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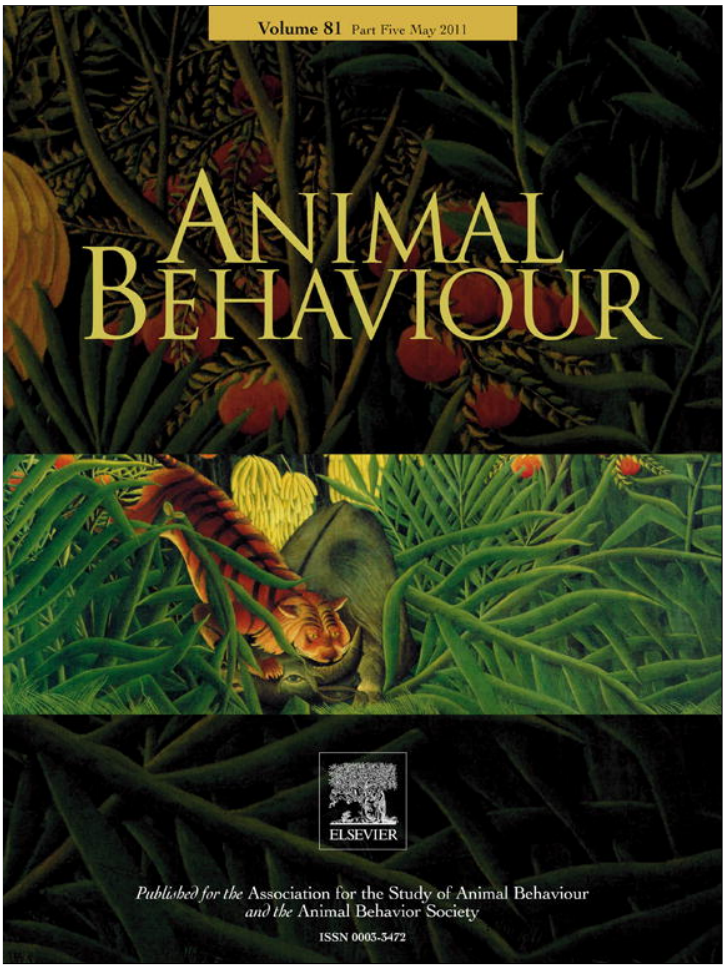

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Eunuchs are better fighters

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Genital amputation, that is, genital damage or loss, seems maladaptive because it renders the amputee functionally sterile, but is nevertheless common in sexually dimorphic spiders. In these species, male genital amputation correlates with plugging of female genitals and with sexual cannibalism. Genital amputation in male spiders may be partial or full; the latter is known as the eunuch phenomenon. We tested two adaptive hypotheses about eunuch behaviour in an orb web spider, *Nephilengys malabarensis*: (1) the plugging hypothesis (i.e. broken male genitals (palps) effectively plug the female genitals) and (2) the better fighter hypothesis (i.e. eunuch males are better fighters than their intact rivals). By staging mating trials, we documented genital amputation (occurrence and frequency), sexual cannibalism and genital organ reuse, morphologically examined plugs to infer their effectiveness, and conducted a series of male–male contests to determine whether eunuch males were better fighters. Copulations always resulted in amputation of the palps: 87.5% of males became eunuchs directly during copulation and plugged females, while 12.5% of males first partially damaged the palps and then severed them after copulation. Sexual cannibalism and plugging effectiveness both reached 75%. Eunuchs guarded females, were highly aggressive and active, and initiated and won contests more often, whereas intact males and half-eunuchs showed significantly lower levels of guarding behaviour, aggression and general activity. Thus, both hypotheses are supported and we conclude that the eunuch phenomenon is adaptive.
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The classical view of overall polygyny (male polygamy) has been challenged by the finding that males practise monogyny without parental care in a number of spider and insect species (Boomsma et al. 2005; Fromhage et al. 2005, 2008; Miller 2007; reviewed in Schneider & Fromhage 2010). However, even in monogynous systems, it is in the interest of males to win in sperm competition, and such selection results in numerous behavioural, physiological and morphological adaptations (Parker 1970). Males can reduce the cost of polyandry (female polygamy) by guarding females, by reducing the willingness of females to remate or their attractiveness to subsequent mates, or by applying mechanical barriers, that is, mating plugs, thereby limiting access to the female's copulatory organs or spermathecae (Parker 1970; Elgar 1998; Simmons 2001; Huber 2005). The latter may involve different mechanisms of male (genital) amputation, ranging from breaking off a part of the male genitals (as is the case in most spiders that exhibit genital damage; reviewed in Uhl et al. 2009), breaking off the entire copulatory organ (ceratopogonid biting midges: Downes 1978; spider genera *Caerostris*, *Deliochus*, *Herennia*, *Nephilengys* and *Phonognatha*: Kuntner 2005, 2007; Kuntner et al. 2008, 2009a, b, c; Kuntner & Agnarsson 2010), to using a body part or the whole body as a plug (queenless ant *Dinoponera quadriceps*: Monnin & Peeters 1998; the spider *Argiope aurantia*: Foellmer & Fairbairn 2003). Yet, such adaptations may also reduce male mating rates because of the time, energy and resources devoted to attempts to monopolize a female. Logically, males with damaged or missing genitals have limited future mating opportunities and thus are fully or almost monogamous by default (Kuntner et al. 2009c). Nevertheless, male monogamy, which is sometimes related to single/double mating with the same female, is favoured in species with a male-biased sex ratio (Fromhage et al. 2005, 2008; Miller 2007).

Despite high costs to the males, mating plugs are not effective in limiting rivals' access to the female's genitalia in all species (Schneider & Elgar 2001, 2002; Kuntner et al. 2009c). Within invertebrates, the spiders *Latrodectus hasselti*, *Argiope bruennichi*, *Nephila fenestrata*, *Herennia multipuncta* and the ant *D. quadriceps* are among the rare species in which broken male genitalia have

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been shown to function as effective mating plugs (Monnin & Peeters 1998; Fromhage & Schneider 2006; Snow et al. 2006; Nessler et al. 2007; Kuntner et al. 2009c). In some of these species, males spontaneously die after the first or second copulation, whereas they may survive copulation in others but subsequently lack their genital organs (palps). We refer to the latter as eunuchs (lacking both palps) or half-eunuchs (lacking one palp; Robinson & Robinson 1978, 1980; Kuntner 2005, 2007; Kuntner et al. 2009a, c). Subsequently, eunuchs commonly employ additional behaviours, for example postcopulatory mate guarding, which may further reduce the risks to their paternity. From an evolutionary viewpoint, a sterile male has no reproductive future and has nothing to lose. Theory predicts that a eunuch guarding a female will respond, to enhance his paternity, with maximal force when faced with an intruder (e.g. Austad 1982, 1983; Watson 1990; Fromhage & Schneider 2005a, b). A potential rival male with functional genitalia (palps), however, has a potentially high future reproductive value, and thus much to lose in a contest. The intact rival is therefore expected to avoid contests and potential injuries prior to mating. In fact, previous studies have suggested that eunuchs aggressively guard females against intact rivals (Robinson & Robinson 1980; Kuntner 2005, 2007; Kuntner et al. 2009a, c). Furthermore, precopulatory removal of the palps in male *Tidarren* spiders renders them more agile, thereby allowing them to win contests against rival males (Ramos et al. 2004). If mate-guarding eunuchs are more aggressive and agile than intact rivals, then the eunuch phenomenon is indeed adaptive.

We studied the mating and postcopulatory behaviour of *Nephilengys malabarensis*, a common South-East Asian nephilid spider with extreme sexual size dimorphism and pronounced sexual cannibalism (Kuntner 2007; Kuntner & Coddington 2009). *Nephilengys malabarensis* has a male-biased sex ratio with males competing for virgin females (Kuntner 2007). Because *N. malabarensis* are entelegyne spiders (i.e. spiders in which females possess separate copulatory and fertilization ducts leading to and from the spermathecae), which presumably exhibit first-male sperm priority (Austad 1982, 1983), it is not surprising that males often guard subadult females in order to be the first to mate (M. Kuntner, personal observation). During copulation, males regularly damage their copulatory organs (palps) within female genitals (Kuntner 2007). Furthermore, these males self-amputate their palps entirely after copulation to become eunuchs (Fig. 1a, b). If males survive copulation, they typically stay with the female to mate-guard her (Kuntner 2007). We tested two adaptive hypotheses that attempt to explain the eunuch phenomenon (Kuntner 2005): (1) the broken genital parts of the male (the embolic conductor or the entire palp) plug the female's copulatory opening and thus prevent rival males' access (henceforth, the plugging hypothesis); and (2) eunuchs are better fighters than their intact rivals, perhaps because they are more agile (they do not have to carry around the relatively large pedipalps) or more aggressive (henceforth, the better fighter hypothesis).

Figure 1. Sexual behaviours in *Nephilengys malabarensis*. (a–b) Males lacking one or both sexual organs (palps) are referred to as (a) half-eunuchs or (b) (full) eunuchs, respectively. (c) Staged mating of a virgin female (left) and a virgin male (right). (d) Plug in female's copulatory opening consists of an entire palpal bulb (arrow). (e) Female's paired genitals internally, showing one broken male embolus (arrow) per spermatheca. (f) Sexual cannibalism: female attacking and devouring her mate.

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METHODS

Study System

We collected *N. malabarensis* spiders between December 2009 and January 2010 in Singapore: Labrador Park (1°15′58″N, 103°48′10″E), Pulau Ubin (1°24′30″N, 103°57′40″E) and Kent Ridge Park (1°17′10″N, 103°47′10″E). To examine rematings using the same genital organ, we needed spiders with known mating histories. Therefore, we collected subadults and reared them to adulthood in the laboratory ($N♀ = 21$, $N♂ = 14$). In addition to subadults, we collected adult females and males for contest trials ($N♀ = 20$, $N♂ = 46$). In the laboratory, we placed the females into glass and Perspex frames ($30 \times 30 \times 30$ cm) to allow them to build webs, whereas males remained in foam-covered plastic vials (0.25 litres). We kept the spiders on a light:dark cycle of 12:12 h, watered them daily, and fed them twice a week with fruit flies, house flies and mealworms.

Experimental Protocols

In staged experimental trials in the laboratory, we first tested the plugging hypothesis. We predicted that broken palps would prevent a female remating by forming a physical barrier inside her genital tract, as in *N. fenestrata* (Fromhage & Schneider 2006) and *H. multipuncta* (Kuntner et al. 2009c). We estimated the importance of male palp amputation for his paternity with respect to the number of insertions by two subsequent suitors. To observe embolic breakage and possible reuse of the copulatory opening, we staged the mating of a virgin female ($N = 21$) and a virgin male ($N = 14$; Fig. 1c). Using a fine paintbrush, we gently placed the male in the female's web 10 cm away from her. During mating trials ($N = 54$), we observed precopulatory behaviour, which palp the male used (left/right/both), how many times the male inserted one or other palp and which copulatory opening he inserted into (left/right/both). In particular, we observed whether males became (half-) eunuchs (amputating one or both palps), and documented how this happened. We also observed whether the female responded aggressively towards the male and whether sexual cannibalism occurred. We considered a female aggressive if she shook her web or her body, or directly attacked a male using her legs and/or biting. If a female did not mate in two subsequent mating trials, we excluded her from further trials.

To test for genital reuse, we aimed to document two successful insertions in the same copulatory opening (female spiders have two copulatory openings) by two subsequent males. We confronted plugged females ($N = 11$) with virgin males or half-eunuchs ($N = 14$) with a functional palp that could only be inserted into the already plugged copulatory opening (insertions were always ipsilateral: left palp into the left copulatory opening, right palp into the right copulatory opening). If remating did not occur in two subsequent trials, we concluded that plugs were effective. After each trial, we gave the spiders at least a 24 h rest. Each trial lasted 90 min. We documented behaviour using hand notes and additionally used video and still photography to document selected sequences.

To test the better fighter hypothesis, we randomly introduced males onto a random female's web, followed by the introduction of the second male using the following combinations: intact + half-eunuch ($N = 28$), intact + full eunuch ($N = 26$), half-eunuch + full eunuch ($N = 14$). In control conditions, we observed contests between two intact males ($N = 37$). To ensure that eunuch males had comparable levels of aggressiveness, we randomly chose virgin males and randomly mated them with virgin females; thus to avoid confounding male aggression and mating success, we allowed eunuchs no previous experience of contests. Some of the males were used in more than one contest (mean = 2.95 times). However, this was accounted for in all analyses involving a generalized linear mixed model (GLMM) by introducing male identity as a random factor.

To see the effect of female presence on male behaviours we observed contests between a full eunuch and an intact male on a female's web in the presence and absence of the female ($N = 27$). During male–male contests, we noted the frequencies of guarding behaviour (walk towards female, touch female), and estimated the distance between both males and the female every 5 min. In addition, we recorded frequencies of male locomotory activity (walk back and forth). We scored male–male antagonism as frequencies of being stationary (score = 0), walking towards another male (score = 1), shaking the web (score = 2), chasing (score = 3) and attacking (score = 4). We then summed the scores to estimate male intensity of aggressiveness. Additionally, we noted frequencies of escapes and falls off the web. At the end of each trial, we noted which male was the initiator of antagonistic interactions, which male was the ultimate winner or loser, and whether any male copulated with the female during the experiment. All trials lasted 60 min. After each trial, we gave the spiders at least a 24 h rest. We filmed selected trials as above.

Morphological Examination

At the end of all trials, we euthanized the spiders and preserved them in 70% alcohol. We excised the epigyna of all mated females ($N = 11$) and further examined them for male genital parts under a Leica MZ16 stereomicroscope following Kuntner et al. (2009c).

Data Analyses

To test the effect of palp amputation on mate guarding, locomotory activity and aggressive behaviours of a male, we used GLMMs. The fixed factors were the number of palps (0 for full eunuch, 1 for half-eunuch, 2 for intact male), the number of the other male's palps (as above) and the presence of a female (present, absent). Because some males were reused, we introduced male identities as random factors. Response variables were behavioural scores. We sequentially deleted fixed terms in order of decreasing significance; only terms with $P \leq 0.1$ remained in the final model. To confirm that the excluded terms did not explain a significant part of the variation, we re-entered them one by one into the final model (Poesel et al. 2006). We present Wald statistics for final models including fixed terms with $P \leq 0.1$ only. Using the above statistical analyses, we tested the effect of palp presence on fight initiation and contest outcome. We tested the effect of contest outcome on copulation occurrence using ANOVA. We analysed the data in PASW version 18 (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

In *N. malabarensis*, a male placed on the female's web usually starts courtship by plucking the threads of the web, and attaching silk to it. If the female shows receptive behaviour, that is, she orients towards the male or comes out of the retreat the male eventually establishes physical contact and climbs onto her dorsal abdomen. The male then switches to her venter and attempts to insert one of the palps (see Supplementary Material, Video and Fig. S1). Figure 2 shows a detailed ethogram of mating behaviours in *N. malabarensis*.

Insertion of a palp always resulted in whole bulb loss, either immediate (direct) or subsequent (indirect). In 14 of 16 (87.5%) palp insertions, the bulb loss was immediate with the broken palp externally stuck in the copulatory opening (Fig. 1d), whereas in two

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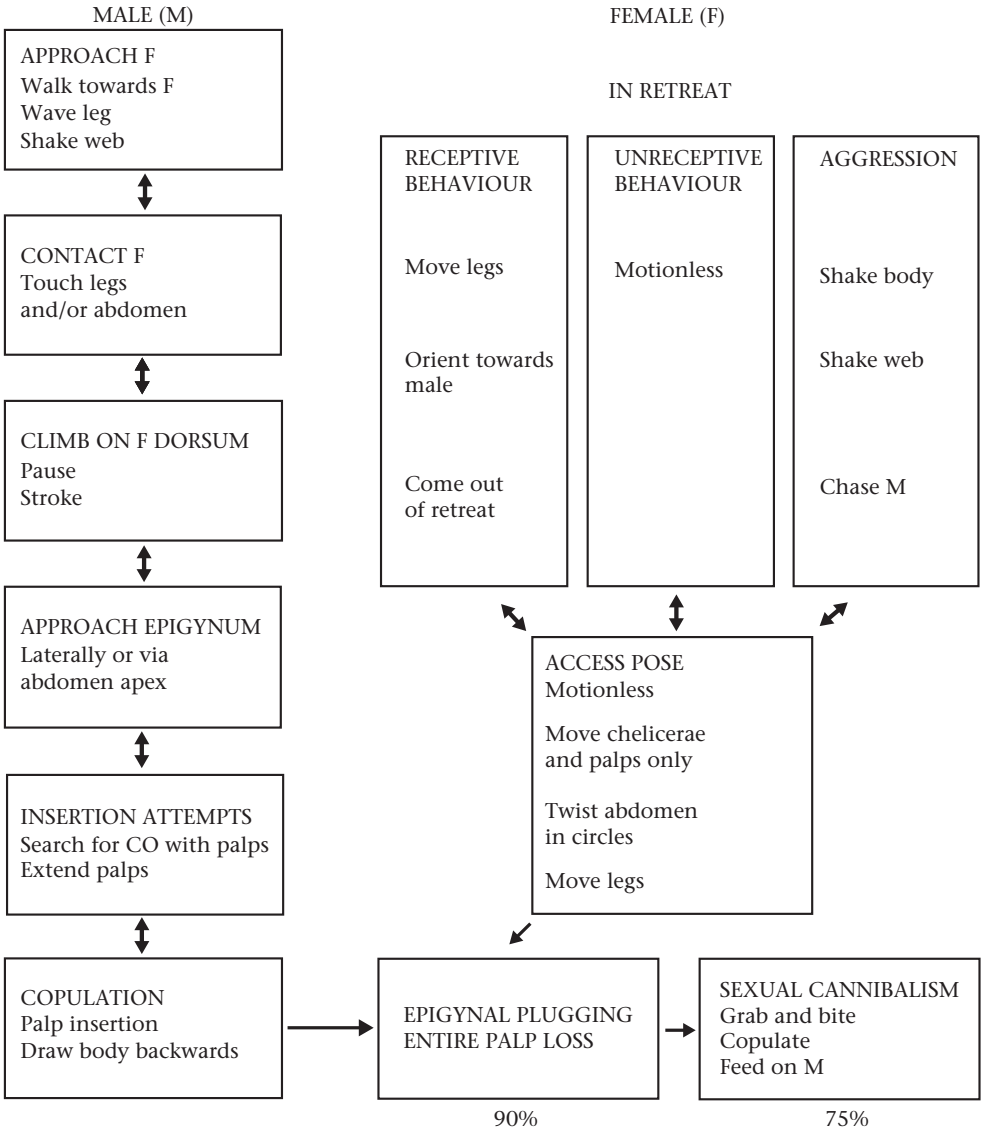

Figure 2. Ethogram of mating sequences in *Nephilengys malabarensis*. Time is on the *Y* axis; arrows show possible directions of behaviours, which may stop at any point. CO = copulatory opening.

cases (12.5%) the palp was first disfigured during copulation and subsequently self-amputated by the male (see Supplementary Material, Video and Fig. S2). The first case is termed here direct amputation (direct severance), and involves the male severing his palp, during or immediately following mating, at the tibia–tarsus joint (see Kuntner et al. 2009a). The second case, that of indirect amputation (severance), first involves damage to the distal sclerite of the palp (embolic conductor, see Kuntner 2007), followed by a period of status quo during which the male persists with his disfigured palp, and by a period of attempts to remove his disfigured organ, which eventually becomes severed. The ultimate morphological outcomes, 'indirect' and 'direct' eunuchs, cannot be anatomically distinguished. In 12 of 16 (75%) successful insertions the female attacked and devoured her mate (Fig. 1e; Supplementary Material, Video and Fig. S3).

Plugging Hypothesis

We confirmed that the broken embolic parts plugged the copulatory openings and reduced access to the used copulatory openings by rival males. Plugging (Fig. 1d), however, did not fully prevent copulation into the used copulatory openings; in three of 11 (27.27%) females with a plugged copulatory opening, another male reused the copulatory opening, producing a second plug, which we externally observed being stacked in the used copulatory openings.

On a closer inspection several hours after plug formation, external parts of plugs were gone ($N = 14$). During trials, we observed two females that had mated once remove their external plugs using their legs (see Supplementary Material, Video and Fig. S4). Our subsequent morphological examinations revealed broken emboli (terminal palpal parts) lodged inside spermathecae in all mated females (Fig. 1e), regardless of the pattern of male genital amputation, with a maximum of one embolus per spermatheca.

Better Fighter Hypothesis

The number of palps in both males significantly affected their behaviour during contests. Full eunuchs were significantly more

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active and aggressive towards rivals compared with half-eunuchs and intact males, whereas half-eunuchs behaved similarly to intact males (Fig. 3; see Supplementary Material, Video and Fig. S5). Full eunuchs moved around the web, walked towards the female and touched her significantly more than half-eunuchs or intact males (Fig. 3a). In the presence of full eunuchs, intact males stayed further from the female in comparison with trials where they were confronted with half-eunuchs or other intact males (Fig. 3b).

The number of male palps significantly affected the intensity and frequency of aggression (Table 1). Full eunuchs most frequently walked towards another male to chase and attack him (Fig. 3c, d; Supplementary Material, Video and Fig. S5). Full eunuchs were also mostly the initiators of antagonistic interactions; (half-) eunuchs, however, initiated aggressive interactions particularly when opposed by intact males. As predicted, full eunuchs won most contests (Table 1). Males escaped or even fell off the web most frequently when attacked by a full eunuch (Fig. 3e). Frequencies of antagonistic interactions varied with the presence of the female: full eunuchs shook the web and chased rivals more frequently and expressed more intense aggressiveness when the female was absent.

Table 1
Statistical results of the final model of the generalized linear mixed models

	Number of palps in ♂ 1	Number of palps in ♂ 2	Presence of a female
Touch female	**0.05**	**0.037**	>0.1
Explore web	**0.002**	>0.1	>0.1
Walk towards female	*0.06*	>0.1	>0.1
Distance to female	>0.1	**0.005**	>0.1
Shake	>0.1	**0.014**	**<0.001**
Walk towards rival	**<0.001**	>0.1	>0.1
Attack	**<0.001**	>0.1	>0.1
Chase	**<0.001**	**0.033**	>0.1
Intensity of aggressiveness	**<0.001**	>0.1	**0.003**
Escape	>0.1	**<0.001**	**<0.001**
Fall off web	**0.01**	**<0.001**	>0.1
Initiator of antagonistic interaction	**<0.001**	*0.07*	NA
Outcome of contest	**<0.001**	**<0.001**	NA
Copulation	**0.025**	*0.1*	NA

The table shows probabilities that fixed factors, i.e. number of palps in male 1, number of palps in the rival male (male 2) and presence of a female, affect the occurrence of behaviours in male 1. NA: not applicable. The last three results were analysed using χ^2. Bold values: $P < 0.05$; italic values: $P < 0.1$.

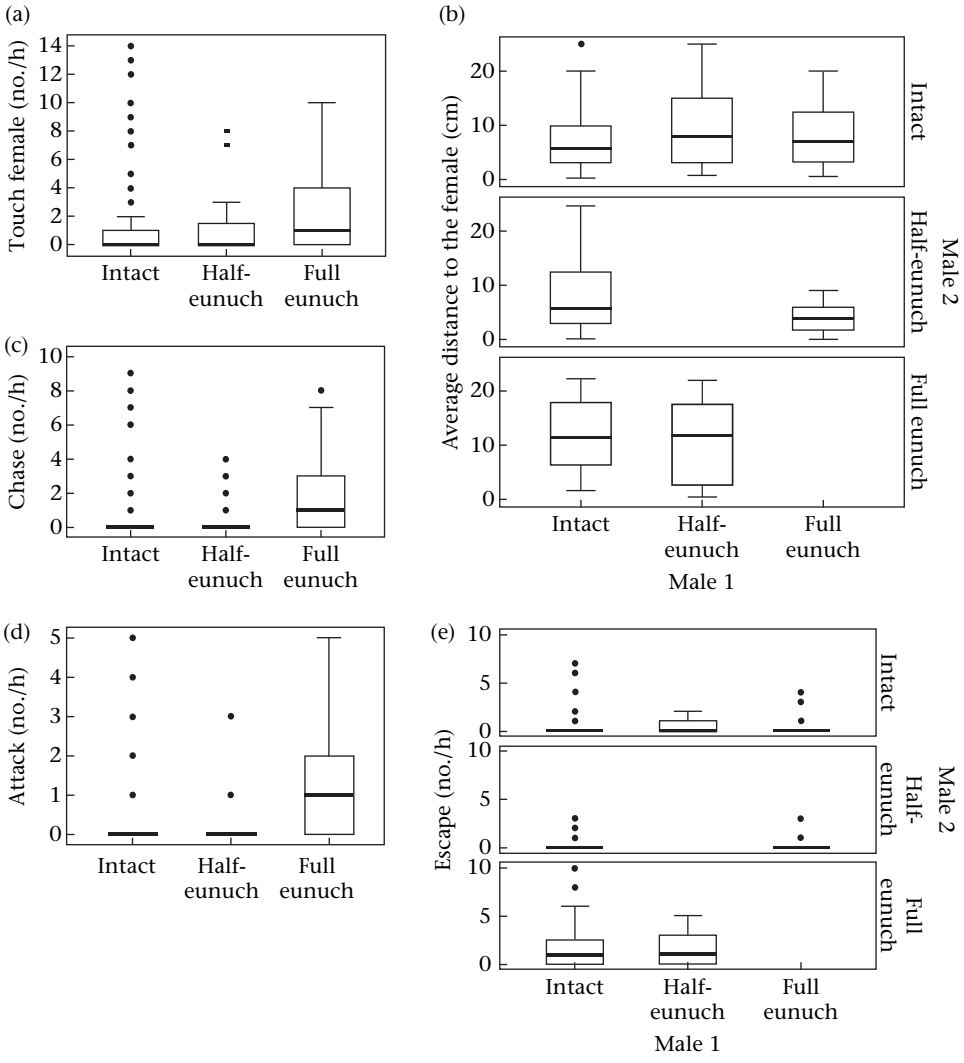

Figure 3. Behaviours of males in male–male contests. (a) Frequency of touching the female; (b) average distance to the female (cm); (c) frequency of chasing; (d) attack frequency; (e) escape frequency. Boxes show the median and upper and lower quartiles, vertical lines indicate values within 1.5 times the interquartile range and circles are outliers.

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Obviously, only intact males and half-eunuchs achieved copulation if not prevented by full eunuchs ($F = 3.903$, $N = 108$, $P = 0.049$). However, copulation with a female was independent of the outcome of the male–male contest ($F = 0.818$, $N = 72$, $P = 0.369$).

DISCUSSION

Male *N. malabarensis* exhibit obligatory damage to their genitals during copulation either through direct or indirect amputation, the latter involving initial sclerite damage followed by subsequent, voluntary palpal removal. Their obligate genital amputation (direct or indirect), also termed the eunuch phenomenon, may be logical from the perspective of very limited sperm in each palp; new research shows that their palps can be charged only once because spermiogenesis in this species, as in *Nephila*, is terminated when males reach adulthood (Michalik & Rittschof 2011). Yet, the effectiveness of broken male genitals functioning as mating plugs had previously been unknown. Our study confirmed the plugging function of broken male genitals within the female's copulatory tract with a 75% prevention of any subsequent copulation. Furthermore, mating and/or damage of male genitals during copulation seem to affect the male's behaviour in an adaptive way that is beneficial for his paternity. Full eunuchs were the most aggressive in the male–male contests on a female's web and thereby most actively guarded females against rival males. Intact males and half-eunuchs were less aggressive and active in the staged male–male contests. In fact, an intact male could achieve copulation during a contest only if not prevented by a full eunuch. Therefore, we found support for both the plugging hypothesis and the better fighter hypothesis.

In *N. malabarensis*, both direct and indirect palpal amputations are possible. Most palps (87.5%) were amputated directly during copulation as whole bulb plugs, similar to *Nephilengys borbonica* (Kuntner et al. 2009a). The remaining 12.5% of males retrieved their damaged palps from copula, but then subsequently chewed them off to become voluntary eunuchs. A similar behavioural pattern has been documented in *H. multipuncta*, in which males first damaged their palps during copulation by breaking the embolic conductor, which became a mating plug, then subsequently chewed off the damaged palp (Kuntner et al. 2009c). Full genital amputation is also known in the theridiid spiders *Echinotheridion* and *Tidarren* (Knoflach & van Harten 2000, 2001; Agnarsson 2006). However, these males self-amputate one of their palps after the penultimate moult, and thus mature already as half-eunuchs. The only other example of full genital amputation known to us is in the ant *D. quadriceps*, in which the female cuts off the male's abdomen during copulation, leaving his genitals inside her (Monnin & Peeters 1998).

Mating plugs may or may not effectively prevent females remating (see Introduction, reviewed in Uhl et al. 2009). *Nephilengys* females are able to remove the whole bulb plug; however, male embolic parts persist within the female's spermathecae (Fig. 1d; Kuntner et al. 2009a). Thus, there seems to be a second breaking point in the palp (Kuntner et al. 2009a). In contrast to *H. multipuncta* where plugging is nearly always effective (Kuntner et al. 2009c), reuse of the same copulatory opening in *N. malabarensis* does occur (25% of cases), which may indicate that the embolic plug is ineffective. However, we recovered only a single embolic plug per spermatheca even in those females that used the same copulatory opening with more than one male. Thus, the second use of female copulatory openings may not necessarily be successful, and if so, embolic plugs in *N. malabarensis* are effective. Nevertheless, our conclusions should be taken cautiously, and the effectiveness of the plug in remated female *N. malabarensis* should be further tested through more direct measures of paternity shares, for example sperm counts or DNA fingerprinting.

Sexual cannibalism is common in highly sexually dimorphic spiders in which male genitalia become damaged or depleted of sperm during or after copulation (Knoflach & van Harten 2000; Kuntner et al. 2009a, c; Nessler et al. 2009). Sexual cannibalism may increase a male's fitness if it prolongs copulation (Andrade 1996; Elgar et al. 2000; Schneider et al. 2000) or increases the female's fecundity and/or reduces her receptivity (Elgar & Nash 1988; Andrade 1996). However, some males attempt to avoid being cannibalized, which leads to certain adaptive male strategies (Schneider & Lubin 1998). For example, surviving nephilid males actively mate-guard after copulation (Kuntner et al. 2009a, c). Cryptic female choice theory (Eberhard 1996) predicts that a female can contribute to male monopolization if he is of superior quality. This may explain why we observed female cannibalism only of copulating males, but never of mate-guarding eunuchs.

In *Nephila* and *Herennia*, mated eunuchs usually win contests against even physically superior rivals. This may be because of increased agility, since their body does not have to carry the relatively large palps, and/or the enhanced investment in attacking rivals when the male has no other reproductive future (Christenson & Goist 1979; Ramos et al. 2004; Fromhage & Schneider 2005a). Our study cannot truly distinguish whether *N. malabarensis* full eunuchs are better fighters because they lack palps or because they have no future mating chances. Either way, increased activity and aggressiveness after copulation seems adaptive as they enhance the eunuch's chances of paternity.

Although virgin *N. malabarensis* males guard subadult females and thus can probably assess female reproductive value prior to copulation (as is the case in other spiders, e.g. *Frontinella pyramitela*: Austad 1982, 1984; Watson 1990; Leimar et al. 1991), eunuchs transferred onto webs of unfamiliar females behaved similarly to when they remained with their mates. The latter suggests a change in physiological mechanisms, which may enhance the eunuch's aggressiveness.

Finally, we suggest another adaptive function of palpal amputation, that is, 'remote copulation'. Owing to short insertion times, and high levels of sexual cannibalism, which males attempt to evade, breakage of the entire bulb in the female's copulatory opening may secure continuous sperm transfer even after the male has been (forcefully) removed from copula or has fallen victim to cannibalism. A similar mechanism was previously suggested for *Tidarren argo* (Knoflach & van Harten 2001). This hypothesis is currently under investigation.

In conclusion, being a eunuch is adaptive for spiders. Genital amputation enhances the eunuch's mate-guarding activity and male–male aggressiveness, which help the male to win contests against rivals. Aggressiveness and functional epigynal plugs together probably increase the eunuch's paternity, and 'remote copulation' may allow continuous sperm transfer after the male has escaped or has been forcefully removed from copula and/or cannibalized.

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Supplementary Material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.anbehav.2011.02.010.

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