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Broken genitals function as mating plugs and affect sex ratios in the orb-web spider *Argiope aurantia*

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ABSTRACT

Background: To curtail competition with their own sperm, males of several spider and insect species mutilate their genitals or sacrifice themselves entirely using their genital parts or their whole dead bodies as mating plugs. The orb-web spider, *Argiope aurantia*, is a species characterized by both male self-sacrifice and extreme female-biased sexual size dimorphism. Plugs may reduce female mating frequency and hence the availability of females to males as mates.

Questions: Do male genital tips that break off during copulation function as mating plugs in *A. aurantia*? Does plugging of females affect sex ratios?

Methods: A series of mating trials to test the mating plug hypothesis and to determine the mating frequencies. I compare experimental results with data from field-collected individuals and estimate the effect on sex ratios.

Results: Genital tips that are broken off do function as mating plugs. However, although males plug females, re-mating by females does occur and females mate on average with 1.6 males. Partly as a consequence of plugging, the operational sex ratio (sexually active males to females) is strongly male-biased in *A. aurantia*. Because males typically insert both pedipalps into the same female, the effective sex ratio (males to females that mate at least once) is also male biased, which supports a recent model for the evolution of monogynous mating systems (males mating with only one female).

Keywords: effective sex ratio, genital damage, mating plugs, monogyny, operational sex ratio, sexual conflict.

INTRODUCTION

Sperm competition is likely to ensue whenever females mate with more than one male during a reproductive season (Parker, 1970; Birkhead and Möller, 1998; Simmons, 2001). Across the animal kingdom, males have evolved a variety of behavioural, morphological, and physiological adaptations to prevent sperm competition or to succeed in it (reviewed in Simmons, 2001;

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Arnqvist and Rowe, 2005). For example, mate guarding, mating plugs and seminal compounds that reduce female re-mating tendencies have probably evolved to avoid sperm competition by preventing other males from mating or at least from successfully transferring sperm (Simmons, 2001). In several spider and insect species, parts of the male copulatory organs break or tear off during copulation and remain in the female's genital tract (e.g. Wiehle, 1967; Levi, 1975; Downes, 1978; Breene and Sweet, 1985). These parts consist of durable sclerotized structures and have most commonly been hypothesized to function as mating plugs (e.g. Levi, 1975; Downes, 1978; Knoflach and Van Harten, 2001; but see, for example, Winston, 1987). Indeed, a mating plug function of broken genital tips has recently been confirmed experimentally for a few spider species [*Nephila fenestrata* (Fromhage and Schneider, 2006), *Latrodectus hasselti* (Snow *et al.*, 2006), *Argiope bruennichi* (Nessler *et al.*, 2007)], although this does not seem to hold for the orb-web spider *Nephila plumipes* (Schneider *et al.*, 2001).

Interestingly, in most of these species genital damage means that males can mate only once, although this is not a prerequisite for male functional sterility after just one mating as males in some species become depleted of sperm (Christenson, 1989), nor does it necessarily impede males from being able to mate multiply in all species (Snow *et al.*, 2006). Orb-web spiders (Orbiculariae) are particularly interesting in this context, as many are apparently characterized by polyandrous mating systems with males that use both their pedipalps (secondary copulatory organs) only once and with the same female (i.e. they are monogamous) in spite of an absence of any paternal investment into offspring (Andrade and Kasumovic, 2005; Fromhage *et al.*, 2005). Recently, the evolution of genital damage in spiders has been found to be correlated with self-sacrificial male behaviour during copulation (Miller, 2007). That is, in species with male genital mutilation, additional terminal investment strategies such as male suicidal behaviour (Grasshoff, 1964; Forster, 1992) or spontaneous death during copulation (Foellmer and Fairbairn, 2003; Knoflach and Benjamin, 2003) are likely to evolve because of the presumed low extra costs (Miller, 2007). Extra costs would be low because once copulatory organs are damaged to a point that they cannot function properly any more, a male cannot achieve further copulations and any additional strategy that increases fertilization success during the accomplished mating should be favoured by selection. Hence, genital parts that break during copulation are expected to entail detectable benefits in terms of paternity protection (Simmons, 2001; Snow *et al.*, 2006; Fromhage *et al.*, 2008).

One potentially important side-effect of mating plugs and other defensive sperm competition adaptations is that they may crucially affect the ratio of males and females sexually active at a given time [the operational sex ratio (Emlen and Oring, 1977; Kvarnemo and Ahnesjö, 1996)] and the total ratio of males and females that mate at least once during a reproductive season [the effective sex ratio (Shuster and Wade, 2003)]. The operational sex ratio is an important factor determining the strength and form of sexual selection on males, especially via effects of local levels of competition over access to females (e.g. Grant *et al.*, 1995; Foellmer and Fairbairn, 2005a). The effective sex ratio has been hypothesized to be relevant for the evolution of male monogamy, in that a male-biased effective sex ratio would lead to a level of competition among males that favours terminal investment strategies in males and hence male monogamy (Fromhage *et al.*, 2005, 2008). Importantly, male-biased adult sex ratios are likely in orb-web spiders with extreme female-biased sexual size dimorphism because of the increased juvenile mortality risk in females due to longer and/or faster growth (Robinson and Robinson, 1980; Roff, 2002; Foellmer and Fairbairn, 2005a; Blanckenhorn *et al.*, 2007; Miller, 2007), but this is not universally so (LeGrand and Morse, 2000). The effects of male sperm competition adaptations on sex ratios through affecting male and female mating frequencies (the degree of polyandry) are poorly understood so far as the relationship is typically analysed from the opposite angle, that is the operational sex ratio affecting sperm competition (Simmons, 2001).

In this paper, I investigate the function of male genital breakage during copulation and examine the consequences for female mating frequencies and sex ratios in the orb-web spider *Argiope aurantia*, a species with extreme female-biased sexual size dimorphism (Foellmer, 2004) that is characterized by spontaneous male death during copulation (Foellmer and Fairbairn, 2003). First, I test the hypothesis that broken-off genital tips function as mating plugs. Second, I experimentally determine female mating frequency (i.e. the degree of polyandry) and the possible limiting effect of genital tips on female mating frequency (i.e. whether plugged females are still receptive towards males, but males are unable to insert). Third, I compare experimentally determined mating frequencies with field estimates to obtain an estimate of the effective sex ratio. Lastly, I put these results into the context of other available data for this species and discuss the consequences for the operational sex ratio and broader evolutionary implications.

METHODS

Study system

In all spiders, the pedipalps (paired appendages anterior to the first pair of walking legs) of males are derived to function as copulatory organs and females have two corresponding genital openings, which lead to separate spermathecae (Foelix, 1996). In *A. aurantia*, the pedipalp's sperm transferring tube (embolus) has an apical sclerotized 'cap' (Levi, 1968), which breaks off frequently during copulation [in ~95% of cases (Foellmer and Fairbairn, 2005a)] (see Fig. 1). The extent to which they remain in females and may function as plugs is not known, however. Males always try to insert both pedipalps, one after the other, into the same female, which requires re-courting the female or at least a re-positioning of the male. They die spontaneously upon insertion of the second pedipalp (Foellmer and Fairbairn, 2003, 2004). Males can pursue two alternative mating tactics. First, if a male encounters a mature female that has recovered from her final moult, he faces a cannibalistic female that he has to re-court after the first insertion (Foellmer and Fairbairn, 2004). Females either kill the male after the first insertion or pull dead males out of their genitalia immediately after the second insertion (Foellmer and Fairbairn, 2004). On the other hand, if males encounter a penultimate female that is close to moulting to sexual maturity, they wait within or in the vicinity of the female's web structures and try to copulate with the female while she is moulting and thus defenceless (Robinson and Robinson, 1980; Foellmer and Fairbairn, 2005a). Most often two or more males are present on the female's web and compete fiercely over access to the female (Foellmer and Fairbairn, 2005a). In this situation, males can insert their second pedipalp by just repositioning themselves and dead males probably function as whole-body mating plugs by preventing other males from inserting their palps, because the freshly moulted female cannot interfere with male activities for about 15–20 min after the moult (Foellmer and Fairbairn, 2003).

I hypothesize that broken embolus caps function as mating plugs at least as soon as the female has recovered from her maturation moult and her cuticle has hardened and any dead males are immediately removed from the genital opening by the female.

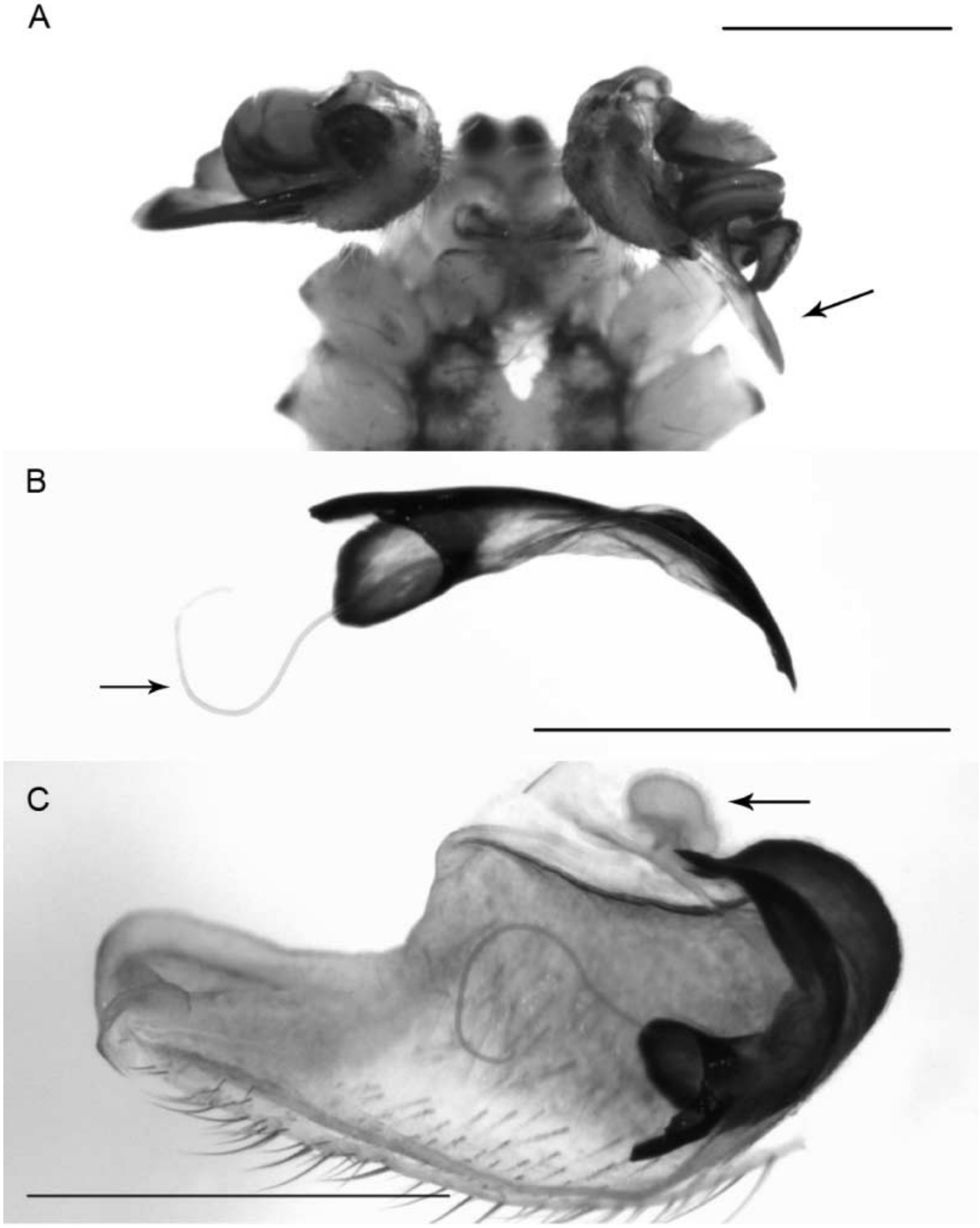

Fig. 1. (A) Ventral view of male pedipalps. The embolus cap of the male's left palp (right in picture) is broken off (arrow). Note that legs are removed for clarity. (B) Close-up of an embolus cap retrieved from a female. Clearly visible is the sperm-transferring tube, the embolus (arrow). (C) Left half of an epigynum after a sagittal cut and maceration in 15% NaOH. An embolus cap is placed so that the tip is situated at the entrance to the spermathecae (arrow). All scale bars = 1 mm.

Experiment 1: Do embolus caps function as mating plugs?

To test the hypothesis that embolus caps function as mating plugs I conducted a double-mating experiment, in which two virgin males were introduced to an initially virgin female in sequence and each male was allowed to insert one pedipalp. I predicted that if embolus caps are mating plugs, then the second male should choose the copulatory duct not used by the previous male. The experiment was conducted over two seasons in 2005 and 2006. In 2005, virgin females and males were obtained from a laboratory population that was reared from egg sacs collected near Bedford, Quebec close to the US border. The rearing procedure followed an established protocol (for details, see Foellmer and Fairbairn, 2004). Adult females were housed in aluminium screen cages (45 × 45 × 15 cm) and fed two large *Tenebrio molitor* larvae three times per week. Adult males do not build catching webs but may scavenge prey present in the female's web. I kept them in individual plastic vials (50 × 23 mm) and provided them with bits of fresh liver once a week. All individuals were provided with water using a water sprayer six times per week. Individuals were chosen randomly and used only once. The mean age [days from maturation moult, ± standard deviation (S.D.)] of females ($n = 19$) was 4.3 ± 2.2 days at the first mating trial and 6.0 ± 3.2 days at the second trial. *Argiope aurantia* exhibits protandry, hence males were on average older. Females' first mates were 10.5 ± 3.6 days old at the time of mating, whereas second mates were 9.2 ± 3.9 days old (paired t-test: $t_{18} = 1.0$, $P > 0.3$). First and second males did not differ in size (mean maximum carapace width, ± S.D.: first males 2.16 ± 0.22 mm; second males 2.08 ± 0.11 mm; paired t-test: $t_{18} = 1.8$, $P = 0.09$). In 2006, penultimate females and mature males were collected in the Annapolis Valley in Nova Scotia, Canada, at the beginning of August. Males were inspected for signs of prior pedipalp insertion using a stereomicroscope to ensure virginity (see Results and Fig. 1); females moulted to maturity in the laboratory. Trial assignment was random. The mean age (days from maturation moult, ± S.D.) of females ($n = 13$) was 2.0 ± 1.0 days at the first mating trial and 2.6 ± 1.1 days at the second trial. First and second males did not differ in size (mean maximum carapace width, ± S.D.: first males 1.63 ± 0.15 mm; second males 1.59 ± 0.16 mm; paired t-test: $t_{12} = 0.6$, $P > 0.5$).

Experimental trials were carried out at ambient room temperature (mean ± S.D.: $2005 = 23.3 \pm 1.3$°C; $2006 = 24.4 \pm 1.8$°C). Each mating trial began when I placed a male carefully into one of the upper corners of a screen cage containing a female without touching the female's web. Males that started walking around usually found web support threads quickly and either approached the female on her web or did not and kept exploring the cage. Mating trials were observed continuously and lasted until males had achieved one insertion or were killed, or until all mating interactions had ceased for at least 30 min (or never ensued within 30 min despite male activity). I immediately removed each male after the first insertion and recorded which pedipalp the male had inserted and whether the embolus cap was broken off using a stereomicroscope. Females were sacrificed and preserved in 70% alcohol. I dissected the epigyna of all females to determine the presence of broken-off embolus caps (see Fig. 1c).

Experiment 2: Female mating frequency when males do not compete over access to females

To determine female mating frequency in *Argiope aurantia*, I introduced virgin males to females sequentially until mating did not result anymore from introducing a male that at least actively explored a given female's cage. I predicted that if caps limit female mating frequency, then a point should be reached in the experiment where females are still receptive, but males cannot or choose not to copulate with the female. Alternatively, females may become unreceptive after a given time window after the maturation moult or after a given number of copulations (e.g. Christenson *et al.*, 1985). I collected 23 penultimate females, 20 adult females, and 51 adult males haphazardly along a roadside in Swanton, Vermont on 31 July and 3 August 2001. Virginity of males was checked under a stereomicroscope. Maintenance of experimental individuals and trial execution followed the same general protocol as in Experiment 1, except that spiders were kept in a greenhouse room under natural light conditions (mean ± S.D.: high temperature = 32.1 ± 2.7°C; low temperature = 20.3 ± 3.2°C). Importantly, males were allowed to insert both pedipalps. I did not allow females to consume any males and instead removed each male immediately from the cage. I recorded pedipalp usage and determined embolus cap breakage under a stereomicroscope.

I conducted two experiments (referred to as Experiments 2a and 2b); the assignment of individuals was always random. Males that did not insert any pedipalp in a trial and that were not harmed by the female were re-used. The aim of Experiment 2a was to clarify whether and to what extent adult females re-mate. Penultimate females were divided into two groups. In the first group ($n = 11$), one male was introduced to each female to induce opportunistic mating [mating during the female's maturation moult (see above and Robinson and Robinson, 1980)] to test whether this has an effect on female mating frequency. In both groups (second group: $n = 12$), one male was introduced to each female on the day following the female's final moult (females that were discovered moulted in the morning were considered to have moulted the preceding day), and every day after. I distinguished the following outcomes: (a) pedipalp insertion(s) occurred, (b) a courting male that was accepted by the female could/chose not to insert any pedipalp, (c) a female was unreceptive to a courting male, or (d) males explored the cage or approached the female but did not court her. If a male did not at least actively explore the cage at a given trial, the male was exchanged with another. Experiment 2b served to determine to what extent mature females collected in the field are receptive to males and how many times they mate under natural conditions. I introduced one male to each of 14 females that were already mature when collected. At the end of each experiment, all individuals were sacrificed and preserved in 70% alcohol. I dissected the epigyna of all females to determine the frequency with which broken-off emboli caps remain in the female (see Fig. 1c).

Experiment 3: Female mating frequency when males compete directly over access to females

This experiment was originally conducted to estimate the strength of sexual selection on male body size and body size components during opportunistic mating with moulting females and is described elsewhere (Foellmer and Fairbairn, 2005a). Briefly, penultimate females ($n = 45$) that were close to their final moult were temporarily caged with all males ($n = 111$) that were originally found cohabiting with them in the field (mean number of males per female: 2.5, range 1–7). In these cages, individuals mated at natural frequencies (Foellmer, 2004; Foellmer and Fairbairn, 2005a). Here I report the mating frequency of females in the context of opportunistic mating when males compete directly over access to females to complement the estimates for the degree of polyandry from Experiment 2. Because the proportion of females mated opportunistically in the field has been estimated (Foellmer and Fairbairn, 2005a), this will allow us to estimate the effective sex ratio for *A. aurantia*.

Non-parametric statistical analyses were used to compare mating and insertion frequencies. Comparisons of age and size of experimental males was done with parametric tests, after checking for normality of variables. All analyses were carried out with SPSS 14.

RESULTS

Experiment 1: Do embolus caps function as mating plugs?

In season 2005, 63% (12/19) of second males inserted into the unused side of the female, compared with 85% (11/13) in season 2006. These frequencies did not differ significantly (Fisher's Exact test, $P > 0.2$), thus the data for the two seasons were pooled for further analyses. First males inserted randomly the left (17/32 = 53%) or the right pedipalp (15/32 = 47%) (binomial test: $P > 0.8$) and the embolus cap broke off in 94% of insertions (30/32). Twenty-three of 32 second males (72%) used a different pedipalp than the first male, which is significantly different from random pedipalp choice ($\chi^2 = 6.03$, $P < 0.02$). Note that I could not determine with confidence the mechanism of choice involved, because insertions proceed very quickly. Based on my observations, it is likely that males can detect caps of a previous male by probing the copulatory duct with their pedipalps (but if so, then obviously not with 100% success). The embolus caps are about 1 mm in length (Fig. 1) and are large compared with other, even congeneric species (see, for example, Nessler *et al.*, 2007). Only in three of 32 cases was there no cap in the female's duct after a first male's insertion. In one of these cases, the second male used the same side as the first male; in the other two cases, they did not. Because of the low frequency of used ducts without male caps, a statistical test of whether the presence of a cap affects the choice of a second male would require a much larger sample size. The caps broke off completely in 84% of insertions (27/32) of second males. The likelihood of a cap breaking off during insertion did not differ significantly between first and second males (Normal approximation test, $z = 1.23$, $P > 0.2$). In total, 53 of 57 broken embolus caps (93%) were found in copulatory ducts. This means that even second males' caps are most often retained in the female copulatory ducts. As is typical for most orb-web spiders (Foelix, 1996), *A. aurantia* exhibits ipsilateral insertion – for example, the left pedipalp always inserts into the left insemination duct of the female.

Experiment 2: Female mating frequency when males do not compete over access to females

In Experiment 2a, 4.4 males on average were introduced per female (S.D. = 2.2, range = 2–11, $n = 23$). Females received a mean number of 2.0 insertions (S.D. = 1.0, range = 1–5, $n = 23$) (Fig. 2) while mating with a mean number of 1.6 males (S.D. = 1.0, range = 1–5, $n = 23$) (Fig. 2). Neither the number of insertions received nor the number of mates differed between females that received at least one insertion during opportunistic mating (insertions: mean = 2.00; mates: mean = 1.45) and those that did not (insertions: mean = 2.10; mates: mean = 1.75; insertions: Fisher's Exact test, $P > 0.7$, $n = 23$; mates: Fisher's Exact test, $P > 0.6$, $n = 23$). Note that in this comparison insertions received during opportunistic mating are included. Seven females (30.4%) became unreceptive to courting males, while seven females (30.4%) were still receptive at the last trial, but in each case the male tried only

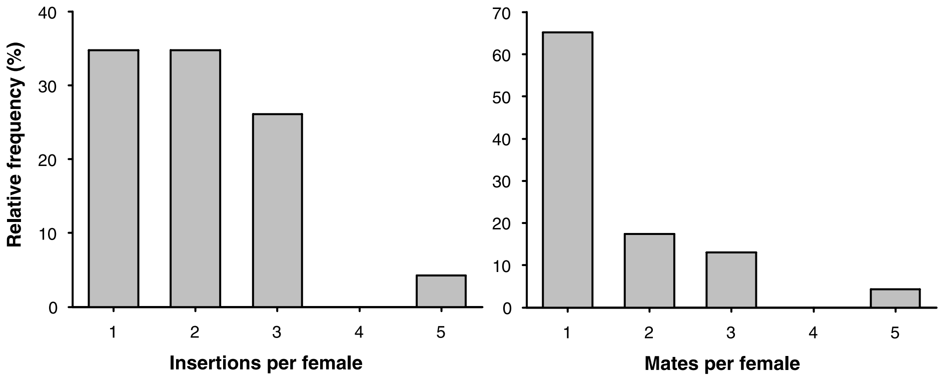

Fig. 2. Relative frequency distributions of the number of pedipalp insertions received by females and the number of mates per female in Experiment 2 (sequential introduction of males).

briefly to insert, and then left the female swiftly without inserting any pedipalp. In the remaining nine cases (39.1%), males did not court the female even though they approached her or at least explored the cage while making ample contacts with the female's web.

In Experiment 2b, which tested for receptivity of field-caught adult females, one of 14 females (7.1%) was receptive and received one insertion, three females (21.4%) were receptive but the male did not insert, nine females (64.3%) were not receptive, and one female (7.1%) was not courted. Hence almost all mature females collected in the field did not appear to be available for males as suitable mates.

Males in Experiment 2a performed a total of 47 pedipalp insertions. The embolus caps of 45 pedipalps (96%) broke; two were still attached through the embolus tube and 43 caps (92%) broke off completely. Forty caps (85% of the total number of inserted pedipalps, or 40/43 = 93% caps not found on a pedipalp) were found in female copulatory ducts. A comparison of females in the two experiments showed that females mated in Experiment 2a contained a mean number of 1.7 embolus caps (S.D. = 1.1, range 0–5, *n* = 23), whereas females collected in the field contained, minus the one insertion received in the experiment, on average 2.3 caps (S.D. = 0.9, range 0–4, *n* = 20), although this difference was not significant (Fisher's Exact test: *P* > 0.1).

Experiment 3: Female mating frequency when males compete directly over access to females

Most pedipalp insertions were not directly observed but inferred from broken embolus caps (Foellmer and Fairbairn, 2005a). Based on broken caps, females received a mean number of 2.6 insertions (S.D. = 1.2) (Fig. 3) and mated with a mean number of 1.6 males (S.D. = 0.8) (Fig. 3). Embolus cap retention rate in female copulatory ducts was not determined in this experiment. However, we can calculate the expected number of retained caps based on the results from Experiment 2a (where the number of mates was not experimentally limited). Forty of 45 broken embolus caps (89%) were retained in female copulatory ducts. Eighty-nine percent of 2.6 insertions equals 2.3 expected caps, which equals exactly the number of caps found in wild-caught females (Experiment 2b).

Because the experiment was conducted over the entire mating season based on natural frequencies of penultimate females close to moult with their associated males, it is possible

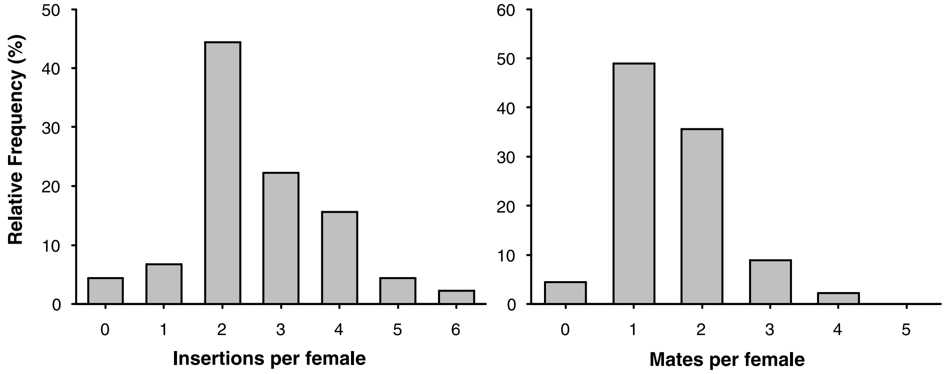

Fig. 3. Relative frequency distributions of the number of pedipalp insertions received by females based on broken embolus caps and the number of mates per female in Experiment 3 (concurrent introduction of males to females close to the maturation moult).

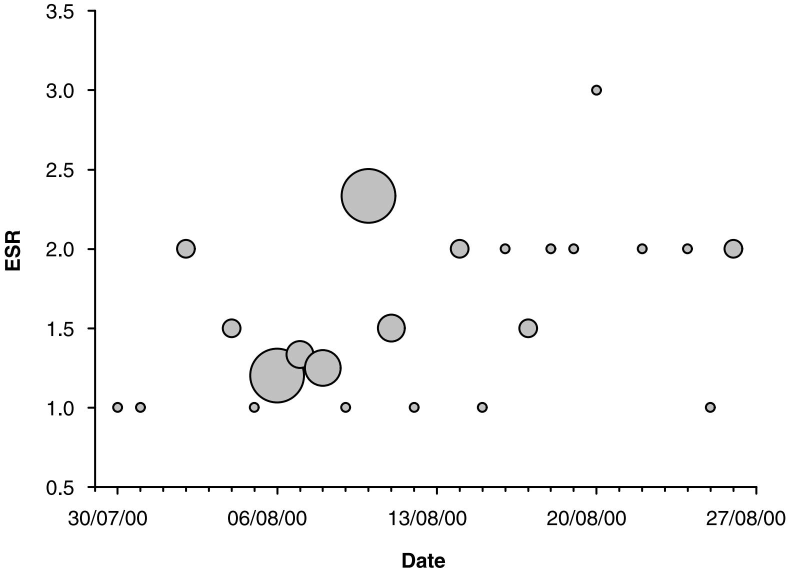

Fig. 4. The local effective sex ratios (ESR) during the mating season in situations when males mate with moulting females (opportunistic mating) and compete directly over access to females. Each bubble represents one female; the size of the bubble is proportional to the frequency of a given effective sex ratio. Males die during insertion of their second pedipalp (copulatory organ); a given male is therefore highly unlikely to appear on more than one web (see text).

to examine any possible change in the effective sex ratio over the season. The local effective sex ratio (number of males that mate with a given female on her web) increases over the season (Spearman's $\rho = 0.366$, $P < 0.02$, $n = 43$) (Fig. 4). Note that the local effective sex ratio is meaningful and is the basis for calculating the total effective sex ratio because males are highly unlikely to mate with more than one female (because they die during copulation).

DISCUSSION

The embolus caps in *Argiope aurantia* are large and break off during almost all copulations. Most broken-off embolus caps (~90%) are retained in the copulatory ducts of females. Most second males that had the choice to insert into a used or into a previously unused copulatory duct in double mating trials inserted into the unused duct, supporting the mating plug hypothesis. Broken male genital tips have probably evolved independently to function as mating plugs in other species (Fromhage and Schneider, 2006; Snow *et al.*, 2006; Miller, 2007; Nessler *et al.*, 2007; but see Schneider *et al.*, 2001). Possibly, these species are subject to similar ecological conditions favouring the evolution of terminal investment strategies (Miller, 2007, and see below).

When female *A. aurantia* were given the opportunity to mate with one male each day from the day of the final moult onwards, most females mated with only one male, and this was independent of whether the first mating occurred opportunistically during the female's moult. About 70% of females became unreceptive to courting males or were not courted by males anymore even though males explored the cage or even entered the female's web and approached her. It is possible that some unreceptive females still emitted pheromones; however, the importance of pheromones for *A. aurantia* is not well understood (Gaskett, 2007). In about 30% of cases, the female was clearly receptive to the male's courtship and assumed the typical acceptance posture. The male, on the other hand, appeared to try to insert but withdrew without inserting any pedipalp. I also observed this behaviour on three occasions in the field. Males abandoned the female's web, although the female had accepted them. I was able to catch the male each time and confirm that none had inserted a pedipalp (personal observation). These females had probably already been mated and plugged.

The fact that many females were still receptive but apparently monopolized by males through plugging suggests that they might not achieve the optimal number of mates. This could lead to sexual conflict over mating frequency and potentially explain sexual cannibalism (Arnqvist and Rowe, 2005); about 80% of mature post-moult females attack males during the first insertion (Foellmer and Fairbairn, 2004). This argument may well hold also for other cannibalistic species (e.g. Uhl *et al.*, 2007). The benefits of polyandry have been demonstrated in three spider species to date (Watson, 1998; Uhl *et al.*, 2005; Maklakov and Lubin, 2006). On the other hand, most female *A. aurantia* collected as adults in the field were clearly not sexually receptive anymore. The number of caps that these females contained was slightly but not significantly higher than the number of caps found in females that had mated with sequentially introduced males and equalled the number of caps expected for females mated exclusively opportunistically during their moult. Hence, females appear to mate on average with 1.6 males regardless of whether mating happens during or after the maturation moult, whereas males are confined to inserting each pedipalp once and they always try to insert both into the same female (Foellmer and Fairbairn, 2003, 2004). Therefore, the effective sex ratio is estimated to be 1.6 for *A. aurantia*. A male-biased effective sex ratio has been identified as an important factor favouring the evolution of male terminal investment in a single mate – that is, a monogynous mating system (Fromhage *et al.*, 2005, 2008).

The chances that a male who inserted only one pedipalp into a given female actually reaches another one are likely to be small (see Vollrath, 1980; Andrade, 2003; Kasumovic *et al.*, 2007), although mortality during mate search has yet to be directly estimated for *A. aurantia*. A high mortality during mate search indicates a low cost of investing terminally into a single mating and is therefore another relevant factor for the evolution of monogyny (Andrade, 2003; Fromhage *et al.*, 2005).

I have shown previously that the ratio of males to females that reach adulthood (the tertiary sex ratio) is male-biased through much of the mating season (see Figure 5 in Foellmer and Fairbairn, 2005a), and it is now evident that even if mortality during mate search is high, the effective sex ratio is still male-biased and may even increase over the season. The latter finding seems surprising and warrants further study. A male-biased tertiary sex ratio is likely a result of the extreme female-biased sexual size dimorphism, because females grow faster and for a longer time than males and thus are prone to suffer higher juvenile mortality than males (Blanckenhorn *et al.*, 2007; Foellmer and Moya-Laraño, 2007). A male-biased effective sex ratio has recently also been found to be at least likely in the sexually dimorphic and monogynous orb-web spider *Nephila fenestrata* (Fromhage *et al.*, 2007). However, note that in species in which monogyny is fixed (all males are monogamous), current sex ratios and sperm preference patterns do not necessarily have to be the same that once favoured the evolution of monogyny, although current conditions can be expected to disfavour the invasion of polygamous mutants.

About 60 % of female *A. aurantia* are mated opportunistically during their final moult in the field (Foellmer and Fairbairn, 2005a). Importantly, most males are found with penultimate females in the field and probably mate opportunistically. The local operational sex ratio (males per female on the web) is male-biased (~ 2 : 1) over the whole mating season (Foellmer and Fairbairn, 2005a). Few males are typically found with mature post-moult females (Foellmer and Fairbairn, 2004, 2005a), and this study shows that most post-moult females are probably not available as mates at any given time, either because they are not sexually receptive anymore or because embolus caps lodged in their genital openings prevent other males from mating. Hence, the overall operational sex ratio is also likely to be male-biased over much of the mating season, leading to strong sexual selection for large body size (Foellmer and Fairbairn, 2005a) as well as for traits that enable efficient mate search such as long legs (Foellmer and Fairbairn, 2005b), because receptive females are scattered in time and space (Foellmer and Fairbairn, 2005a).

In this study, actual sperm transfer and paternity were not assessed and this will be necessary to fully understand the payoffs of the different mating tactics of males (opportunistic mating with moulting females vs. mating with post-moult females) in *Argiope aurantia*. Interestingly, in the congener *A. trifasciata*, second males do not insert preferentially into unused copulatory ducts, although inserting into a used one results in significantly lower fertilization success (Maupin, 2005). It is unclear, however, whether and to what extent genital tips break off during copulation in *A. trifasciata* (Maupin, 2005). Another aspect that was not evaluated here was whether copulation duration is dependent on whether a male inserts into a used or unused opening, as has been shown, for example, for *Nephila madagascariensis* (Schneider *et al.*, 2005). This could indicate an additional effect of caps. However, copulation duration is very short in *A. aurantia* (median = 3.5 s) and is not correlated with fertilization success (Foellmer and Fairbairn, 2004). Therefore, sperm appear to be transferred in an all-or-nothing fashion in this species (Foellmer and Fairbairn, 2004). Similarly, in *A. trifasciata*, no significant difference in copulation duration between insertions into used versus unused spermathecae was observed (Maupin, 2005).

In conclusion, I have shown that in the monogynous orb-web spider *Argiope aurantia*, male genital mutilation probably evolved as a mating effort as broken-off embolus caps function as mating plugs, at least during copulation with post-moult females. The embolus caps may limit the number of males a female can mate with, but this does not prevent polyandry in all cases. Females mate on average with 1.6 males, and this is independent of whether males mate opportunistically with females during their final moult or mate with post-moult females. The degree of polyandry is in part determined by females becoming unreceptive to males, and in part by males being able to monopolize females by leaving the mating plugs behind in the female copulatory duct. Because males mate with only one female, the effective sex ratio is male-biased in this species (male to females = 1.6), which supports a recent model for the evolution of monogyny.

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