Males sacrifice their legs to pacify aggressive females in a sexually cannibalistic spider

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Monogynous male mating strategies have repeatedly evolved in spiders along with female-biased sexual size dimorphism (SSD) and extreme male mating investment. As a manifestation of sexual conflict, male African golden-silk spiders, *Trichonephila fenestrata*, are regularly attacked by females during copulation, and sexual cannibalism is common. Curiously, attacked males actively cast off (autotomize) their front legs and copulation continues while the female is feeding on these legs. Since the loss of legs is costly in reducing males' ability to mate guard, it should yield significant benefits. In a series of experiments, we investigated the behavioural mechanism of male leg ejection and tested three hypotheses. First, we performed feeding experiments to test whether conspecific male legs are particularly attractive for females and act as a sensory trap. Second, we conducted mating experiments with sibling and nonsibling pairings to test whether males preferentially invest in high-quality mates. Third, by offering male legs during copulations, we tested whether male leg ejection serves to distract and pacify females. In support of the female pacifier hypothesis, our results confirm a significantly reduced probability of attacks in females that had been offered a male leg, but we found no relationship between simulated leg ejection and male survival. While there was no evidence for special properties of male legs, females accepted male legs significantly more often than insect food. The degree of male leg sacrifice did not depend on male−female relatedness, but large males lost more legs than small males, and small males achieved more copulations. However, total copulation duration was unrelated to male size. Male leg sacrifice in *T. fenestrata* may represent a rare example of an evolutionary transition in which the antipredation behaviour of autotomizing body parts has changed its function into a sexual context, here to pacify females and to facilitate undisturbed copulations.

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Monogynous mating systems are characterized by males that invest maximally in paternity with a single female without providing paternal care (Fromhage, Elgar, & Schneider, 2005). The maximum reproductive success a monogynous male can achieve is to sire all offspring of a single female. The male's prospects of gaining exclusive paternity will depend on his ability to find a virgin female and to defend her against rivals. However, monopolization by a single male may not coincide with female interests. Indeed, many studies have shown that females benefit from polyandry by gaining direct and indirect fitness benefits (Arnqvist & Nilsson, 2000; Slatyer, Mautz, Backwell, & Jennions, 2012). It is also well established that sexual conflicts over mating rate can spark an antagonistic coevolution of female resistance and male persistence (Arnqvist & Rowe, 2005; Rice, 1996).

Monogynous mating systems occur in diverse taxa and have evolved several times independently in spiders along with extreme reversed sexual size dimorphism (SSD). The tiny males mate with only one or very few females and possess special traits to monopolize paternity with a single female (Miller, 2007; Schneider & Fromhage, 2010). One such trait is the plugging of female genital openings with parts of the male copulatory organs. These chitinous plugs are broken-off fragments of the sperm-transferring structures; hence mate plugging will usually render the copulatory organ dysfunctional after mating (Fromhage & Schneider, 2006). Such 'one-shot genitalia' occur in various spider families, including the Nephilidae (Kuntner, Agnarsson, & Li, 2015).

A typical female behaviour in monogynous spider species is the killing and consuming of males during or after copulation. The question why females kill their mates has been debated since Darwin (Darwin, 1871; Elgar, 1992) and sexual cannibalism has

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been called the pinnacle of sexual conflict (Elgar & Schneider, 2004); however, whether or not cannibalism is costly for the male depends on whether it occurs before copulation, or after his first or second copulation. Males that have achieved their maximum possible insemination rate in their final copulation may be complicit and actively sacrifice their body to the female (Andrade, 1996; Andrade, Gu, & Stoltz, 2005; Schwartz, Wagner, & Hebets, 2014). Accordingly, male and female interests may diverge not simply over the event, but also over the timing of the male's death, so that the timing of cannibalism can be considered a trait under sexually antagonistic selection (Fromhage & Schneider, 2006).

Male counter-adaptations to sexual cannibalism are manifold and comprise reducing harm during attempted cannibalism by means of a protective abdominal constriction (Andrade et al., 2005), feigning death (Bilde, Tuni, Elsayed, Pekar, & Toft, 2006), binding the female with silk (Zhang, Kuntner, & Li, 2011), mating with subadult females (Biaggio, Sandomirsky, Lubin, Harari, & Andrade, 2016), remote copulation (i.e. continued sperm transfer by an autotomized mating organ; Li, Oh, Kralj-Fiser, & Kuntner, 2012), and mating with a moulting, and hence defenceless (Uhl, Zimmer, Renner, & Schneider, 2015), or a feeding and hence occupied and distracted female (Fromhage & Schneider, 2005a).

Mating while the female is feeding has been experimentally shown to reduce sexual cannibalism in the African golden-silk spider, *Trichonephila fenestrata*, resulting in significantly higher paternity (Fromhage & Schneider, 2005a). (The name of the genus has recently been changed from *Nephila* to *Trichonephila*, Kuntner et al., 2018.) Males in this species possess typical one-shot genitalia that break off during copulation to produce mating plugs (Fromhage & Schneider, 2006). However, these plugs do not always prevent successive copulations and rivals still pose a significant threat to a male's paternity. It is therefore important for males to survive mating and to defend the female against rivals as a measure of paternity protection (Fromhage & Schneider, 2005a). Consistent with this notion, males of *T. fenestrata* are not complicit in sexual cannibalism. However, copulating males regularly lose their front legs, which females then feed on while the copulation continues. Males actively cast off these legs in reaction to attacks by a female which may serve to prevent a single male monopolizing her (Fromhage & Schneider, 2006). Autotomy of appendages is a common antipredation behaviour in spiders (Foelix, 2011), but copulatory male leg ejection in *T. fenestrata* is the only known example of autotomy of extremities during ongoing copulations (Fromhage & Schneider, 2006).

Cases of females feeding on male body parts in the absence of obligatory cannibalism are rare. A well-studied example is females feeding on the fleshy hindwings of male sagebrush crickets, *Cyphoderris strepitans*, during copulation. While this nuptial gift serves to prolong copulation, the resulting loss of haemolymph reduces males' future mating success (Sakaluk, Campbell, Clark, Chadwick-Johnson, & Keorpes, 2004; Sakaluk & Ivy, 1999). More common nuptial gifts are body secretions, such as the spermatophylax of crickets, the salivary droplets produced by male scorpionflies (Engqvist & Sauer, 2001) and the nutritional liquid produced inside the cephalic hump of male *Oedothorax* dwarf spiders (Kunz, Garbe, & Uhl, 2012). In the latter cases, the amount of nutrients to be gained for the female is often positively correlated with the duration of copulation and the number of sperm transferred. Male front legs in *T. fenestrata* are probably of low nutritional value for the female, because males are very much smaller than females in this species. However, they could be particularly attractive for females and act as a sensory trap (Vahed, 2007).

Sacrificing one or more legs is a costly behaviour, as it may generally reduce male locomotion and manoeuvrability (Gerald,

Thompson, Levine, & Wrinn, 2017; Wrinn & Uetz, 2008). Previous experiments with *T. fenestrata* have shown that male competitive ability was affected by the number of legs lost, as males with fewer legs were less successful in male–male contests and post-copulatory mate guarding (Fromhage & Schneider, 2005b). Based on the idea that copulatory male leg ejection (and female occupation with feeding on the legs) serves to reduce the risk of sexual cannibalism, another study compared the frequency of cannibalism between males that had their front legs removed before copulation and control groups in which males had either two other legs or no legs removed (Fromhage & Schneider, 2006). Although males missing legs were cannibalized more than twice as often as intact males, the differences were not significant. However, a potential relationship between leg ejection and cannibalism may have been obscured by a high degree of variation and limited sample sizes.

Expanding on our hypothesis regarding the function of copulatory male leg ejection, we propose that males sacrifice their legs as an investment in paternity success with a given female. We performed a series of experiments to test the following hypotheses: (1) the sensory trap hypothesis: conspecific male legs should be particularly attractive food items for females (possibly by means of special cuticular substances); (2) the mating investment hypothesis: males should preferentially invest in high-quality mates; and (3), the female pacifier hypothesis: feeding on a male leg should reduce female aggression and resistance behaviour to the benefit of the male. We first compared female handling duration between male legs and normal insect prey items, as well as between sanitized (ethanol-washed) and untreated male legs, to test for specific properties of the latter. Second, we staged mating trials with sibling and nonsibling pairings to vary relative mate quality and genetic compatibility. We predicted males would invest more in unrelated, higher quality females and hence would eject more legs in non-sibling trials. Third, we experimentally simulated copulatory male leg ejection by offering male legs to females during mating and expected the probability of female attacks and cannibalism to be reduced significantly compared to a control group.

METHODS

Study Animals

We collected 10 gravid females at Mawana Game Reserve, Zululand District, KwaZulu-Natal, South Africa in 2012 (permit OP 990/2012 from EZEMVELO KZN WILDLIFE PERMITS OFFICE). F1 offspring reared from eggsacs produced by these females were used for experiment 2. F2 offspring derived from pairings of unrelated F1 pairs were used for experiments 1 and 3. The spiderlings were separated after approximately two additional moults and housed individually in air-vented plastic cups. We maintained the spiders on a diet of *Drosophila* and *Calliphora* flies (see Schneider, Lucass, Brandler, & Fromhage, 2011 for a detailed description of housing and rearing). After the experiments, the study animals were killed at -80 °C and preserved in ethanol. The experiments were carried out between November 2012 and October 2013.

Mating Behaviour

The mating behaviour of *T. fenestrata* is well known from previous studies. Females build large orb-webs between bushes and trees and usually sit in the hub of their web. Mature males do not build their own orb-webs but search for females (Fromhage, Jacobs, & Schneider, 2007). Females often attack the male during copulation and up to 30% of males were cannibalized after mating in experimental mating trials (Fromhage & Schneider, 2005a); hence males are generally reluctant to mate and often wait motionless in

the upper part of the female's web. However, they are more willing to mate when the female has captured prey and is engaged in feeding. Such 'opportunistic matings' (Robinson & Robinson, 1980) have been shown to prevent sexual cannibalism (Fromhage & Schneider, 2005a). Male spiders possess two copulatory organs, located at the tip of their pedipalps. Correspondingly, females have two copulatory openings at the venter of their opisthosoma, each of which leads to a separate sperm storage organ (spermatheca; Uhl, 2002; Uhl & Vollrath, 1998). In *T. fenestrata*, males insert one pedipalp at a time into one of the female's genital openings; thus, two separate copulations are necessary to inseminate both female sperm stores. Copulations are generally long, but their duration is highly variable (25 min, on average; this study). The male generally terminates copulation by slowly withdrawing his pedipalp from the female's opening. In this procedure, the tip of the male's copulatory organ usually breaks off, leaving a mating plug in the female's opening. Owing to genital mutilation, males are restricted to two copulations at most (Fromhage & Schneider, 2006). A male may either dismount the female after his first copulation or immediately copulate a second time without separating from her. While the mating plugs in *T. fenestrata* significantly reduce copulation success of rival males with the same female, they do not completely prevent additional copulations (Fromhage & Schneider, 2006). Therefore, males guard their female against rivals after mating (Fromhage & Schneider, 2005b). The female may attack the copulating male by kicking, pushing and pressing him with her hindlegs and bending her body inwards with jerky movements in an attempt to seize his body with her chelicerae. Males regularly cast off (autotomize) one or more of their front legs in reaction to an attack. The female then feeds on these legs while the copulation continues (Fromhage & Schneider, 2006).

Experimental Procedure and Data Recording

Experiment 1: Leg and prey handling

We designed this experiment to investigate whether conspecific male legs possess special properties, for example nutritional substances on the cuticular surface, which could make them especially attractive for females. Females were offered either a leg that had been sanitized (i.e. washed with 95% ethanol to remove potential substances from the cuticle) or an untreated leg. We recorded female acceptance and handling duration and predicted that females would be occupied with untreated male legs for longer than washed legs. In addition, we carried out tests in which females were offered *Carabus* beetle legs or *Gryllus* cricket body parts in order to compare female handling duration between conspecific male legs and normal insect prey of web-building spiders.

We randomly allocated females to one of two treatments: (1) the male leg handling treatment in which females were offered an untreated leg on one day and a washed leg of the same male on a different day (12 females), or (2) the insect prey handling treatment in which females were offered a *Carabus* beetle leg and a body part of a *Gryllodes sigillatus* cricket in the same manner (nine females). Insect prey items were cut to resemble the average mass of a male leg (accepting a 0.5 mg tolerance). Objects were offered in random order in both treatments.

To test females' handling behaviour with respect to conspecific male legs, it was necessary to obtain fresh legs in a controlled procedure. Spiders, in general, are able to autotomize any leg at the basal leg joint (coxa-trochanter joint) with little impairment aside from the loss of the extremity itself. This behaviour is often used as a measure of last resort to escape from a predator (Foelix, 2011). We seized the front legs of each male with forceps and the male ejected his legs immediately (see Fromhage & Schneider, 2006). Male legs were obtained from males unrelated to the female within 15 min before starting the trial.

The trials were conducted with females normally positioned at the hub of their webs. To be able to reach the female's mouthparts, we cut a hole approximately 2 cm in diameter into the female's web prior to each trial using fine scissors (females showed little or no signs of being disturbed by web manipulation, but were given a 10 min rest period before a trial was started). We used fine forceps to pass the food items to the female through the hole in the web. Each male leg or insect prey was brought in contact with the female's mouthparts up to three times (with 10 s intervals), so that the female was able to grasp it with her chelicerae. Female handling duration was defined as the period of time a female was occupied with the item, irrespective of whether the spider was actually feeding on it or not. Each trial was carried out 5–10 days after the female's final moult to ensure that her exoskeleton had completely hardened. Females were not fed between the day of maturation and handling trials.

Experiment 2: Male mating investment

In line with the mating investment hypothesis, we based this experiment on the assumption that males prefer mating with unrelated females (i.e. higher quality mates in terms of genetic compatibility) over mating with sibling females. We therefore expected increased male mating investment in nonsibling females. Because females may counteract copulation attempts by attacking the male, strong male mating investment (i.e. prolonged copulations and/or second copulations) should be reflected in an increased number of legs ejected in a mating trial. In addition, we also assessed a potential female counter-adaptation, namely whether female attacks during first copulations reduce males' chances of a second copulation with the same female.

The mating trials in this experiment were staged as less risky 'opportunistic matings' (Robinson & Robinson, 1980) with feeding females. When the female wraps prey items during copulation, the male's front legs may occasionally become entangled in silk threads and this can lead to rare cases of male leg ejection in the absence of a female attack. However, apart from sexual cannibalism, feeding females, as well as males copulating with them, generally show the same behavioural repertoire as can be observed in matings with nonfeeding females.

We arranged 30 trials in which males and females were related (i.e. siblings from the same maternal lineage) and 32 trials in which mates originated from unrelated maternal lineages. In some cases, *T. fenestrata* males do not separate from the female and the male pedipalp remains inserted for several hours. Mating trials with overlong insertions (≥ 2.5 h) were excluded from the analyses (two nonsib trials). Males weighed a mean $\pm$ SE of 24.82 ± 0.9 mg at maturation and females 353.33 ± 10.96 mg. The study animals' adult age (the number of days passed from the date of maturation) was a mean $\pm$ SE of 24.87 ± 0.88 days in males and 27.9 ± 1.22 days in females.

Before being used in a mating trial, each female was transferred to a 40 x 40 cm and 10 cm deep Perspex frame and given several days to build a normal orb-web inside. At the beginning of each trial, we introduced the male by randomly positioning him either on the left or on the right upper frame threads of the female's web. The preset time for a trial was 3 h and all pairs mated within this time (observations and data recording were extended for ongoing copulations). We fed the female one *Calliphora* fly 3 min after starting the trial and a second fly after another 5 min if the male did not mount the female. If the spiders copulated, the feeding of the second fly was delayed until the copulation had ended. A third fly was fed when the female had finished feeding. The male performed one or two copulations within a trial; observations ended with the

end of a second copulation. We recorded the occurrence of copulatory female attacks (i.e. the female bent her body inwards and at the same time kicked, pushed and pressed the copulating male with her hindlegs) and sexual cannibalism, copulatory male leg ejection, the number of copulations and copulation duration (the time between the inflation of the haematodocha, a balloon-like organ generating haemolymph pressure for sperm transfer, and the collapsing of the organ). Pairing attempts lasting less than 1 min were not considered as copulations (such attempts always left the male pedipalp intact and this was often used for a regular copulation afterwards).

Experiment 3: Simulated copulatory male leg ejection

We aimed to test the effect of conspecific male front legs offered to females during copulation and manipulated the regular mating procedure by offering a male leg independently of initial female aggression. We predicted this treatment would inhibit female aggression towards the male, thereby reducing the probability of copulatory female attacks and sexual cannibalism.

We randomly allocated 50 males and 50 females to either the male leg ejection simulation treatment or the control group, where no food item was offered. The study animals used in each trial were unrelated to each other. All males had their front legs removed before being used in the experiment. Each male was allowed a single copulation. If copulation did not occur within the preset time (1.5 h), the trial was repeated on the following day. If that also failed, the trial was excluded from the study (nine control trials). A proportion of males did not separate from the female after the first copulation and immediately inserted the second pedipalp into the opposite copulatory opening. As we focused on first copulations in this experiment, trials were terminated after the first copulation had ended. Males weighed a mean ± SE of 20.84 ± 0.86 mg at maturation and females 282.2 ± 10.47 mg. The study animals' adult age was a mean ± SE of 18.89 ± 0.94 days in males and 18.18 ± 0.76 days in females.

We removed the front legs of males as described for experiment 1. Males were given at least 15 min for recovery afterwards. The legs offered in the mating trials were obtained from males that were unrelated to the focal male and female. Before a trial was initiated, we cut a hole into the female's web as described for experiment 1. Mating trials were started as described for experiment 2. To clearly determine the potential effect of male leg ejection, we offered the leg after 14 min of copulation, approximately 4 min before the average time of initial female aggression in preliminary observations (R. Neumann, personal observations). The leg was offered up to three times for 10 s (using 10 s intervals) by bringing it in direct contact with the female's mouthparts. In the event of an earlier female attack, the leg was offered immediately. We recorded the occurrence of copulatory female attacks, cannibalism and copulation duration.

Statistical Analyses

All analyses were conducted in JMP Pro 13 (SAS Institute Inc., Cary, NC, U.S.A.). Data from experiment 1 were analysed using a paired t test for normal data with equal variances (indicated by Shapiro and Bartlett's tests), nonparametric Wilcoxon tests and a G test. We ran generalized linear models (GLMs) to analyse data from experiment 2, using the following dependent variables: the number of male legs ejected, the number of copulations performed (one or two) and total copulation duration. Explanatory variables were male–female relatedness (sib–sib/sib–nonsib mating), male and female adult weight (a proxy of adult size) and age, copulatory female attack (yes/no) in the first copulation (used only with number of copulations as the dependent variable), female attack

(yes/no) in the first and/or second copulation and the number of copulations (used only with total copulation duration and number of male legs ejected as dependent variables). GLMs to analyse data from experiment 3 were specified with the following dependent variables: female copulatory attack (yes/no), sexual cannibalism (yes/no) and copulation duration. As explanatory variables, we used the leg ejection simulation treatment, male adult weight and age, and female condition (the ratio between the female's weight at the day of the trial and her adult weight to incorporate the increase of female body mass after maturation). We specified models with a binomial distribution and logit link function for binary variables, such as number of copulations (one or two), the occurrence of female attacks (yes/no) and cannibalism (yes/no), a Poisson distribution with log link function for count data, and a normal distribution with identity link function for continuous numerical data (log-transformed to improve model fit). Models were simplified by stepwise removal of nonsignificant effects. Effect tests for individual variables in JMP are based on ANOVA model comparisons between the full model and a reduced model lacking the respective variable. Nonsignificant P values correspond to the time a variable was removed from the model. Significant P values denote variables that remained in the final model. Additional statistical tests are given in the Results section. Sample sizes within experiments may differ owing to missing data.

Ethical Note

All study animals were reared specifically for study purposes, using offspring of a minimum number of wild-collected females. This research did not require approval by an institutional or governmental regulatory body. Autotomy of appendages is a common antipredation behaviour in spiders, which possess a special mechanism to immediately seal the wound (Foelix, 2011). We used this natural mechanism for our experiments and induced autotomy by simulating an attack on the leg. Autotomy occurred immediately and there was no decline in the study animals' condition. Spiders mainly feed on insects, but generally do not refrain from cannibalism. Hence, feeding on a conspecific leg is a natural behaviour.

RESULTS

Experiment 1: Leg and Prey Handling

All 12 females accepted the untreated as well as the sanitized male leg and fed on the food items, whereas in the insect prey treatment, only five of nine females accepted the beetle leg or the cricket body part. All females that accepted the cricket body part rejected the beetle leg, and vice versa. Comparing female acceptance of beetle legs with untreated male legs (excluding values for sanitized legs to account for the paired design), we found that male legs were significantly more attractive (G test: $\chi^2 = 8.09$, $N = 21$, $P = 0.005$). Female handling duration did not differ between sanitized male legs (mean ± SE = 1051.08 ± 428.19 s) and untreated male legs (1084.50 ± 519.43 s; paired t test: $t = -0.22$, $N = 12$, $P = 0.83$). We recorded a mean handling duration ± SE of 921.11 ± 1311.4 s for beetle legs and 268.11 ± 464.06 s for cricket body parts (including zero values from trials in which the leg was not accepted). Considering only those trials in which the female accepted the food item, handling duration was 1658 ± 1383.02 s for beetle legs and 482.6 ± 548.92 s for cricket body parts. The difference was not significant (Wilcoxon test: $Z = -1.04$, $N = 10$, $P = 0.30$), which was also the case when comparing handling durations between untreated male legs and beetle legs (including zero values; Wilcoxon test: $Z = -1.18$, $N = 21$, $P = 0.24$).

Experiment 2: Male Mating Investment

Contrary to predictions, our models showed that male–female relatedness had no effect on measured parameters of male mating investment. Whether males mated with sibling or nonsibling females did not predict the number of legs a male ejected in a mating trial (nonsib trials: mean $\pm$ SE $= 0.77 \pm 0.23$; sib trials: 1.1 ± 0.26, $N = 60$), the number of copulations performed (nonsib trials: mean $\pm$ SE $= 1.63 \pm 0.09$; sib trials: 1.63 ± 0.09, $N = 60$) or the total copulation duration (nonsib trials: mean $\pm$ SE $= 2120.18 \pm 160.71$ s; sib trials: 2716.93 ± 361.62 s, $N = 58$; Table 1).

We recorded copulatory female attacks in eight of all first copulations (13.3%) and 10 of all second copulations (26.3%). Copulatory male leg ejection occurred in 15 of 60 trials (25%). Males ejected significantly more legs in trials in which the female attacked the male (Fig. 1, Table 1) and large males lost more legs than small males (adult weight used as a proxy of size; Fig. 2, Table 1). Of 60 males, 38 copulated twice (63.3%). Whether or not males copulated a second time did not depend on the occurrence of female attacks during first copulations (Table 1). Despite large males' higher frequency of leg ejection, they were less likely to achieve two copulations than small males (Table 1). The model also revealed that two copulations occurred more often in trials with relatively small and young females (Table 1). Total copulation duration in trials where females attacked the male significantly exceeded copulation duration with quiescent females, and copulation duration was positively correlated with female adult age and the number of copulations in a trial (Table 1). However, although small males copulated more often than large males, total copulation duration was unrelated to male size (Table 1). Three males in this experiment were cannibalized (5%).

Experiment 3: Simulated Copulatory Male Leg Ejection

Females that had been experimentally offered a male leg during copulation were significantly less likely to attack males than those not offered a leg. Of 25 females, 14 (56%) in our leg ejection simulation treatment showed copulatory attacks, whereas 12 of 13 females (92.3%) attacked the copulating male in the control group (Fig. 3, Table 2). However, three treatment females did not accept the offered leg and nevertheless remained quiescent during mating. If we exclude these trials from the model for a more conservative test, the treatment effect is still significant ($\chi^2 = 4.01$, $N = 35$, $P = 0.045$).

All males had their front legs removed, but some also ejected their own second-pair legs (two in the leg ejection simulation treatment and five in the control group). Male second-pair leg autotomy invariably occurred in reaction to female attacks and thus
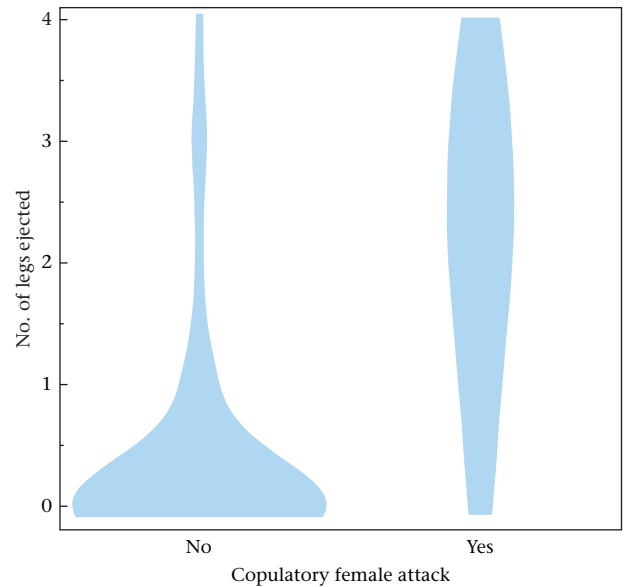
Figure 1. Relationship between male leg loss and the occurrence of copulatory female attacks in mating trials (experiment 2).
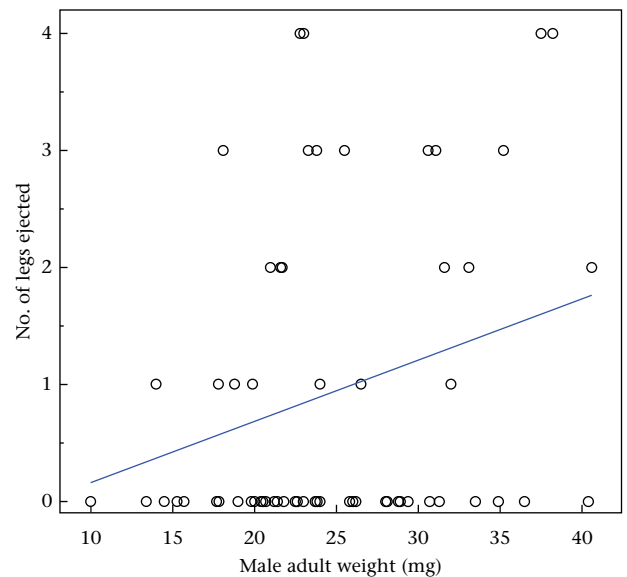

Figure 2. Relationship between male adult weight (a proxy of body size) and the number of male legs ejected in mating trials (experiment 2).

Table 1
Summary of generalized linear models to test for effects of male–female relatedness on male mating investment and to analyse correlations between male and female traits and male mating success

Explanatory variable	No. of male legs ejected in trial ($N = 60$)			No. of copulations in trial ($N = 60$)			Total copulation duration ($N = 58$)		
	χ^2	P	df	χ^2	P	df	χ^2	P	df
Male–female relatedness	1.16	0.281	1	0.11	0.744	1	0.008	0.928	1
Male adult weight	4.46	**0.035**	1	9.57	**0.002**	1	0.31	0.577	1
Male adult age	0.03	0.865	1	<.0001	0.997	1	0.26	0.609	1
Female adult weight	2.06	0.151	1	5.45	**0.02**	1	1.71	0.191	1
Female adult age	0.23	0.633	1	5.51	**0.019**	1	5.57	**0.018**	1
Female copulatory attack in first copulation	–	–	–	0.53	0.465	1	–	–	–
Female copulatory attack in first and/or second copulation	37.03	**<.0001**	1	–	–	–	16.03	**<0.0001**	1
No. of copulations in trial	0.13	0.72	1	–	–	–	18.32	**<0.0001**	1

Trials were staged as 'opportunistic matings' with feeding females. Significant P values are shown in bold. Total copulation duration was log-transformed for the analysis.
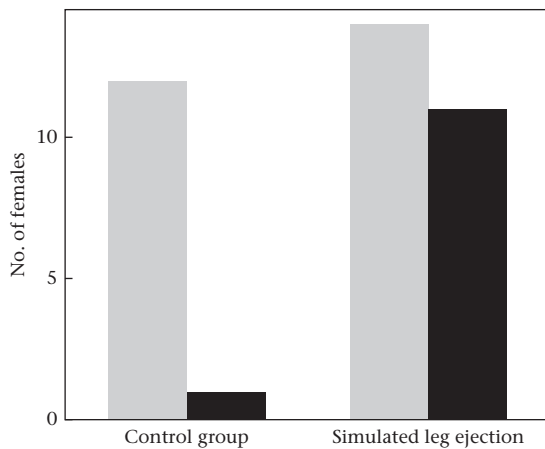
Figure 3. Numbers of females that attacked the male (grey bars) and nonaggressive females (black bars) in the leg ejection simulation treatment and in the control group (experiment 3).

Table 2
Summary of generalized linear models to test predictions related to simulated copulatory male leg ejection

Explanatory variable	Female copulatory attack ($N = 38$)			Sexual cannibalism ($N = 31$)			Copulation duration ($N = 37$)		
	χ^2	P	df	χ^2	P	df	χ^2	P	df
Leg ejection simulation	6.05	**0.014**	1	1.18	0.277	1	0.6	0.439	1
Male adult weight	0.75	0.387	1	0.3	0.584	1	0.59	0.442	1
Male adult age	0.32	0.571	1	0.31	0.577	1	2.52	0.112	1
Female condition	0.73	0.392	1	0.63	0.426	1	6.24	**0.013**	1

Significant P values are shown in bold. Female condition was calculated as female weight at the day of the trial/female adult weight. Copulation duration was log-transformed for the analysis.

could not prevent initial attacks. Therefore, autotomy did not confound the predicted effect of our treatment to inhibit female aggression completely in a proportion of mating trials. On the other hand, male second-pair leg ejection could potentially have influenced the probability of sexual cannibalism, and we excluded these trials from the respective model. Relatively few males in this subset did not survive mating. Males were cannibalized by three of 23 females in the leg ejection simulation treatment (13%) and two of eight females in the control treatment (25%), but the difference was not significant (Table 2). All three cannibalistic females in the leg ejection simulation treatment had previously accepted the offered leg and cannibalism was not preceded by female attacks in two of these trials. The two cases of cannibalism in the control group occurred in trials where the female had previously attacked the male. We also analysed potential correlations between our explanatory variables and copulation duration, using the full data set. Mating trials in this experiment were terminated after a single copulation, for which we recorded a mean duration ± SE of 1530.7 ± 82.05 s ($N = 37$). The model indicated that copulation duration was positively correlated with female condition (Table 2).

DISCUSSION

Our results corroborate the female pacifier hypothesis, as significantly fewer females attacked the copulating male when they had been experimentally offered a male leg during mating. However, we found no support for a protective function with regard to sexual cannibalism. As the number of legs a male ejected did not

depend on whether he mated with an unrelated or a sibling female, we could not confirm our predictions in favour of the mating investment hypothesis either. Finally, there was no evidence for special properties of male legs, which could make them especially attractive for females. Nevertheless, females invariably accepted and fed on male legs in our handling experiments, whereas insect prey items were rejected at a significant rate. Male legs were handled for 17 min, on average, suggesting that an ejected leg can effectively distract the female.

Females often grasped a male front leg autotomized during mating and refrained from further attacks. Indeed, so-called 'opportunistic matings' with females engaged in prey capture and feeding or directly after the female has moulted to maturity are common in spiders, including *Trichonephila* (Foellmer & Fairbairn, 2003; Robinson & Robinson, 1980). Males in *Trichonephila plumipes* as well as in the autumn spider, *Metellina segmentata*, even specialize exclusively in such opportunistic matings where the risk of injury or cannibalism is significantly reduced (Elgar & Fahey, 1996; Prenter, Elwood, & Montgomery, 1994; Robinson & Robinson, 1980). However, such opportunities are probably rare, given that few spider females can be observed feeding at the same time in field studies (Higgins & Goodnight, 2011; Venner & Casas, 2005). In addition, a male-biased operational sex ratio generates intense competition for receptive females in many species showing extreme reversed SSD. In general, males in most of these species should therefore engage in a risky mating with a potentially dangerous female, particularly if the female is unmated (Fromhage & Schneider, 2005a) or of high reproductive value. Copulatory male leg ejection may thus serve to distract and pacify especially those females that are not occupied with self-caught prey and become aggressive towards the male.

Female attacks occurred frequently across mating experiments, but sexual cannibalism was much less common. We recorded only five cases of cannibalism (16%) in experiment 3, three of which occurred in the treatment group where females had been offered a male leg. Interestingly, all but one male victim in our study were cannibalized directly after their first copulation, leaving one of the female's sperm storage organs empty and unplugged. Hence, from the female's perspective, the act of cannibalism safely eliminated the possibility of another mating attempt and subsequent mate guarding by the first male.

Under natural conditions, the female may practise a trading-up choice strategy and prioritize gaining sperm during her first copulation to secure reproduction. If the first male was of relatively low quality, the female may prevent him from remating in favour of another male of higher quality (Jennions & Petrie, 1997; Kempenaers et al., 1992; Welke & Schneider, 2009).

In many animal species, copulation duration is related to sperm transfer and is therefore an important predictor of male fertilization success (Simmons, 2001). Particularly, males in highly specialized terminal investing species with one-shot genitalia should evolve mechanisms to optimize copulation duration (Schneider & Elgar, 2001; Schneider, Gilberg, Fromhage, & Uhl, 2006). Female aggression towards males may thus be interpreted as a measure to reduce copulation duration in order to prevent a male monopolizing the female as well as to reduce the general costs of mating (Edvardsson & Canal, 2006). For example, female attacks significantly reduce copulation duration in the black widow spider, *Latrodectus tredecimguttatus* (Neumann & Schneider, 2011). In contrast, female attacks were instead related to prolonged copulatory insertions in *T. fenestrata*. As males are able to monopolize a female most efficiently by copulating twice with her, we asked whether female attacks could be adaptive in restricting males to a single copulation. However, whether a male performed a second copulation with a female did not depend on her aggressive

behaviour during his first copulation. Nevertheless, female enforcement of leg ejection through copulatory attacks is associated with the cost of impaired mobility (Wrinn & Uetz, 2008) and reduced mate-guarding ability in males (Fromhage & Schneider, 2005b), which probably also prevents the male monopolizing the female.

We expected males to invest differentially in females, preferring mates of relatively higher reproductive value (Welke & Schneider, 2010), and presented males with either an unrelated or a sibling female; the latter potentially bearing a risk of fitness costs through negative effects of inbreeding. However, the relatedness between male and female had no effect on male mating investment and performance. Fitness effects of inbreeding in spiders have been shown, for example, in the wasp spider, *Argiope bruennichi*, where sibling matings resulted in lower hatching rates (Zimmer, Krehenwinkel, & Schneider, 2014). Inbreeding also affected fecundity and hatching rates in the dwarf spider, *Oedothorax apicatus*, but these findings were reported for inbreeding over three generations (Bilde, Maklakov, & Schilling, 2007). First-generation inbreeding in *T. fenestrata* might not cause significant effects in terms of the offspring's fitness; hence selection for an avoidance mechanism may be weak.

Instead of assessing female compatibility, males prolonged copulation with females of large size and high body mass, which are indicators of increased fecundity (Higgins, 1992; Hirt, Ruch, & Schneider, 2017; Neumann, Ruppel, & Schneider, 2017). In addition, copulation duration was positively correlated with female age, probably because older females are close to their maximum body mass and to laying eggs. This also entails a decreased risk of reproductive failure through prereproduction mortality in these females (Rittschof, 2011).

Our analyses revealed interrelations between male and female traits and mating performance that may hint towards size-related male mating strategies and trade-offs involved in them. Note, however, that these considerations are derived from analyses of mating trials with feeding females. Risky matings with nonfeeding females are likely to be more common in nature, and such different settings are known to have a strong impact on male mating strategies (Fromhage & Schneider, 2005b, 2006). Similar to many other spider species showing extreme reversed SSD (Neumann & Schneider, 2015; Schneider, Herberstein, De Crespigny, Ramamurthy, & Elgar, 2000; Wilder & Rypstra, 2008), male and female body size in *T. fenestrata* has a significant influence on the process of mating, including copulatory male leg ejection. Less risky matings with feeding females still involved loss of legs in 25% of all trials, and large males ejected significantly more legs than small males. A reason may be that large males present an easier target for female attacks. Because of their longer appendages and larger body, it is probably easier for females to reach and grasp parts of their body. Furthermore, we found that two copulations occurred more often in pairings of small males and females; hence large males more often left one of the female's copulatory tracts unplugged. Although large males are generally more successful in guarding their female against rivals, a higher number of legs lost compromises this ability. Small males, on the other hand, may often increase their paternity by copulating twice without sacrificing legs. Having achieved their maximum mating rate, they remain functionally sterile and engage in especially vigorous mate guarding (Fromhage & Schneider, 2005b). On the other hand, fitness prospects of this mating strategy may be limited because two copulations typically occurred with young and small females, which are generally of lower reproductive value than larger and/or older females (see above).

Our findings suggest that the behavioural mechanism of copulatory male leg ejection in *T. fenestrata* differs in its function from other reported cases of nutritional donations, where males provide females with special substances or parts of their own body during mating (Kunz et al., 2012; Sakaluk et al., 2004; Vahed, 1998). In our study species, conspecific male front legs probably do not serve as a vector for nutritional substances that females could in turn reward with longer copulation duration or increased sperm storage. In contrast to many spiders (Herberstein et al., 2011; Schneider et al., 2006; Zhang et al., 2011), copulation duration in *T. fenestrata* seems to be largely under male control (Fromhage & Schneider, 2005a). This is because the male's pedipalp stays firmly attached to the female's genital opening during copulation and is rather slowly withdrawn at the end of copulation.

The adaptive value of copulatory male leg ejection cannot be understood without considering both male and female interests. The occurrence of female attacks was associated with a higher number of male legs ejected, but also with prolonged copulation durations. This suggests that males have to pay for copulations exceeding the preferred duration from the female's perspective by sacrificing legs and hence mate-guarding ability. While the adaptive significance of prolonged copulations in *T. fenestrata* is unclear (Fromhage & Schneider, 2006) copulations lasting longer than required for transferring sperm can increase male reproductive success in other spider species, for example by fulfilling an extended mate-guarding function (Linn, Molina, Difatta, & Christenson, 2007) or biasing paternity in postcopulatory competition (Bukowski & Christenson, 1997; Snow & Andrade, 2004). The underlying mechanisms in the latter cases remain unresolved, but studies on insects provide evidence for the transfer of accessory substances to the female genital tract that take up space the female could use to store other males' sperm, manipulate female receptivity or induce egg laying before another mating takes place (reviewed in Arnqvist & Nilsson, 2000). Single copulations in *T. fenestrata* lasted 25 min, on average, and often much longer, thus clearly exceeding the time required for sperm transfer. These observations raise the question whether males in this species transfer nongametic accessory substances in addition to sperm to maximize paternity. The pacifying effect of male leg ejection may aid in implementing such a mechanism.

In conclusion, male leg sacrifice in our study species may represent a rare example of an evolutionary transition of a widespread antipredation behaviour to another function. The more or less voluntary separation of an animal's own body parts that are then left to the predator for the sake of survival occurs not only in arthropods and other invertebrates (Fleming, Muller, & Bateman, 2007), but also in reptiles (Maginnis, 2006) and even in a small number of mammals (Dubost & Gasc, 1987; Seifert et al., 2012). The autotomized male legs in *T. fenestrata*, however, do not serve to increase the male's chances of escaping from cannibalistic females. *Trichonephila fenestrata* differs from all other species in its genus in that males show the most extreme specialization to monopolize a single female (Kuntner et al., 2018). This strategy involves male control over copulation duration by means of a special coupling mechanism of the copulatory organ and a modified mating position in which the female's body is tightly embraced with the posterior pairs of legs. Under lasting sexual conflict, the ancestral function of leg ejection seems to have changed along with the male mating strategy from an anticannibalism trait to the female pacifier which facilitates undisturbed copulations to the benefit of the male.

Data availability

The data are available at: Schneider, J. M. & Neumann, R. (2019). Males sacrifice their legs to pacify aggressive females. Mendeley Data, V1, https://doi.org/10.17632/th7xdcjm6x.1.

Acknowledgments

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