Males of the orb-web spider *Argiope bruennichi* sacrifice themselves to unrelated females

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Costs of inbreeding can lead to total reproductive failure and inbreeding avoidance is, therefore, common. In classical sex roles with no paternal care, the selective pressure to avoid inbreeding is mostly on the female, which carries the higher costs. In some orb-web spiders, this situation is very different because females are polyandrous and males are monogynous or at most bigynous. Additionally, females of many entelegyne orb weavers are thought to bias paternity post-copulatorily towards a desired mate. This increases the selective pressure on males to adjust their investment in a mating with regard to the compatibility to a female.

Here, we examine whether genetic relatedness influences mating behaviour in the orb-web spider *Argiope bruennichi*. We mated either a sibling or a non-sibling male to a female in single copulation trials and compared copulation duration, cannibalism rate and female fecundity.

Our experiment revealed that males prolonged their copulation duration and were cannibalized more frequently when mating with a non-sibling female. Males mating with a sibling female were more likely to escape cannibalism by copulating briefly, thus presumably increasing their chances of re-mating with a more compatible female. This suggests that males can adaptively adjust their investment relating to the compatibility of a female.

Keywords: sexual cannibalism; male self-sacrifice; inbreeding avoidance; *Argiope bruennichi*

1. INTRODUCTION

Inbreeding occurs if closely related individuals mate and increase homozygosity in their offspring (Pusey & Wolf 1996). This carries a number of risks that can reduce reproductive success up to the total loss of offspring (Charlesworth & Charlesworth 1987; Hoogland 1992).

Classical sex roles imply that females suffer most from inbreeding and that selection should favour mechanisms of inbreeding avoidance in females (Jennions & Petrie 2000). Pre-mating adaptations in this context include, for example, kin recognition through social learning (Dobson *et al.* 1997), olfactory imprinting (Gerlach *et al.* 2008) and sex-specific dispersal (Cockburn *et al.* 1985). Polyandry creates an opportunity for post-copulatory mechanisms to reduce

the costs of inbreeding for females, even in the absence of kin recognition (Cornell & Tregenza 2007). This has been shown in a number of taxa, including mammals (Firman & Simmons 2008), birds (Foerster *et al.* 2003), insects (Arnqvist & Nilsson 2000) and spiders (Welke & Schneider 2009).

In spiders, sedentary species may be particularly prone to inbreeding as eggs are laid in large clutches and often hatch synchronously. While aerial dispersal (ballooning) will distribute the sexes widely, it is not an obligate strategy, as shown for *Argiope bruennichi*, and a proportion of young always stays near the egg sac (Walter *et al.* 2005). Additionally, silk-related dispersal is known to be reduced by inbreeding (Bonte 2009). While avoidance of kin-mating has not been demonstrated in spiders, polyandry can reduce its negative effects up to a total rescue (Bilde *et al.* 2007).

Whereas females avoid inbreeding or reduce its negative consequences, males with an unlimited sperm supply and no paternal investment experience low costs if they mate with a sister (Bateman 1948). This is different in mono- and bigynous mating systems, in which males invest heavily in any single mating and inbreeding may hence carry a large opportunity cost. Monogynous mating systems are taxonomically widespread but are particularly common among spiders (Schneider & Fromhage 2010). Males possess paired mating organs, the pedipalps, and in many monogynous spiders, they are restricted to use each pedipalp only once, leading to a maximum of two copulations in their lifetime (Herberstein *et al.* 2005). Thus, males should be under strong selection to exercise mate choice.

In the cannibalistic and polyandrous orb-web spider *A. bruennichi*, the much smaller males are invariably attacked during their first copulation and cannibalized in up to 80 per cent of the cases (Fromhage *et al.* 2003; Fromhage & Schneider 2005; Schneider *et al.* 2006). All males die after their second copulation, a pattern also known for other *Argiope* species (Sasaki & Iwahashi 1995; Foellmer & Fairbairn 2003). Whether a male survives its first copulation or not depends on the duration of genital contact: males that jump off early (less than 5 s) have a chance of surviving while males that copulate longer (greater than 10 s) invariably die (Schneider *et al.* 2006). Cannibalism and its associated prolongation of copulation positively influence sperm transfer and relative paternity (Schneider *et al.* 2006).

In this study, we examine whether genetic relatedness between mates influences their mating strategies. We predict that males should be less inclined to sacrifice their life when copulating with a sister.

2. MATERIAL AND METHODS

(a) Study animals

We collected egg sacs of *A. bruennichi* on meadows near Hamburg in the Nordheide and Buxtehude, Germany, in February and March 2008. To minimize the risk of collecting more than one egg sac per female, we chose a minimum distance of 50 m between individual egg sacs (females place their egg sac within a radius of 1–2 m).

Egg sacs were placed in plastic containers (15 × 12 × 7 cm) in which the spiderlings hatched. We fed them with *Drosophila* sp. once a week and sprayed with water daily. Once spiderlings built their own webs they were placed in individual plastic cups (250 ml). We watered them 5 days a week and fed them *Drosophila* sp. twice a week. Female and male spiders are easy to distinguish before their final moult because of the male's bulbous copulatory organs (pedipalps). As soon as we could sex females, they were

transferred to bigger plastic cups (500 ml). Males stayed in their cups until we used them for mating experiments. Spiders were all weighed after their final moult. Adult females were transferred into Perspex frames (30 × 30 × 6 cm) in which they built normal-sized webs. Mating trials were conducted in those frames.

(b) Mating procedure

Only virgin spiders were used for the experiment. We defined sibling spiders as individuals that originated from the same egg sac, even though they could be half-siblings. Non-siblings were derived from different egg sacs.

Ninety-one females from 14 families were mated with either one sibling ($n = 45$) or one non-sibling ($n = 46$) male after a preset schedule and a split design. Each trial consisted of a single copulation. Males were weighed before the start of a trial because of sexual cannibalism and the loss of legs during mating. We introduced males with a soft brush on one of the frame threads of the female's web. Males were given a time limit of 2 h to copulate, which none of them exceeded and the mean duration until copulation was equal between the treatments (non-siblings: 22.4 ± 2.9 min; siblings: 24.2 ± 3.7 min). All experimentally matched pairs copulated regardless of the treatment. During a mating trial we documented copulation duration and cannibalism attempts.

Cannibalized males were removed from the female fangs; survivor males were killed by hypothermia.

We weighed females after copulation to minimize disturbance and to avoid web damage prior to the mating trials. After weighing, females were returned into their Perspex frames to build egg sacs. Egg sacs were weighed and then stored in gauze-covered plastic vials. Hatching rate, clutch weight and clutch size were determined for all first egg sacs.

Statistical analyses were performed with JMP 7.0.2. Data were checked for normal distribution using the Kolmogorov–Smirnov test. We used parametric and non-parametric tests as appropriate. Sample sizes differ between analyses because not all data were available for all trials. All values are given as mean $\pm$ s.e.

3. RESULTS

Siblings escaped sexual cannibalism more often than non-siblings (siblings: 46.7%; non-siblings: 21.7%; $\chi^2 = 6.3$, $p = 0.01$, $n = 91$; figure 1). Copulation duration was shorter for siblings (5.81 ± 0.55 s) than for non-siblings (9.03 ± 1.27 s; Mann–Whitney U test: $Z = -2.53$, $p = 0.01$, $n = 89$; figure 2). Corroborating previous results, cannibalism significantly prolongs the duration of copulation duration (logistic regression: $\chi^2 = 30.17$, $p < 0.0001$, $n = 89$). A logistic model (whole model: $r^2 = 0.22$, $\chi^2 = 25.52$, $p = 0.04$, $n = 91$) showed a significant influence of treatment ($\chi^2 = 8.07$, $p = 0.005$) on cannibalism but no influence of female family ($\chi^2 = 19.12$, $p = 0.16$).

Females in the two treatments did not differ in days from final moult until death (non-siblings: 39.36 ± 2.42 days; siblings: 40.95 ± 2.54 days; pooled t -test: $t_{42} = 0.45$, $p = 0.65$). Fifteen females died before they produced an egg sac but the mating treatment had no effect on female mortality ($n = 91$, $\chi^2 = 2.13$, $p = 0.14$). Treatment did not influence any of the following parameters: number of egg sacs produced (Mann–Whitney U test: $n = 91$, $Z = 0.36$, $p = 0.72$); total weight of first egg sac (non-siblings: 161.9 ± 10.36 mg; siblings: 170.91 ± 11.55 mg; pooled t -test: $t_{74} = 0.58$, $p = 0.56$); silk weight of first egg sac (non-siblings: 32.65 ± 1.95 mg; siblings: 34.72 ± 2.11 mg; pooled t -test: $t_{63} = 0.72$; $p = 0.47$); combined weight of eggs in first egg sac (non-siblings: 132.85 ± 61.79 ; siblings: 129.93 ± 53.04 ; pooled t -test: $t_{62} = -0.2$, $p = 0.84$); hatching rate (non-siblings: $40.79 \pm 5.73\%$; siblings: $41.64 \pm 6.45\%$; pooled t -test: $t_{68} = 0.1$, $p = 0.92$).

Figure 1. Males mated to a non-sibling female were cannibalized significantly more often than males mated to a sibling.

Figure 2. Copulations between unrelated pairs had a longer duration than copulations between siblings.

4. DISCUSSION

In accordance with our prediction, relatedness between mating partners influenced their mating behaviour: copulation duration and the risk of sexual cannibalism were significantly lower in sibling matings than in non-sibling matings.

One possible explanation for these results is that females react differently towards brothers, by interrupting copulations earlier to limit sperm uptake and/or by letting them survive because their brothers' future mating success would add to the females' inclusive fitness. However, this seems unlikely because females stereotypically attack any male independently of their degree of relatedness and independently of male courtship behaviour (Schneider & Lesmono 2009). Males cling to the genital opening and can actively modulate the timing of their escape (Nessler *et al.* 2009). The only reliable predictors of sexual cannibalism in *A. bruennichi* are copulation duration and male mating status. The female's influence, by contrast, appears to be limited (Fromhage & Schneider 2005).

The potential for polyandrous females to bias paternity cryptically towards a more compatible male (Welke & Schneider 2009) may reduce the value of a sister as a mating partner for a male. Male investment in a mating event is reflected by two alternative mating tactics. They can either be monogynists (first copulation: long, second copulation: none or first copulation: short and second copulation: long with the same female) or bigynists (first copulation: short, second copulation: long, with another female (Fromhage *et al.* 2008)).

In our study, almost all of the non-sibling males were monogynous. Although re-mating with the same or another female would be possible, males sacrificed themselves to the female after a long first copulation. By prolonging their copulation, males increase their paternity share with a female but they also risk being cannibalized (Schneider *et al.* 2006). High mortality during mate search in addition to an increased paternity share and benefits for the offspring could make it beneficial for males to be monogynists (Buskirk *et al.* 1984; Andrade 2003). Thus, if a male has found a compatible female, his optimal strategy may be to perform a long first copulation and invest maximally instead of copulating briefly and risking death during further mate search.

Half of the males mated to sibling females succeeded in cannibalism avoidance and thus in a natural setting would potentially be bigynous. But why do these males mate with their sisters at all? One possibility is that high male mortality during mate search selects for acceptance even of sub-optimal mating opportunities, but not at the cost of losing all other mating opportunities. This would explain why males tend to copulate briefly enough to survive (less than 5 s). Being monogynous with a sister would be disadvantageous because females might re-mate and post-copulatorily bias paternity towards a more compatible suitor and a sibling male would have sacrificed itself in vain.

In conclusion, our results support the idea that males adaptively modulate their mating tactic through selective self-sacrifice or escape behaviour.

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