Mate plugging via genital mutilation in nephilid spiders: an evolutionary hypothesis

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Abstract

Nephilid spiders are known for gigantic females and tiny males. Such extreme sexual dimorphism and male-biased sex ratios result in fierce male–male competition for mates. Intense sperm competition may be responsible for behaviors such as mate guarding, mate binding, opportunistic mating, genital mutilation, mating plugs and male castration (eunuchs). We studied the mating biology of two phylogenetically, behaviorally and morphologically distinct south-east Asian nephilid spider species (*Herennia multipuncta*, *Nephila pilipes*) in nature and in the laboratory. Specifically, we established the frequencies and effectiveness of plugging (a plug is part of the male copulatory organ), and tested for male and female copulatory organ reuse. Both in nature and in the laboratory, plug frequencies were higher in *H. multipuncta* (75–80% females plugged) compared with *N. pilipes* (45–47.4%), but the differences were not significant. Plugs were single and effective (no remating) in *H. multipuncta* but multiple and ineffective (remating possible) in *N. pilipes*. In *Herennia*, the males plugged when the female was aggressive and in *Nephila* plugging was more likely when mating with previously mated and larger females. Further differences in sexual biology are complete palpal removal and higher sexual aggressiveness in *Herennia* (sexual cannibalism recorded for the first time), and mate binding in *Nephila*. Thus, we propose the following evolutionary hypothesis: nephilid plugging was ancestrally successful and enabled males to monopolize females, but plugging became ineffective in the phylogenetically derived *Nephila*. If the evolution of nephilid sexual mechanisms is driven by sexual conflict, then the male mechanism to monopolize females prevailed in a part of the phylogeny, but the female resistance to evade monopolization ultimately won the arms race.

Introduction

Sexual selection traditionally emphasizes precopulatory male–male competition and female choice (Darwin, 1871), but females are often polygamous and males monogamous; thus, post-copulatory sexual selection is important (Eberhard, 1996; Arnqvist & Danielsson, 1999; Zeh & Zeh, 2003; Snook, 2005; Andersson & Simmons, 2006). Post-mating sexual selection (sperm competition, cryptic female choice and sexual conflict) may influence genital morphology and sexual behaviors (Parker, 1970; Eberhard, 1985, 1996, 2004; Arnqvist, 1998). In polyandrous mating systems, sperm competition results in a range of behavioral and physiological adaptations, such as mate guarding, opportunistic mating with freshly molted or feeding females (Fromhage & Schneider, 2005) and mate plugging (Arnqvist & Rowe, 2005). Male-produced mating plugs occur in diverse animals

such as mammals, snakes, insects, acanthocephalan worms (Parker, 1984; Schwartz, McCracken & Burghardt, 1989) and spiders (Wiehle, 1967; Knoflach & van Harten, 2000, 2001; Schneider, Thomas & Elgar, 2001; Schneider, Fromhage & Uhl, 2005; Agnarsson, 2006). In spiders, plugging via breakage of the male palpal (sperm-transferring organ) structures evolved independently in several lineages (Austad, 1984; Miller, 2007). Here, we investigate an extreme case of plugging via sexual mutilation, in the nephilid spiders, and focus on its prevalence, effectiveness and evolutionary implications.

Nephilid spiders (genera *Nephila*, *Nephilengys*, *Herennia* and *Clitaetra*; Kuntner, 2006) are sexually dimorphic (Vollrath & Parker, 1992; Kuntner, Coddington & Hormiga, 2008) and variable in size (Uhl & Vollrath, 2000). *Nephila* females are evolutionary giants (Fig. 2a; Coddington, Hormiga & Scharff, 1997; Hormiga, Scharff & Coddington,

2000; Higgins, 2002), being 10–100 times heavier than the tiny males. The evolution of sexual size dimorphism in nephilids progressed from moderate to extreme (female gigantism) and this gradual shift may affect sexual biology and web size (Ramos *et al.*, 2005; Kuntner *et al.*, 2008). Because of longer female maturation, extreme sexual size dimorphism results in strongly male-biased sex ratios and fierce male–male competition for mates. As a consequence, characteristic sexual behaviors have evolved, including sexual cannibalism (Elgar & Fahey, 1996; Uhl & Vollrath, 1998; Schneider & Elgar, 2001, 2002; Elgar *et al.*, 2003; Elgar & Schneider, 2005), male genital mutilation and removal (Fig. 1a–c; Robinson & Robinson, 1978, 1980; Kuntner, 2005, 2007; Kuntner *et al.*, 2008) and plugging of female genitals (Fig. 1b and c; Kuntner, 2005, 2007; Fromhage & Schneider, 2006; Kuntner *et al.*, 2008). Males of certain species practice monogyny, but females of other species are extremely polyandrous (Fromhage, Elgar & Schneider, 2005; Kuntner *et al.*, 2008). Further, *Herennia* and *Nephilengys* males emasculate themselves by removing their palpal bulbs entirely to become effectively sterile

eunuchs or *castratos* (Fig. 1d; Robinson & Robinson, 1978, 1980; Kuntner, 2005, 2007).

In nephilids, plugging via genital mutilation occurs in *Herennia* (Fig. 1a and b; Kuntner, 2005), most *Nephila* species (Fig. 1c; Schneider *et al.*, 2001, 2005; Fromhage & Schneider, 2006; Kuntner *et al.*, 2008) and *Nephilengys* (Kuntner, 2007). Single and complex plugs (*Herennia*: Fig. 1b, *Nephila fenestrata*), which appear to be functional, contrast with thin and multiple plugs (*Nephila pilipes*: Fig. 1c), which appear to be non-functional. Nephilid phylogeny suggests a single origin of plugging in the common ancestor of *Nephila*, *Nephilengys* and *Herennia* (Kuntner, 2006; Kuntner *et al.*, 2008). The male palpal morphology (and thus plug shape) evolved from complex (*Herennia*) to simple (*Nephila*; Kuntner *et al.*, 2008). Genital damage and plugging frequencies differ: whereas 96% of palps are damaged during copulation in *N. fenestrata* (the phylogenetically intermediate nephilid with effective mating plugs; Fromhage & Schneider, 2006), the frequencies are much lower in most other nephilids (Kuntner *et al.*, 2008). Here, we examine different sexual strategies in phylogenetically distant
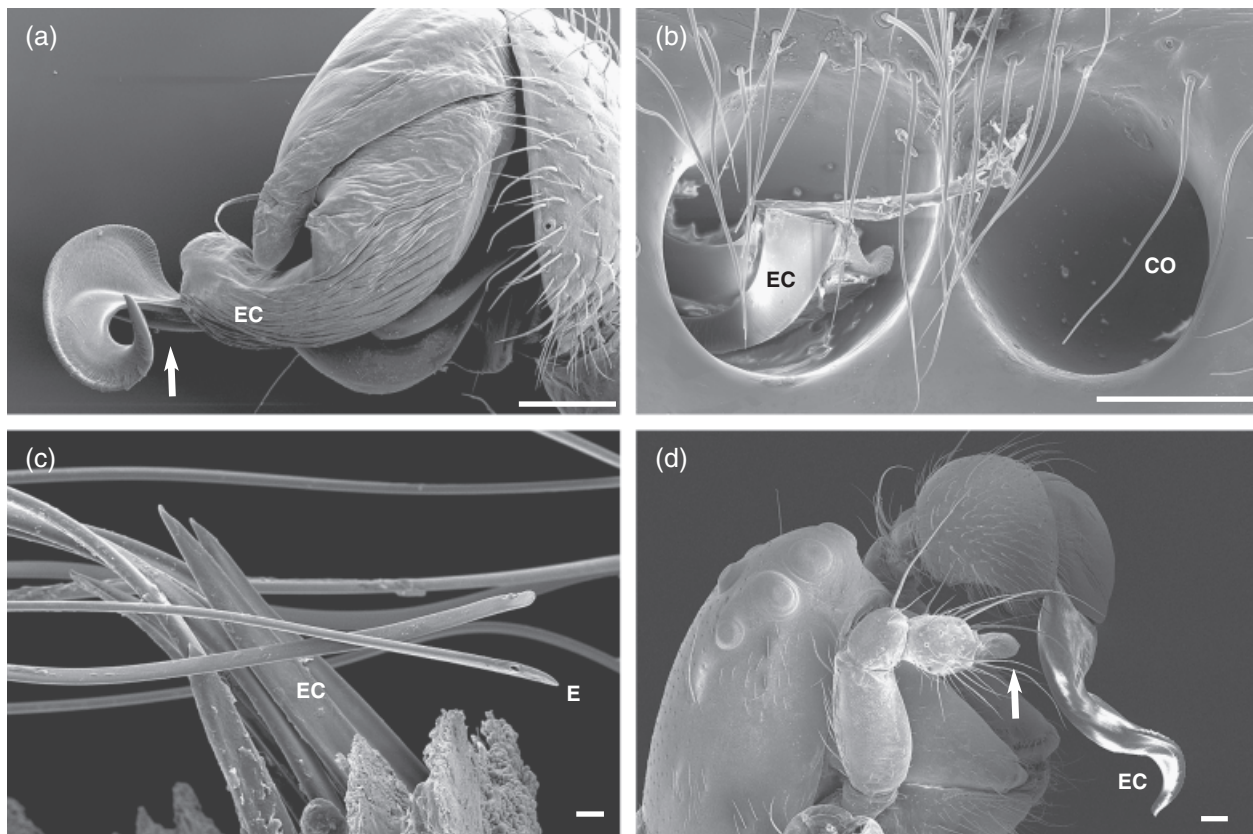

Figure 1 Nephilid genital morphology and damage: a–b, *Herennia multipuncta*; a, intact male palp, the arrow indicates the typical breaking point of the distal embolic conductor (EC); b, female epigynum, ventral, with the left copulatory opening (CO) intact and the right CO plugged with male palpal sclerite, EC; c, *Nephila pilipes*, detail of removed amorphous epigynal plug showing numerous male EC plus emboli (E) lodged in a single CO; d, *Nephilengys malabarensis* male half eunuch, lacking the right palpal bulb (arrow); identical bulb severance is also common in *Herennia*. Scale bars = 100 μm, except c = 10 μm.

nephilids and focus on the effectiveness and evolutionary implications of plugging during mating.

Plug frequency and effectiveness were assessed in two nephilid species, which are phylogenetically scattered relative to *N. fenestrata*. *Herennia multipuncta* and *N. pilipes* exhibit different sexual strategies: *H. multipuncta* males produce single embolic plugs with a massive embolic conductor (Fig. 1a and b) and then sever palps to become eunuchs (Fig. 1d; Kuntner, 2005). In contrast, *N. pilipes* females may be multiply plugged with thin emboli and short parts of embolic conductors (Fig. 1c), and males do not sever palps (Kuntner *et al.*, 2008). Preliminary morphological data from museum specimens (Kuntner, 2005, 2006, 2007; Kuntner *et al.*, 2008) suggest that organ damage in *Herennia* is nearly universal, but is rare and less extreme in *Nephila*. Based on these morphological data, in addition to knowledge about variation in plug effectiveness in two other *Nephila* species (Schneider *et al.*, 2001; Fromhage & Schneider, 2006), we predicted high plugging frequencies and effective embolic plugs in *H. multipuncta*, because remating with a previously damaged organ [broken palp in male; plugged epigynal copulatory opening (CO) in female] should be precluded. We also predicted a lower frequency of embolic plugs in *N. pilipes*, and plug ineffectiveness, because remating (and thus polygamy) should be possible due to thin embolic plugs. Note that both male palps (Fig. 1d) and female COs (Fig. 1b) are paired structures that are used independently. Hence, plugging one CO or damaging one pedipalp leaves a second mating possible with the same or a different partner.

Materials and methods

Study animals

Spiders were collected in April and May 2007 in Singapore (Pulau Ubin, Sentosa, Central Catchment Area) and Malaysia (Pulau Tioman). To manipulate spiders with known mating histories, we collected subadults from their own webs and reared them to adulthood, but also took additional live adults of both sexes for behavioral trials. In total, we kept 33 captive laboratory animals (*H. multipuncta*: $n♀ = 7$, $n♂ = 8$; *N. pilipes*: $n♀ = 11$, $n♂ = 7$). Between 28 April 2007 and 13 May 2007, laboratory spiders were kept on a light–dark cycle of 12:12 h, watered daily and fed twice a week with *Drosophila* and mealworms. Additionally, we recorded spiders and their behavior in nature in and around female webs and preserved them in 70% ethanol for morphological examination (*H. multipuncta*: $n♀ = 8$, $n♂ = 7$; *N. pilipes*: $n♀ = 19$, $n♂ = 7$).

Experimental protocol

In staged mating trials in the laboratory, we studied behavioral patterns of mating, occurrences of genital damage and rematings in *H. multipuncta* and *N. pilipes*. We placed females into Perspex frames to allow them to build webs, whereas males remained in foam-covered plastic cups. Only females that had built a web were used in mating trials. Mating was staged by gently placing a male (or two males; see below) in the female web, *c.* 15 cm away from her. In 16 trials of *H. multipuncta*, precopulatory behavior occurred in 12 (in six of the 12, it was followed by mating) and in four cases no precopulatory behavior and no mating occurred. In 19 trials of *N. pilipes*, precopulatory behavior occurred in 15 (in nine of the 15, it was followed by mating); two further cases lacked both precopulatory behavior and mating and in two cases mating occurred without precopulatory behavior. During copulation (which occurred in 51.4% of trials), we recorded which palp the male used (left/right/both), how many times the male inserted each palp, which CO he inserted into (left/right/both), whether the female responded aggressively towards the male and whether sexual cannibalism occurred or not. A female was considered aggressive when she shook the web, directly attacked a male by biting or shook her body and brushed off a male using her legs. Observations of two insertions in the same CO in females and two insertions with the same palp in males would confirm female and male genital reuse. To test whether a mated male can remate with the same palp, we confronted him with an unplugged female. To test whether the female can remate in the same CO, a previously mated female (with and without plugged CO) was mated with a male with intact palps. We also recorded copulatory behaviors when both, male and female, had mated previously and even confronted two males: one palped and one palpless (eunuch) with a female. Not all females mated (Table 2).

Observations lasted at least 1 h (in case of successful reuse of one palp) and at most 4 h. After a trial the spiders were given at least a 24-h rest. At the end of all trials, the spiders were euthanized and their first tibia plus patella length was measured.

Morphological examination

All adult specimens (from mating trials and those fixed directly) were measured under a Leica MZ16 stereomicroscope (Leica Microsystems, Wetzlar, Germany) and examined for external genital damage. External palpal morphology reveals damage of only certain sclerites, for example, of the embolic conductor (Kuntner, 2005, 2006, 2007), but not necessarily of the embolus. Therefore, all palps were macerated in concentrated KOH overnight to make them transparent and expandable in distilled water. All epigyna were excised and further examined externally from an extreme caudal view. Each epigynal preparation was macerated in concentrated KOH overnight, and then carefully cleaned in distilled water with needles. This technique exposed the dorsal epigynal anatomy (Kuntner, 2005, 2006, 2007; Kuntner *et al.*, 2008). Spermathecae became translucent in most cases, rendering emboli lodged inside visible under a stereo or a compound microscope. To improve the visibility of male genital parts within females (in particular within the strongly sclerotized copulatory ducts),

epigynal preparations were treated with methyl salicate for 1–4 h. In the extreme cases, where even this technique failed to result in translucent spermathecae and ducts, the spermathecae were cut open using a fine scalpel, to expose their lumen.

Ethograms

We used the existing ethograms for *Herennia papuana* (as *Herennia ornatissima*; Robinson & Robinson, 1973, 1976, 1980; Robinson, 1982) and *N. pilipes* (as *Nephila maculata*; Thakur & Tembe, 1956; Robinson & Robinson, 1973, 1976, 1980; Olsen, 1993).

Statistical analyses

We assessed differences in tibia + patella I length between spiders with and without genital damage using the Mann–Whitney test (all collected spiders), and tested whether the occurrence of a plug/broken palp could be predicted by multiple use of the same CO/palp or by female aggression (spiders from mating trials) with logistic regression. The analyses, performed with SPSS version 13, were performed for males and females separately. The results were corrected for 'experiment wide error rate' and considered significant at probability 0.025 ($\alpha/2$).

Results

Field data

In nature, males *H. multipuncta* and *N. pilipes* occur in webs of subadult and adult females (*H. multipuncta* average 0.7 males in 11 webs; *N. pilipes* 0.88 males in 19 webs). In webs of subadult female *H. multipuncta*, we found four males: two were intact (with undamaged palps), one was half eunuch (one palp lost) and one full eunuch (both palps lost); the latter two cohabited in the web (Fig. 2c). In webs of adult female *H. multipuncta*, we found three males: one was intact and two were half eunuchs. Males in webs of *N. pilipes* were intact (85.8%) or had one palp damaged (14.2%). Mate binding (male deposition of silk on female carapace, legs and abdomen; Fig. 2a) is an obligate component of the precopulatory repertoire in *N. pilipes*. The frequencies of genital plugs and damage in the field-collected spiders were higher in *H. multipuncta* than in *N. pilipes*, but the differences were not statistically significant (Table 1; difference in mating plugs in females – Mann–Whitney $U = 56.5$, $P = 0.269$, $N = 27$; difference in damaged palps in males – Mann–Whitney $U = 16.5$, $P = 0.194$, $N = 14$). Epigyna of female *N. pilipes* may be completely covered with hard amorphous matter (Fig. 1c; Robinson, 1982; in our study, $N = 5$), which may have an additional plugging function.

Laboratory data

In all mating encounters, insertions were ipsilateral (left palp inserted into left CO, and right palp into right CO) as is common in araneoid spiders (Knoflach, 1998). In both species, the female/male reused the same CO/palp in successive copulations with the same partner (Table 2). In *N. pilipes* (but never in *H. multipuncta*), the same female CO was used by more than one mate, even if previously plugged (Table 2). Females may be plugged in one or both CO. Although no statistically significant difference was detected (Mann–Whitney $U = 25.5$, $P = 0.407$, $N = 17$), plug frequencies were higher in *H. multipuncta* compared with *N. pilipes* (Figs 3 and 4). Males of both species never attempted to insert damaged palps. Likewise, full eunuchs (*H. multipuncta*) never attempted to copulate ($N = 3$).

Summary ethograms derived from mating trials (Figs 3 and 4) show substantial differences in approach, mating and acceptance/rejection behaviors as well as in frequencies of genital damage and plugging between the two species. In *Herennia*, the approach to the epigynum is lateral from the female dorsum, but in *Nephila* it is either apical from the dorsum or direct from the web. Mate binding is absent in *Herennia*. Receptive *Herennia* females facilitate male access by moving their abdomen out of the hub-cup, but *Nephila* females remain motionless if receptive. *Herennia* males copulate by twisting their body sideways into a perpendicular position relative to the female axis, but *Nephila* males remain parallel to the female while copulating. Female aggressiveness in *Nephila* precluded or ended copulation, but in *Herennia* it led to plugging and cannibalism.

In *H. multipuncta*, an extensive distal part of the male palp blocks the female CO (Fig. 1a and b) and maximally one plug per CO was observed. Palpal breakage ($\chi^2 = 12.365$, $P < 0.001$, $N = 10$) as well as plugging ($\chi^2 = 10.585$, $P = 0.001$, $N = 8$) were significantly more likely when the female was aggressive towards the male during copulation, but not when she multiply reused the same CO (Table 3). Three males removed their disfigured palps after copulation and became eunuchs.

In *N. pilipes*, the embolus always broke close to the embolus base, leaving the base attached to the bulb and most of the thin embolus broken off. Thin male palpal sclerites broke off and remained in female CO (Fig. 1c). Neither multiple palp reuse nor female aggression during copulation could predict palpal breakage (Table 3). Females had up to three plugs per CO. Larger females were more likely to have at least one plug (Mann–Whitney $U = 13$, $P = 0.004$, $N = 21$, Fig. 5). Plugging was significantly correlated to multiple CO reuse, but not to female aggression (Table 3). Emasculation was never observed in *N. pilipes* (Table 2).

Discussion

We tested the prediction that male genital damage and the resulting frequency and efficiency of plugging the female would differ in two sympatric south-east Asian nephilid species that differ in their relative phylogenetic position,

Figure 2 Nephilid sexual biology (in nature): a, *Nephila pilipes* male (small) resting on female dorsum in between copulation bouts; note silk deposited during mate binding on the female prosoma and the right male palp being broken at the tip (Sentosa, Singapore); b–d, *Herennia multipuncta*; b, half-eunuch male (small) resting on the female dorsum after copulation (P. Ubin, Singapore); c, cohabitation of subadult female in her web with a half- (right) and a full eunuch, left (Tioman, Malaysia); d, adult male in his web, presumably just molted and thus a virgin (Bogor, Indonesia).

Table 1 Genital plug and palpal damage frequencies in spiders collected in nature

	Female plugs			Male palpal damage		
	None	In one CO	In both CO	None	One	Both
Herennia multipuncta ($n♀=8$, $n♂=7$)	25% ($n=2$)	37.5% ($n=3$)	37.5% ($n=3$)	42.9% ($n=3$)	42.9% ($n=3$)	14.3% ($n=1$)
Nephila pilipes ($n♀=19$, $n♂=7$)	52.6% ($n=10$)	31.6% ($n=6$)	15.8% ($n=3$)	85.7% ($n=6$)	14.3% ($n=1$)	0% ($n=0$)

CO, copulatory opening.

Table 2 Conclusions from mating trials

	Herennia multipuncta	*Nephila pilipes*
Number of mating trials	16	19
Number of trials with at least one copulation	6	11
Palp reuse, CO reuse same partner	Yes ($n=3$)	Yes ($n=8$)
Palp reuse, different partner	Yes ($n=1$)	Yes ($n=4$)
CO reuse, different partner	No	Yes ($n=2$)
Palp breakage	Yes ($n=4$)	Yes ($n=5$)
Palp removal (eunuchs)	Yes ($n=3$)[a]	No
Successful copulation in previously plugged CO	No	Yes ($n=2$)
Female aggressiveness towards male	Yes ($n=5$)	Yes ($n=10$)
Female cannibalism upon male	Yes ($n=2$)	No

[a]Two males with a broken palp were cannibalized before palpal removal could occur.
$n=$number of trials, where event was observed.
CO, copulatory opening.

their behavior, morphology and natural history. Both in nature and in the laboratory, plug frequencies were higher in *H. multipuncta* (75–80% females plugged) compared with *N. pilipes* (45–47.4%), but the differences were not significant (Table 1, Figs 3–4). Our data confirm that plugs in *Herennia* are effective barriers to subsequent mating and allow males to achieve monopolization of the given CO. In *H. multipuncta*, the only indication of female remating (two males, same CO) came from a field-collected female with two plugs in one CO. Although more than half of the males produced a plug, usually only females that reacted aggressively towards a male received a plug. Female aggressiveness may thus facilitate breakage of the palp by forcing the male to retract and sever the distal sclerites. Alternatively, female aggressiveness may be a signal to a male that his investment is safe and that the high costs of emasculation are justified.

In *N. pilipes*, fewer males plugged the females and the plugs were numerous and apparently ineffective. In contrast to *H. multipuncta*, females of *N. pilipes* received copulations from several males into the same CO, which was in accordance with the frequent finding of several plugs inside a single CO in nature. Thus, in *N. pilipes* male plugging is ineffective in preventing female polyandry. Female multiple organ reuse (polygamy) predicted plugging (Table 3) and larger females had higher plug frequencies (Fig. 5). The

pattern suggests a male preference for large, maximally fecund females. Looking at the broader pattern, plugging seems to be ineffective in phylogenetically relatively distal *Nephila* species (*N. pilipes*, *Nephila plumipes*; Schneider *et al.*, 2001), but effective in the relatively basal *N. fenestrata* (Fromhage & Schneider, 2006). Because plugging in nephilids is homologous, its ineffectiveness in distal *Nephila* spp. is derived.

Sexual aggression is common in female *H. multipuncta* and may result in sexual cannibalism, which was recorded in *Herennia* for the first time. Aggressive females tend to receive plugs (Table 3). Plugging may be the male's attempt to monopolize the female genital opening before aggression terminates in dismount or cannibalism. Unlike in black widows (Andrade, 1996), male nephilids do not sacrifice themselves but rather attempt to escape the cannibalistic female.

Apart from plugging, nephilid sexual behaviors remain poorly understood. Mate binding (Fig. 2a) was consistently present in *N. pilipes*. Although Robinson & Robinson (1980) reported mate binding in *H. papuana* (as *H. ornatissima*), the behavior is not present in *H. multipuncta*. In our study, female *N. pilipes* were never aggressive during mate binding, which suggests that the behavior might have a stimulating function (Robinson & Robinson, 1980), perhaps to counter female cannibalism.

Complete palpal severance (the eunuch phenomenon), unknown in *Nephila* and *Clitaetra*, is common in *Nephilengys* and *Herennia* (Kuntner *et al.*, 2008). In *H. multipuncta*, males become eunuchs by emasculating their palpal bulb(s) after dismount following sexual mutilation. The adaptive significance of the eunuch phenomenon remains elusive (Robinson & Robinson, 1978, 1980; Kuntner, 2005, 2007; Kuntner *et al.*, 2008). Kuntner (2005) introduced three related hypothesis discussing the stages of becoming eunuch. The first hypotheses (that broken embolic parts function as mating plugs) was directly tested and supported by our data. The second hypothesis (that bulb severance prevents hemolymph loss) was not explicitly tested but remains plausible. The third hypothesis (that the eunuch protects his parental investment by fighting off rival males) is supported by our data (eunuchs do remain in female proximity and mate guard). Here we propose a fourth, more precise and testable prediction, named the 'gloves off' or 'male eunuch advantage' hypothesis. Male's post-copulatory interest is to defend 'his' female from remating. Because a mated male with a disfigured palp is likely to find himself at a cumbersome disadvantage fighting intact males, he
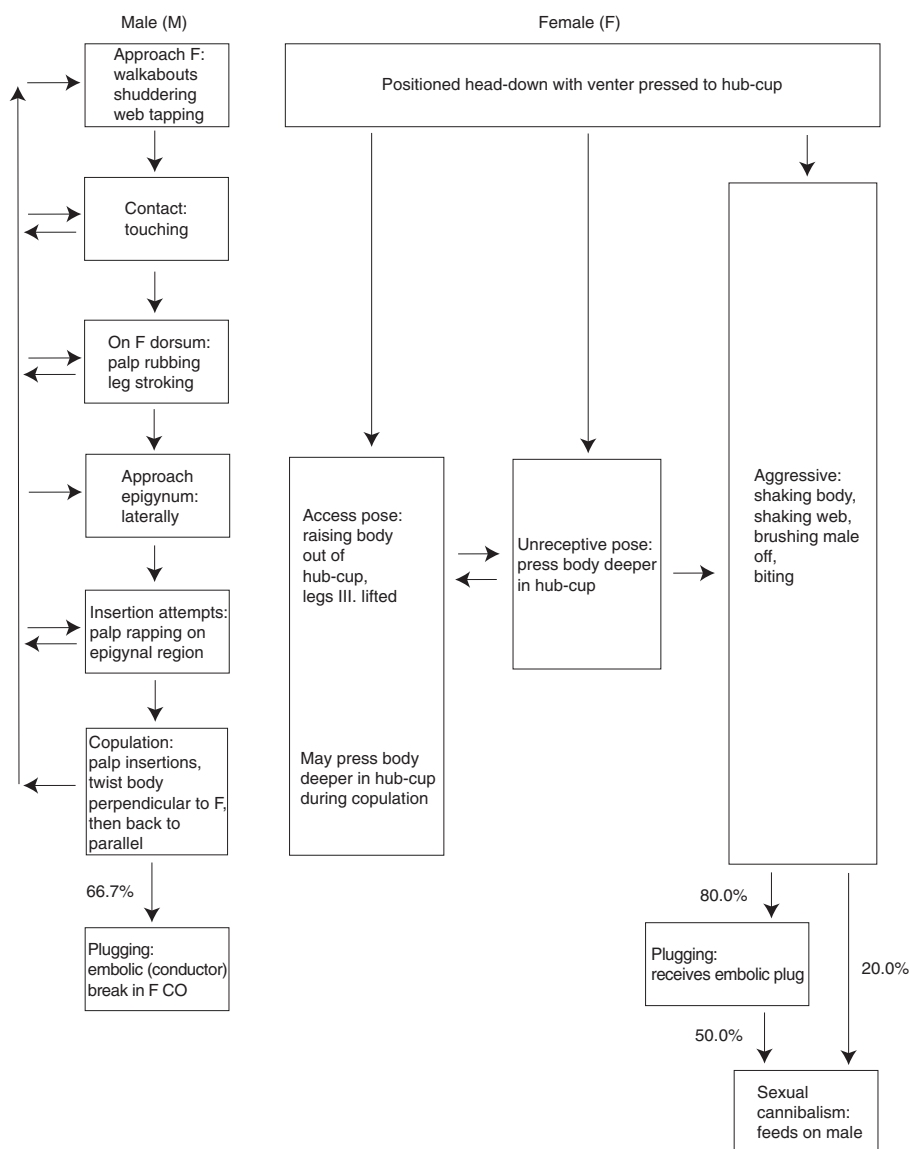
Figure 3 Ethogram of mating sequences in *Herennia multipuncta*. Time is on the *y* axis; arrows show possible directions of behaviors, which may stop at any point. The frequencies of final outcomes are according to laboratory mating trials.

chooses self-emasculation. This is analogous to the precopulatory self-emasculation in male theridiid spiders *Echinotheridion* and *Tidarren* (Knoflach & van Harten, 2000, 2001; Agnarsson, 2006), a behavior shown to increase male mobility during sexual encounters (Ramos, Irschick & Christenson, 2004). To a nephilid eunuch, emasculation poses no costs because his disfigured palp cannot be recharged or reused (he is effectively sterile), but his potential benefit may be a more efficient fight with rival males. Eunuchs are always aggressive towards rival males whenever a female is present (virgin or mated), the most paradoxical case being the guarding of a subadult, virgin female by a full eunuch (Fig. 2c).

What are the evolutionary implications of our results? In nephilids, two phylogenetically distinct mating strategies are apparent. Promiscuity (both polygyny and polyandry) operates in *N. pilipes* (and in other distal *Nephila*) in spite of embolic plugs. On the other hand, monogyny and monandry, enforced by male plugging, are prevailing mating strategies in *Herennia* (as in *H. multipuncta*) as well as in *N. fenestrata* (Fromhage & Schneider, 2006). Nephilid plugging was ancestrally successful as it enabled males to monopolize females, but accumulating evidence suggests that it became ineffective in phylogenetically derived *Nephila*. Our results imply that in nephilid spiders, both females and males may have evolved strategies to overcome
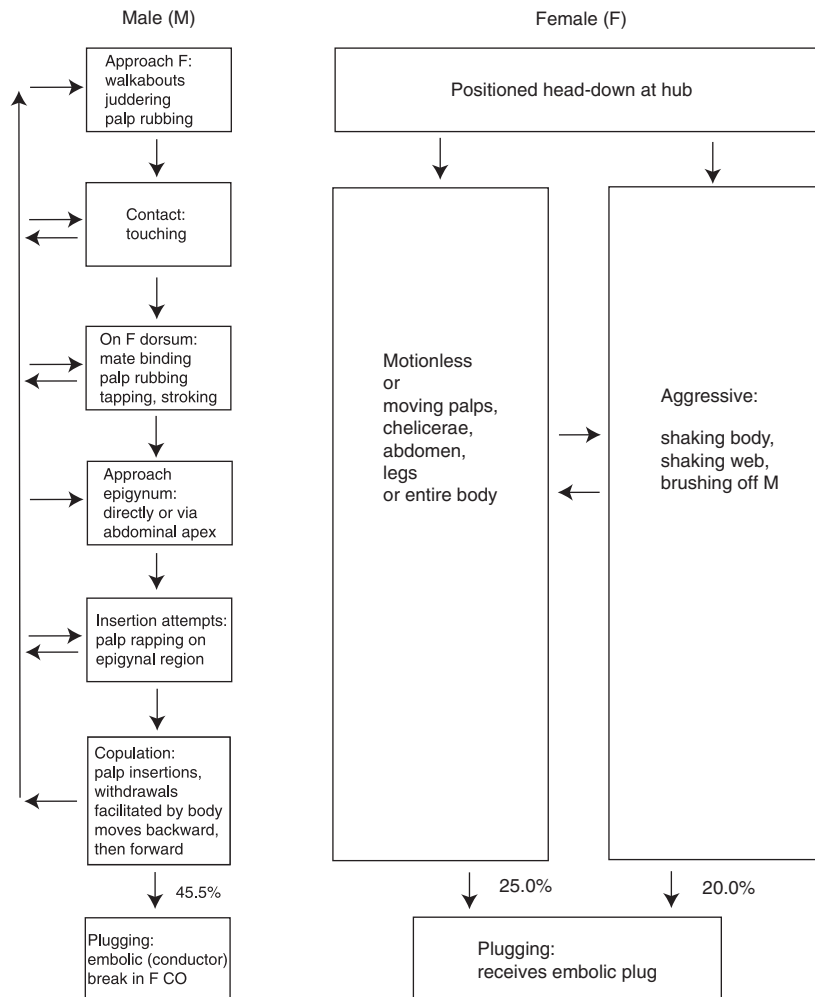
Figure 4 Ethogram of mating sequences in *Nephila pilipes* (see the explanation in Fig. 3).

Table 3 Estimated probability (using logistic regression) for the occurrences of a plug/broken palp predicted by multiple use of the same CO/palp or by female aggression during a mating trial

	Herennia multipuncta			*Nephila pilipes*		
	χ^2	P	N	χ^2	P	N
Multiple organ use						
Palpal damage	0.228	0.633	10	2.522	0.112	15
CO plug(s)	0.541	0.462	8	6.904	**0.009**	16
Female's aggression during copulation						
Palpal damage	12.365	<**0.001**	10	0.187	0.666	15
CO plug(s)	10.585	**0.001**	8	0.287	0.592	16

CO, copulatory opening; (bold = significant).

monopolization. Future research may reveal whether these changes are the result of an arms race scenario based on a sexual conflict over mating rate (Arnqvist & Rowe, 2005).
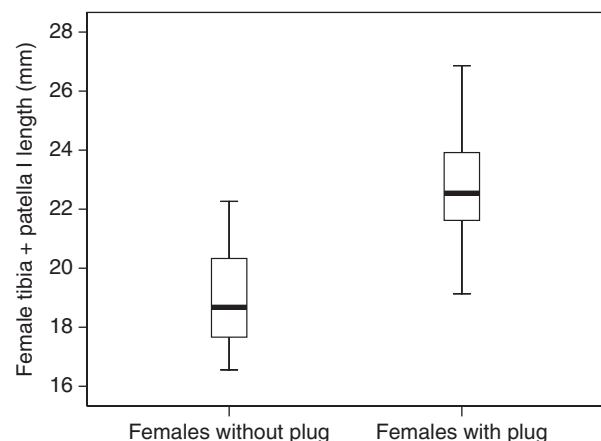

Figure 5 In *Nephila pilipes*, larger females were more likely to be plugged (Mann–Whitney $U = 13$, $P = 0.004^*$, $N = 21$).

Acknowledgments

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