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Spontaneous male death during copulation in an orb-weaving spider

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Males of some cannibalistic species of spiders and insects appear to sacrifice themselves by allowing the female to eat them, and the adaptive significance of such drastic terminal reproductive investment has recently been demonstrated for a spider. Typically, the female has to kill the male, but it has been suggested that males of some species in the cannibalistic orb-weaving spider genus *Argiope* may die in copula without female 'collaboration'. Here, we provide the first experimental evidence to our knowledge of programmed sudden death after onset of copulation in males of the spider *Argiope aurantia*. Our observations reveal that males invariably die during the insertion of their second pedipalp, regardless of whether they mate with newly moulted, defenceless females or with older mature females that often attack them. We determined experimentally that the death of males is triggered immediately upon insertion of the second palp, when males become unresponsive, and heartbeat ceases within minutes of insertion. We discuss the possible adaptive significance of programmed death during copulation, and argue that male death has evolved in a context other than sexual cannibalism.

Keywords: male self-sacrifice; whole-body mating plug; sudden death; sexual selection; sexual cannibalism; *Argiope aurantia*

1. INTRODUCTION

Mating can be deadly for males. Examples of sexual cannibalism where the female devours the male during or after copulation are well known, especially from many species of spiders and praying mantids (Elgar 1992). In many cases, cannibalism is probably maladaptive for the males, and males try vigorously to escape the cannibalistic female (Elgar 1992; Arnqvist & Henricksson 1997; Maxwell 1998). However, in some species of orb-weaving spiders (Araneoidea) and ceratopogonine midges, males apparently follow an adaptive strategy by sacrificing themselves and allowing the female to eat them (Blanke 1975; Downes 1978; Forster 1992). In this context, sexual selection for male complicity in sexual cannibalism has been demonstrated for the redback spider *Latrodectus hasselti* (Andrade 1996, 2000; Andrade & Banta 2002).

Whether males assist or not, all of these cases have in common that males are killed by females. By contrast, anecdotal accounts suggest that males of some species in the cannibalistic orb-weaving spider genus *Argiope* may die spontaneously during copulation, without female collaboration (Gerhardt 1933; Robinson & Robinson 1980). Sasaki & Iwahashi (1995) showed that in *Argiope aemula*, males do not try to escape from the female after mating and die soon (often within 1 day) even when females are prevented from eating them.

Here, we demonstrate that males of the orb-weaving spider *Argiope aurantia* experience programmed sudden death after the onset of copulation. We show that all males exhibit the typical dead posture just after they insert their second pedipalp and before the female can bite them. Furthermore, we determine that this signals rapid cessation of heartbeat, and thus death, without female complicity.

2. MATERIAL AND METHODS

(a) *The mating behaviour of* A. aurantia

In spiders, the pedipalps (the pair of extremities posterior to the fangs) are morphologically derived to function as copulatory organs (Foelix 1996). Each palp inserts into one of the female's paired genital openings, which lead to separate sperm storage organs. Upon insertion, the distal bulb of the palp is inflated through increased haemolymph pressure, which leads to the coupling of the palp with the female's genital plate. In *A. aurantia*, males pursue two alternative mating strategies, depending on whether they encounter a juvenile or a mature female in the field (Robinson & Robinson 1980). If they encounter a penultimate female (a juvenile just one moult from adulthood) they often cohabit with the female on her web and wait for her to undergo the maturation moult. They then try to copulate (i.e. achieve two pedipalp insertions) with the female during her moult, when she is defenceless and has no overt control over mating (opportunistic mating). If males encounter an already mature female, they court her and, if the female accepts, the male inserts the first palp. He then has to withdraw and re-court the female to achieve the second insertion. However, in this situation the female may attack the male at any time.

(b) *Experimental observations*

We conducted a series of observations to determine how frequently males die spontaneously during the second insertion, and whether males die regardless of the state of the female.

We observed 16 opportunistic matings during which males achieved two insertions. Two matings were observed naturally in the field. Ten cases involved caged individuals in a field experiment, and in four instances field-caught individuals mated in the laboratory. Two of the latter matings were videotaped. These observations involved a total of 13 females. Two females had one male present on their web at the time of mating, the other 11 had two or more males on their web.

We also videotaped 99 staged matings between mature virgin females and males (laboratory reared). Each individual was used only once. Mean adult age (days from maturation moult) ± 1 s.d. was 5.5 ± 1.7 days for females and 17.2 ± 2.2 days for males (males are protandrous). Mean adult size (prosoma width at broadest point) ± 1 s.d. was 4.0 ± 0.3 mm for females and 2.1 ± 0.2 mm for males. Females were allowed to build a new orb web in a mating cage (aluminium screen cages, 45 cm × 45 cm × 15 cm) before they were used in a trial. At the beginning of each trial, we placed males carefully into an upper corner of the cage without touching the female's web. Upon finding the female's web structures, males usually approached and courted the female. Mating trials lasted until males had achieved two insertions or were killed, or until all mating interactions had ceased for at least 30 min. We separated males from females after mating to prevent females from eating the males. We then inspected the males under a dissecting microscope.

(c) *Experimental manipulation of individuals*

Nearly half of the palps inserted second were severely damaged (see § 3). If palpal damage causes the death of males (pedipalps do not autotomize), experimentally detaching the palps might yield the same result and clarify the trigger of death. To test the hypothesis that detaching the palps causes the death of males and to describe the symptomatic pattern of male death, we compared males that had inserted both their palps with males that had their palps experimentally removed. We used field-collected individuals, all virgins at the time of the experiment. Males were collected as adults and checked for virginity by inspecting their palps. Females were collected as pen-

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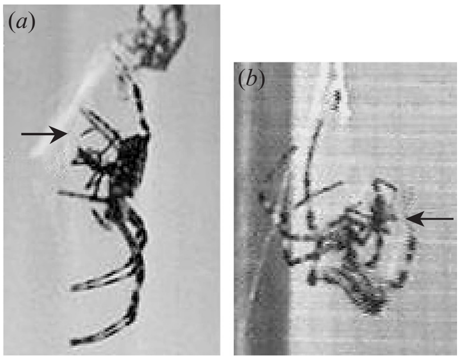

Figure 1. The appearance of males after insertion of the second palp. The arrows point to males in dead posture with legs folded under the body. (*a*) Mating with a moulting female: the female has come to sit on the web after the moult and the male's pedipalp is still anchored in the female's genital opening. At the top right corner is the female's exuvia visible. (*b*) Mating with an older mature female: the male has just been pulled out by the female and she is wrapping him with silk.

ultimates and allowed to moult to maturity prior to the experiment. Individuals were assigned randomly to four treatments (two main effects and a double control). Males in the first treatment were allowed to insert both palps, but were separated from the female within 5 s after inserting the second palp, before she could bite them ($n = 7$). Males in the second treatment were held gently between two fibreglass screens glued to circular plastic frames. We then detached both palps using fine tweezers ($n = 8$). We held males of the third treatment similarly between fibreglass screens and quickly cut the first pair of legs through the femur or tibia to control for haemolymph leakage resulting from palp detachment ($n = 6$). Males of the fourth treatment (handling control) were held between the screens for a similar amount of time (2 min; $n = 7$). After their specific treatment, we placed each male in a Petri dish, poked him with tweezers to provoke a reaction and recorded his behaviour. We observed each male under the dissecting microscope to check for movement and heartbeat. These observations were recorded immediately after the respective treatment, and we checked again for heartbeat after 5, 15 and 30 min.

3. RESULTS

During opportunistic mating, all males that achieved two insertions assumed the typical dead posture with legs folded under the body while still in copula (figure 1*a*). Males competed over access to the female, and often attacked any male that had inserted a palp. They tried to pull out dead males that were anchored by means of their second inserted palp, but they succeeded in only three out of 11 cases. After 15–25 min females came to sit on the web and eventually pulled out the apparently dead male stuck in their genital opening. They did not eat males right away, probably because their cuticle has to harden first. Thus, none of the males survived opportunistic mating, even though females could not attack them.

During the staged matings with already mature females, all males vigorously tried to escape from the female by jumping off her body after a very short insertion of the first palp (median of 3.5 s, range of 1–27 s, $n = 97$). However, upon insertion of the second palp all males ($n = 44$) became completely motionless, and were pulled out by the female after a median duration of 8 s (range of 1–55 s). Males exhibited the dead posture (figure 1*b*), and this was evident before the female could bite them (the female has to move the male towards her fangs to bite him). None of these males reacted when poked with tweezers. Note that females attacked males with similar frequency during the first and second insertion (77%, $n = 97$, and 82%, $n = 44$, respectively; $\chi^2 = 0.4$, d.f. = 1 , $p > 0.3$), and 38% (17 out of 44) of males that inserted twice had visible bite marks. Thus, in these cases the motionless males had been bitten before we separated males and females. Forty-five per cent (20 out of 44) had one palp ruptured, that is haemolymph was leaking out of a wound probably caused by torn membranes of the pedipalp. None had both palps ruptured. Ruptured palps were not found in any of the 22 males that were caught by the female after insertion of the first palp, and the difference between these groups is significant ($\chi^2 = 13.9$, d.f. = 1, $p < 0.001$). This suggests that only the palps inserted second were ruptured, either because females have to pull males out forcefully after the second insertion, or because second palps are damaged during intromission.

In our experiment with manipulated individuals, treatment type had an obvious effect on male response. All males in the 'palps inserted' and 'palps detached' treatments exhibited the typical dead posture, whereas this occurred in only 33.3% and 0% of individuals in the 'legs detached' and 'handling control' treatments, respectively (Fisher's exact test, $p < 0.000\,01$). When poked with tweezers, no males that had inserted their palps reacted, whereas all males of the other treatments showed a reaction (Fisher's exact test, $p < 0.000\,01$). All individuals except those in the 'palps inserted' treatment maintained a heartbeat over the entire examination period. By contrast, the hearts of all males in the 'palps inserted' treatment stopped beating within 15 min of insertion, and the heartbeat of these males was generally slower than that of males in the other treatments. Heartbeat could not be detected for one male (14%) at the first scan, for three males (43%) after 5 min, and for the remaining three males after 15 min. Thus, although detaching the palps results in some of the symptoms characteristic of males after the second insertion, insertion of the second palp itself with its accompanying processes (increase of haemolymph pressure to inflate the distal bulb of the palp) appears to be necessary to initiate the full cascade of events leading to the death of male *A. aurantia*.

One peculiar additional observation involved a male who inserted his first palp in a female, but re-courted a carcass of a mealworm beetle (*Tenebrio molitor*) larva that was present in the web instead of re-courting the female. This male inserted his second palp in the carcass. Immediately upon inflation of the distal bulb of the palp, the male assumed the dead posture. The palp was not ruptured. As the female was not in contact with the male, it is obvious that male death occurs even without contact with the female.

4. DISCUSSION

Our results clearly indicate that male *A. aurantia* die as a consequence of inserting their second palp. Females do not appear to be complicit in their death. With insertion of the second palp, a physiological process commences that leads to immediate (i.e. within seconds) lack of responsiveness and to certain death (as defined by the cessation of the heartbeat) within 15 min. The death of the male who mated mistakenly with the mealworm beetle carcass suggests that this process initiates when the distal bulb of the palp is inflated, and requires neither actual contact with the female nor rupturing of the palp.

Determining the adaptive significance of sudden male death will be difficult, given that it appears to be a fixed trait (i.e. it occurred in all males sampled). Male self-sacrifice during copulation is seen as a form of terminal reproductive investment, and is expected to evolve when the benefits of this investment outweigh the cost of foregoing future mating opportunities (Buskirk *et al.* 1984; Johns & Maxwell 1997). Males may benefit for two reasons. First, males may contribute their soma to increase the quantity and/or quality of the female's offspring. In *A. aurantia*, such an effect is possible, but it is probably not a major factor given the small size of males compared to females (see Andrade 1996; Elgar *et al.* 2000). Second, males may increase their paternity, because females copulate longer with them and/or are less likely to re-mate. When mating with a mature female, males cannot gain extra copulation time, because females pull them out. However, males may gain a paternity advantage by dying during opportunistic mating. Males fight over access to the moulting female, and try to dislodge any male that has a palp inserted. Because the palps of dead males are fixed in the inflated state and are therefore harder to remove, dead males act as whole-body mating plugs, often preventing other males from copulating. On average 60% of females are mated opportunistically in the field (M. W. Foellmer, unpublished data). Opportunistic mating is thus an important component in the evolution of the mating system of this species, and we postulate that spontaneous male death has evolved in that context.

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