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EXTREMELY SHORT COPULATIONS DO NOT AFFECT HATCHING SUCCESS IN *ARGIOPE BRUENNICHI* (ARANEAE, ARANEIDAE)

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ABSTRACT. Females of the orb-weaving spider *Argiope bruennichi* are very cannibalistic and regularly terminate copulations by aggressively attacking the male. Few males survive mating and they escape only if they mate no longer than 8 seconds on average. We speculated that the brief copulations of surviving males will not result in complete fertilization of all of a female's eggs and that multiple mating is necessary to compensate for that. Surprisingly, we found no difference in the proportion of hatched young in clutches of females that were experimentally assigned to mate once or twice. Even females that mated with one male for less than 10 seconds produced clutches with hatching rates no different than treatments with two matings. The question remains why males risk their lives by prolonging copulation duration. Possible causes and functions in the context of sexual selection are discussed.

Keywords: Sexual cannibalism, sexual conflict, orb-weaving spiders, mating behavior

Theory suggests that male fitness increases with the number of females with whom they mate (Bateman 1948). However, it is less clear why females mate with more than one male in many animal species. A considerable body of recent literature investigates the question why females mate multiply and which kind of direct (Arnqvist & Nilsson 2000) or indirect (Jennions & Petrie 2000) benefits could be responsible. One such potential benefit is the avoidance of unfertilized eggs due to sperm limitation (Arnqvist & Nilsson 2000). A consequence of multiple mating by females is that the sperm of different males competes for the fertilization of a female's eggs. Sperm competition selects for male strategies that enhance the relative success of males (Elgar 1998; Simmons 2001). The duration of copulation is an important trait in this respect because it may, for example, determine the quantity of sperm transferred, aid removal of sperm from previous males, increase the probability that females will store and use the sperm or, for long copulations decrease the opportunities for females to copulation with another male. Males are thus under strong selection to achieve an optimal duration of copulation. However, mating can be costly to females. For example males can manipulate female reproductive behavior (Chapman et al. 2003) or even cause direct physical harm to females (Crudgington & Siva-Jothy 2000). In these cases, we expect a sexual conflict over the duration of copulation where females will often attempt to end copulation earlier than the males (Stockley 1997).

In spiders, the duration of copulation varies largely across taxa (Elgar 1995; Stratton et al. 1996) and is relatively short in the orb-web spiders, particularly in the genus *Argiope* Audouin 1826. Orb-weavers show a high frequency of post-mating sexual cannibalism and in a comparative analysis, Elgar (1995) found that within the Araneidae, sexually cannibalistic genera have shorter copulation durations than other taxa.

From the female perspective, the timing of cannibalism during mating seems an ideal instrument to control the duration of copulation. In *Argiope keyserlingi* Karsch 1878, females can adjust the relative paternity of two males by selectively timing copulation duration through attacking the male (Elgar et al. 2000). It was therefore suggested that cannibalism evolved under sexual conflict over the control of mating (Elgar et al. 2000; Schneider & Elgar 2001; Schneider et al. 2000).

In *Argiope bruennichi* Scopoli 1772 as in other *Argiope* species, the female often terminates copulation aggressively by attacking the male. Most such cannibalistic attacks are fatal to the male. Unlike some other spiders (Forster 1992; Andrade 1996), *Argiope* males will try to escape, at least after their first copulation (Sasaki & Iwahashi 1995; Elgar et al. 2000). Surviving males usually lose at least one of their legs in the attempt to escape the fangs of the female (Fromhage et al. 2003). An experimental study showed that surviving males copulated less than 10 seconds while cannibalized males remained attached to the females up to 8 times longer (Fromhage et al. 2003). If the brief copulations with surviving males are not sufficient to allow sperm transfer, what appears to be post-mating cannibalism will be classified more appropriately as pre-mating cannibalism.

Here, we explore this possibility by measuring hatching success in relation to sexual cannibalism and in relation to the number and duration of copulations. We allowed females to mate with one or with two males and compared hatching success of three successive egg sacs. We expected that a single short copulation would not result in complete fertilization of a female's eggs. In double mating trials we expected that females which received a very brief first copulation would compensate for this by acquiring an increased sperm supply from a following male.

METHODS

Subadult females (80) and adult males (about 120) of *A. bruennichi* were collected in July and August 2002, from dense populations in patches of grassland within the city of Bonn, Germany. Voucher specimens are deposited at the Museum Alexander Koenig in Bonn, Germany. Females were housed in individual plastic cups (400 ml) where they were watered 6 days per week and fed about 3–4 *Calliphora* sp. flies every 2–3 days. Adult females were housed in separate Perspex frames (30 cm x 30 cm x 6 cm), where they built typical orb-webs. After mating, they were retransferred to plastic cups where they were checked for egg sacs 6 days per week. Egg sacs were stored in individual plastic vials and preserved in alcohol after 26–29 days of incubation at 25 °C. Hatchlings and undeveloped eggs were subsequently counted under the microscope. Clutch size was the combined number of the number of hatchlings and the number of undeveloped eggs. After the natural death of a female, we used calipers to take the tibia-patella length of a first foreleg as a measure of its fixed body size. We randomized the choice of left or right leg but used the intact leg if one of the front legs was obviously shorter than the other or the second leg. We used body mass divided by tibia-patella length as an estimate for condition, ensuring the requirement that the relation between these parameters was largely isometric within the range of our data. Since females were weighed after their final molt and after mating, we could quantify their mass gain. In order to correct this mass difference for body size, we used condition. We obtained condition at maturity as mass at maturity divided by fixed body size, and condition at mating as mass at mating divided by fixed body size (=tibia-patella length). Males were of unknown mating status since they were collected in the field as adults. The majority of these males possessed all eight legs. Given the high mortality rate during copulation and a high probability of survivors to have lost at least one leg (Fromhage et al. 2003) and the observation that each pedipalp is used only once (unpub. data), we assume that most of these males were virgin. In the lab, they were maintained in individual cups (150 ml) on a diet of *Drosophila*. Shortly before mating, each male was weighed and the tibia-patella length of a foreleg was measured while he was immobilized by covering him with plastic film.

Females were randomly assigned to one of two different mating treatments: they were either presented with a single male that was allowed one insertion or with two males in succession each allowed a single insertion. There were 38 females in each of the two treatments. The mean duration of the 1^{st} copulation did not differ between treatments (Mann-Whitney-U-Test, $Z = -1.48$, $P = 0.14$). Matings were staged by placing a male near a support thread of the females' orb-web. When the male entered the web, the female would typically assume a distinctive posture with its body lifted from the web, often swaying slightly. The male then traversed the web to the hub and ran over the female a few times before he inserted one of his pedipalps. Time to copulation was measured with a stopwatch from the moment the male entered the web until the beginning of copulation. We measured copulation duration from pedipalp insertion until withdrawal.

Data analyses were carried out using JMP 4.02. Not all data were available for each mating trial and therefore sample sizes may differ between analyses. To fit a normal distribution, copulation duration, total copulation duration and male mass were log-transformed when parametric statistics were performed. We used nonparametric statistics where normally distributed residuals could not be obtained, or where variances were unequal. The use of Spearman correlations is indicated by the symbol r_s for the correlation coefficient, the use of Chi-square tests by indication of χ^2_1.

RESULTS

Clutch size and hatching success.—Mated females in the laboratory (all treatments combined) laid an average of 2.1 ± 0.2 clutches (mean ± SE, $n = 76$). The average clutch size was 170.7 ± 5.2 (clutch 1–3, $n = 133$) and did not differ significantly between 1st, 2nd and 3rd clutches (Oneway-Anova: $F_{2,\,130} = 2.01$, $P = 0.14$). If clutch sizes of successive clutches are compared considering individual females as block effects, we also found no significant difference (Oneway-Anova: $F_{2,\,68} = 2.68$, $P = 0.076$).

The proportion of hatched eggs differed significantly between successive clutches ($\chi^2_2 = 23.84$, $P < 0.0001$). Pairwise comparisons of the means using Dunns method for nonparametric comparisons with unequal sample sizes (Zar 1999) revealed a significantly lower percentage of hatched eggs in third clutches (0.42 ± 0.06, $n = 35$) than in first (0.60 ± 0.05, $n = 53$) and second (0.73 ± 0.05, $n = 45$) clutches ($P < 0.05$). Egg sacs collected from the habitat of the source population contained an average of 271.7 ± 20.0 eggs with a proportion of hatched eggs of 0.98 ± 0.02 ($n = 20$).

Size of the first clutch was significantly related to female fixed body size ($r_s = 0.54$, $P < 0.001$, $n = 55$), female condition at mating ($r_s = 0.38$, $P = 0.021$, $n = 36$), but not with female condition at maturation ($r_s = 0.25$, $P = 0.11$, $n = 43$).

None of these female body parameters was correlated with the proportion of hatched eggs in the 1st clutch (female fixed adult size (tibia-patella length): $r_s = -0.08$, $P = 0.6$, $n = 55$; female mass at maturity: $r_s = -0.11$, $P = 0.44$; female condition at maturation: $r_s = -0.07$, $P = 0.67$, $n = 43$; female condition at mating: $r_s = -0.15$, $P = 0.41$, $n = 36$). Clutch size and hatching success were not correlated for 1st and 3rd clutches although we found a positive correlation for 2nd clutches (1st egg sac: $r_s = 0.21$, $P = 0.14$; 2nd egg sac: $r_s = 0.45$, $P = 0.002$; 3rd egg sac: $r_s = 0.22$, $P = 0.20$).

Sperm availability and copulation duration.—There were no indications that sperm availability is a limiting factor of female reproductive success. Neither the proportion of eggs hatched nor the number of hatchlings significantly depended on the number of matings that a female experienced (number of matings vs. % hatched eggs: 1st egg sac: $\chi^2_1 = 0.22$, $P = 0.64$, 2nd egg sac: $\chi^2_1 = 1.86$, $P = 0.17$, 3rd egg sac: $\chi^2_1 = 1.98$, $P = 0.16$ (Fig. 1); number of matings vs. number of hatchlings: 1st egg sac: $\chi^2_1 = 0.12$, $P = 0.73$, 2nd egg sac: $\chi^2_1 = 0.02$, $P = 0.89$, 3rd egg sac: $\chi^2_1 = 2.78$, $P = 0.10$).

Similarly, the total duration of copulation that a female experienced was not correlated with the absolute number of hatchlings nor with relative hatching success of clutches: non-parametric correlations of total copulation duration vs. % hatched eggs: 1st egg sac: $r_s = -0.14$, $P = 0.32$, $n = 53$ (Fig. 2), 2nd egg sac: $r_s = 0.01$, $P = 0.94$, $n = 45$, 3rd egg sac: $r_s = -0.04$, $P = 0.83$, $n = 35$; total copulation duration vs. number of hatchlings: 1st egg sac: $r_s = -0.03$, $P = 0.86$, $n = 55$, 2nd egg sac: $r_s = 0.25$, $P = 0.10$, $n = 48$, 3rd egg sac: $r_s = 0.03$, $P = 0.85$, $n = 36$).

Copulation duration of cannibalistic matings was much longer than in copulations with surviving males, as reported elsewhere (Fromhage et al., 2003). While cannibalized males mated for a median of 23s (upper quartile = 35.9s, lower quartile = 10.27s, $n = 148$), survivors copulated for a median of only 7.8s (upper quartile = 11.5s, lower quartile = 6s, $n = 40$) (median is given because the data are not normally distributed).

Regarding cannibalistic first matings (with two outliers excluded to obtain a normal distribution), the duration of copulation was pos-

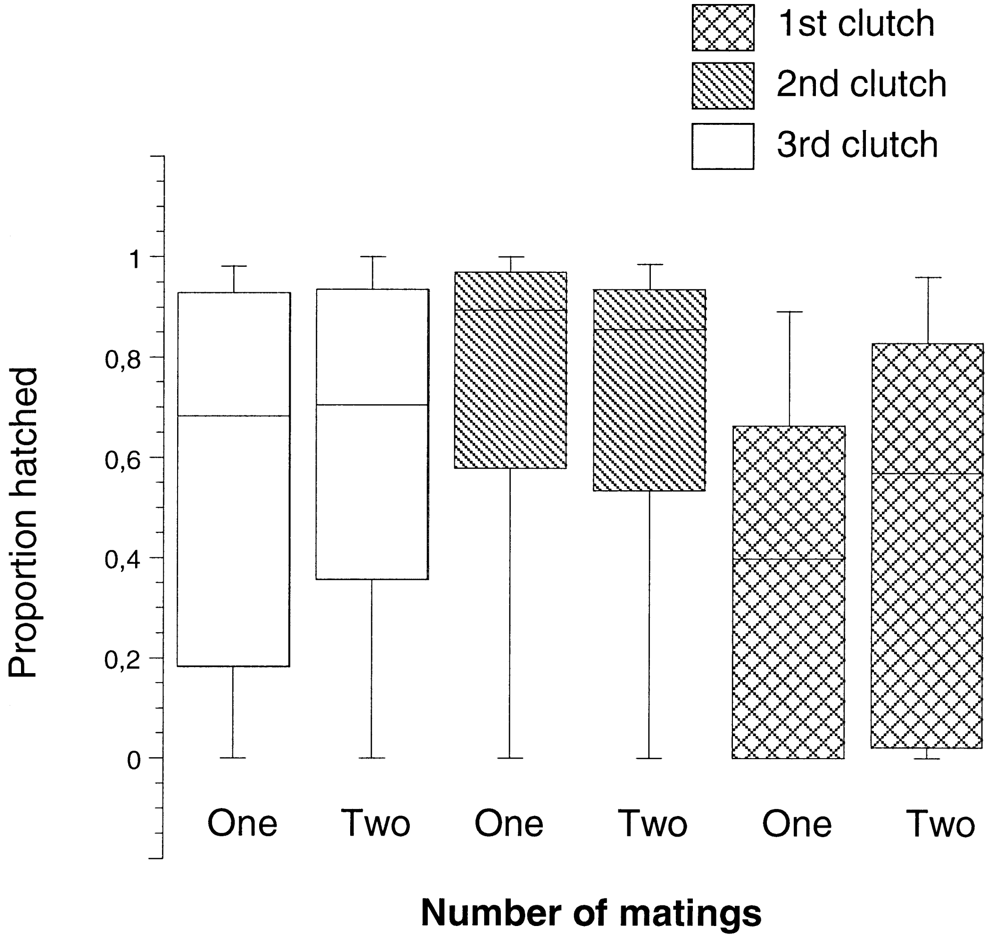

Figure 1.—Proportion of young hatched in three successive egg sacs of females that mated with one or two males. Box plots show median, interquartiles and range.

itively predicted by fixed male body size (linear regression: $r^2 = 0.09$, $P = 0.03$, $n = 58$) but not by male body mass ($r^2 = 0.04$, $P = 0.15$, $n = 59$) nor time to copulation (linear regression: $r^2 = 0.01$, $P = 0.61$, $n = 59$). In 25 cases, where females were cannibalistic towards both of her mates, the difference in body size of the males did not correlate with the difference in copulation duration ($r_s = -0.1$, $P = 0.58$).

First and second matings of the same females revealed no significant difference regarding copulation duration when compared with the Wilcoxon signed rank test for matched pairs ($Z = -0.43$, $P = 0.68$, $n = 38$). Females that received short non-cannibalistic 1st matings ($N = 7$) did not copulate longer with the 2nd male than 31 females that cannibalized their 1st mate (mean ± SE, cannibalistic: 20.45 ± 4.1, non-cannibalistic: 25.86 ± 8.58 ; t-test: $t = 0.57$, $P = 0.57$). In addition, females whose first mating was shorter than 10s (independent of cannibalism) did not mate longer with a 2nd male than females with a longer 1st copulation (22.37s ± 3.5, $n = 26$ long vs 19.45s ± 8.9, $n = 12$ short copulations; Wilcoxon Test: $Z = -1.13$, $P = 0.25$).

Large females produced more eggs, but female size did not influence the duration of her 1st ($r_s = 0.013$, $P = 0.9$) or 2nd ($r_s = 0.29$, $P = 0.1$) copulation.

DISCUSSION

Argiope bruennichi females are very cannibalistic and aggressively attack most males

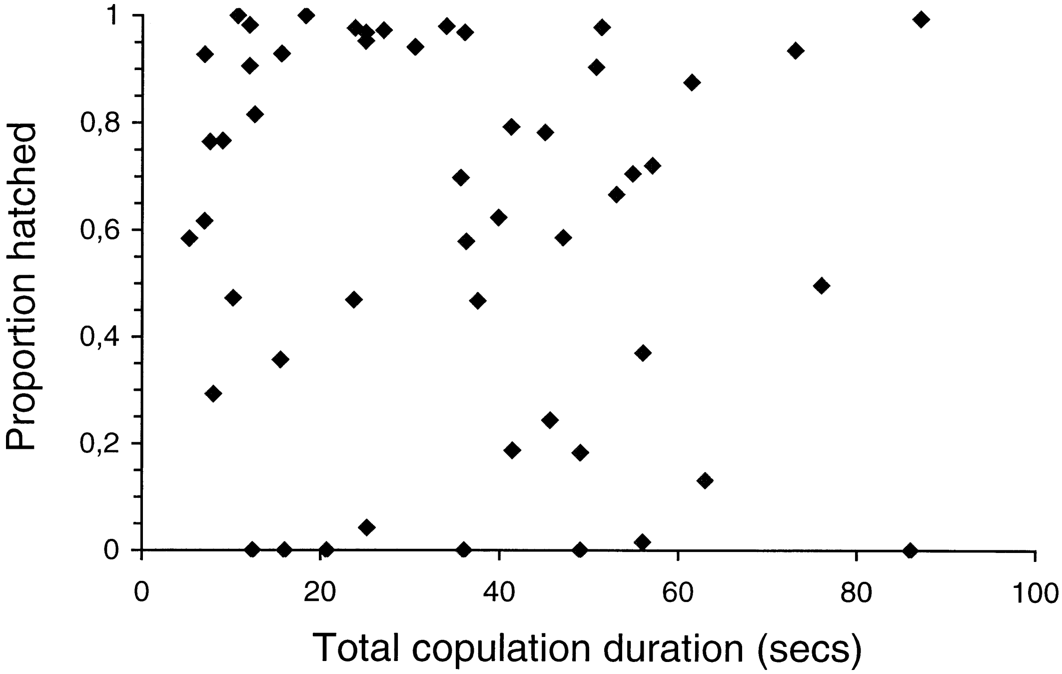

Figure 2.—The proportion of young hatched in the 1^{st} clutch as a function of the total duration of copulation a female experienced.

that mate with them. In 80% of all cases, the female kills and consumes the male, thereby terminating copulation. The duration of copulation strongly depends on the fate of the male, with copulations of survivors being extremely short (median of 8 seconds). We speculated that sperm transfer in extremely brief matings would not be sufficient to fertilize all eggs in a female's successive clutches.

To our surprise, we did not find any relationship between the duration of copulation and hatching success. Eggs of females who copulated once for only 5 seconds achieved hatching rates comparable to females who mated twice, and/or experienced those with much longer copulations. We did not detect any predictor of hatching success: hatching success was not related to female body size and mass nor to the size of the clutch. However, we found a positive correlation between clutch size and hatching rate for 2^{nd} clutches. As known for many spider species (Marshall & Gittleman 1994), clutch size in *A. bruennichi* is a function of female size and condition at mating. This may suggest that large females with large clutches achieve higher fertilization rates because males mate longer and/or are more willing to risk their lives when mating with more fecund females. However, female size, mass, and condition were not correlated with the duration of copulation (Fromhage et al. 2003).

Hatching success in the laboratory was lower than in the field, which is likely a consequence of the conditions that we provided. However, conditions were controlled and the same for all the females. It is therefore unlikely that our results are affected by the generally low hatching success. A factor that we cannot exclude is that females in the field mated with many more than two males and that more males are necessary to guarantee complete fertilization. However, in 18 1^{st} clutches we found a hatching success above 90% and among these females, 50% mated once, one female for only 7 seconds.

Although 3^{rd} clutches had lower hatching rates than 1^{st} clutches, this is not explained by the mating experience of the female. Fertilization rates may simply go down with time, perhaps through loss or aging of stored sperm cells. In this case, polyandry in *A. bruennichi* Scopoli 1772 may serve as a strategy to reduce this cost through repeated transfer of viable sperm.

Our results suggest that males can transfer enough sperm within a few seconds to ensure complete fertilization. Why then would males mate longer and risk their lives? Given the high risk of cannibalism for males that mate longer than a few seconds, a benefit of prolonged copulation is predicted that may offset the costs of losing all future mating chances. There are a number of possibilities that need to be investigated in future research projects. Firstly, sperm transfer may continue and even though additional sperm is not required for the purpose of fertilization, increased sperm numbers may be advantageous in sperm competition (Simmons 2001). This is likely if the outcome of sperm competition is determined by the relative quantity of sperm of rival males much like in a fair raffle (Parker 1990; Wedell et al. 2002). Such mechanisms have been demonstrated in insects (e.g. Dickinson 1986; Keller & Passera 1992). In a congener of our study species, *A. keyserlingi*, relative copulation duration of two competing males determines their relative paternity (Elgar et al. 2000), and similar relationships have been detected in *Nephila edulis* Labillardiere 1799 (Schneider et al. 2001) and *Pholcus phalangioides* Fuesslin 1775 (Schäfer & Uhl 2002). However, in none of these studies has it been determined whether the transfer of sperm was responsible for the advantage in sperm competition. In fact, the linear relationship between copulation duration and sperm transfer that has been demonstrated for many insects may not be valid for spiders. Several studies on spiders found no such relationship (Bukowski & Christenson 1997; Christenson & Cohn 1988; Willey Robertson & Adler 1994, Uhl unpubl. data), indicating that large parts of spider copulatory behavior serve functions other than sperm release (Eberhard 1994, 1996).

Given that under sperm competition copulation duration influences relative paternity, males will have an interest to prolong copulation while females may benefit by selectively terminating copulation. If copulation duration is largely under female control, males will best serve their interest by speeding up the mating procedure. This in turn will feed back on the female behavior. A conflict over copulation duration may therefore drive an antagonistic co-evolution, with copulations becoming shorter and shorter until an absolute minimal duration sets a limit to the evolutionary race.

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