized by the second male, P_2 , is significantly greater if he copulates for relatively longer than the first male ($r = 0.447$, $n = 22$, $p < 0.04$). The duration of copulation is determined by the onset of sexual cannibalism, and thus is under female control. The correlation remains significant when both males are cannibalized ($r = 0.483$, $n = 18$, $p < 0.05$).

The correlations between relative male size, relative duration of copulation and share of paternity were not evident among copulations in which females did not cannibalize the male and hence did not have unambiguous control over the duration of copulation. There was no correlation between the difference in the size of the males and the difference in duration of copulation when the second male was not cannibalized ($r = 0.125$, $n = 26$, $p > 0.5$).

There was no evidence of an inherent advantage of small male size in escaping cannibalism and thereby prolonging copulation. In fact, small males tended to be more frequently cannibalized than large males (first male, $t_{64} = 1.73$, $p = 0.07$; second male, $t_{64} = 1.85$, $p = 0.09$). Furthermore, the variation in the duration of copulation was not explained by the size of either the first ($r = 0.084$, $n = 40$, $p > 0.6$) or the second male ($r = 0.224$, $n = 33$, $p > 0.2$) when either the first or second male was cannibalized, respectively.

Finally, it is possible that the males assessed the size of a rival when both males were on the web simultaneously and adjusted the duration of copulation accordingly. However, whether males were presented simultaneously or consecutively had no significant effect on how long the second male mated ($F_{1,30} = 0.13$, $p > 0.7$) or on the difference in the duration of copulation ($F_{1,30} = 0.84$, $p > 0.3$). Male size was not a significant covariate in either model.

4. DISCUSSION

Females delay cannibalizing their second mate if he is relatively smaller than the first, which results in the second male fertilizing a larger proportion of her clutch of eggs. Females adjust the paternity of a preferred male through the timing of cannibalism. Females that captured

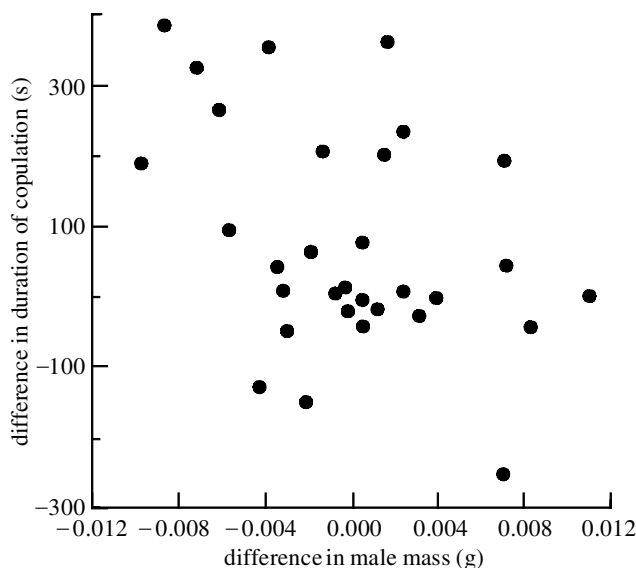


Figure 2. In matings where the second male is cannibalized, relatively smaller males copulate for relatively longer; the difference in the duration of copulation between the first and second male is significantly correlated with the difference in their size ($r = -0.397$, $n = 31$, $p < 0.03$).

two males cannibalized the second male after a relatively longer copulation if she preferred him to the first male. Confounding effects can be largely excluded in our study because the observed difference was only apparent in paired comparisons when the female cannibalized both males. For example, the duration of copulation did not differ between virgin and mated females and small males did not necessarily copulate for longer than large males. Thus, there was no covariance between the preferred male trait and the choice mechanism. More significantly, there was no correlation between male size difference and copulation duration in the absence of cannibalism, and male size *per se* was not associated with the duration of copulation or the likelihood of cannibalism.

Females do not consume the captured male while he is copulating, unlike other sexually cannibalistic spiders (e.g. Andrade 1996), but first wrap him in silk. This causes an immediate dislocation of the intromittant organ of the male from the genital opening of the female. Thus, sexual cannibalism allows female *A. keyserlingi* to exert sequential mate choice by unambiguously terminating copulation in a remarkably efficient manner. It is important to note that the data revealing female control of paternity include only cases where the female cannibalizes the male. Thus, these data reflect the females' decision when, not whether, to cannibalize their mate.

Several further lines of evidence indicate that the timing of sexual cannibalism in *A. keyserlingi* is under female control and is not an adaptation favouring male fitness. First, unlike other species (Andrade 1996; Sasaki & Iwahashi 1995), there was little indication of male complicity, since males invariably struggled to escape capture. Out of a total of 52 males that survived copulation, 41 lost at least one leg, either as they ceased copulating or while dismounting from the female. Moreover, there was no evidence that sexual cannibalism *per se* increased the share of paternity of cannibalized males (cf. Andrade 1996), or that it was more common

under conditions of sperm competition and perhaps, therefore, functioning as a sperm protection device.

There was no evidence that sexual cannibalism in *A. keyserlingi* represents a female foraging strategy to gain nutrients for egg production (see Newman & Elgar 1991; Andrade 1998; Maxwell 2000). The fecundity of females in our experiments was not increased by the consumption of one or two males. It is possible that the feeding regime of these spiders was sufficiently high that it masked any nutritional gains from eating males. However, the number of eggs per egg sac of our captive females (mean = 260 ± 14 eggs, $n = 48$) is well within the range of that of females collected in the field (mean = 296 ± 46 , $n = 26$; Bradley 1993). These data also indicate that cannibalism provides no benefit to the male through offsetting any loss of future mating opportunities by increasing the fecundity of his cannibalistic mate (Buskirk *et al.* 1984; Elgar 1998). The negative correlation between relative male size and relative duration of copulation (figure 2) could be interpreted as a female foraging strategy, if females were more motivated to capture and consume larger males. However, this is unlikely because there was no significant correlation between absolute male size and the timing of cannibalism by females mating with either their first or their second male. Finally, the cannibalistic behaviour of females of *A. keyserlingi* is not influenced by their previous feeding experience (M. E. Herberstein, J. M. Schneider and M. A. Elgar, unpublished data).

Our experimental data therefore meet each of the three criteria proposed by Birkhead & Møller (1998) for demonstrating cryptic female choice. First, the proportion of eggs fertilized by each male in double matings was highly variable, with neither the first nor second male consistently obtaining greater fertilization success. Second, females unambiguously control part of the variation in P_2 by adjusting the timing of cannibalism and thus the duration of copulation. Finally, females base their choice on a male character, which is male body size in *A. keyserlingi*.

The mating advantage for small male spiders is surprising because larger body size is more typically favoured by sexual selection either through male–male competition or female choice (Andersson 1994; Elgar 1998; Simmons & Siva-Jothy 1998). The reasons that females of *A. keyserlingi* prefer small males may be similar to those that have driven the evolution of relatively small male size in this species (Elgar 1991, 1992, 1998; Vollrath & Parker 1992; Head 1995; Elgar & Fahey 1996; but see Coddington *et al.* 1997; Prenter *et al.* 1997, 1998). The sexual conflict over the control of fertilization in *A. keyserlingi* appears to be won by the females, thereby suggesting a sequential female choice benefit to polyandry (see Elgar 1998). This result is unusual because theory predicts that selection to control fertilization will be stronger on males than females (Parker 1984). Our study reveals that certain characteristics of the mating system, such as sexual cannibalism, may predispose the female to decide the battle of the sexes in her favour. Of course, this may not be true of all sexually cannibalistic species because the conflict between the sexes is dynamic (e.g. Schneider & Lubin 1998; Lessels 1999), and males may have evolved strategies that overcome female dominance in other sexually cannibalistic mating systems.

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