Remote copulation: male adaptation to female cannibalism

Daiqin Li1,2,*, Joelyn Oh1, Simona Krailj-Fišer3 and Matjaž Kuntner2,3,4

1Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Republic of Singapore
2College of Life Sciences, Hubei University, Wuhan 430062, Hubei, China
3Institute of Biology, Scientific Research Centre, Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia
4National Museum of Natural History, Smithsonian Institution, Washington, DC, USA
*Author for correspondence (dhighq@nus.edu.sg).

Sexual cannibalism by females and associated male behaviours may be driven by sexual conflict. One such male behaviour is the eunuch phenomenon in spiders, caused by total genital emasculation, which is a seemingly maladaptive behaviour. Here, we provide the first empirical testing of an adaptive hypothesis to explain this behaviour, the remote copulation, in a highly sexually cannibalistic orb-web spider Nephilengys malabarensis. We demonstrate that sperm transfer continues from the severed male organ into female genitals after the male has been detached from copula. Remote copulation increases the total amount of sperm transferred, and thus probably enhances paternity. We conclude that the mechanism may have evolved in response to sexual cannibalism and female-controlled short copulation duration.

Keywords: sexual conflict; sexual cannibalism; coevolution; genital emasculation

1. INTRODUCTION

Sexual conflict, divergence between the reproductive interests of males and females, can lead to antagonistic coevolution in which adaptations in each sex select for counter-adaptations in other [1]. Sexual cannibalism by females and the associated male behaviours are examples of antagonistic coevolution driven by sexual conflict [1–5]. In many highly sexually cannibalistic spiders, males adopt a monogamous mating strategy, typically mating with one or two females (spiders have paired genitals) [4]. However, in spite of mono- or bigyny, polyandry may continue to serve interests of females [5]. To counter female polyandry, however, males have evolved mate guarding, male sacrifice to a cannibalistic female, genital mutilation resulting in mating plugs, and mechanisms that increase the quantity of sperm transferred [3,4,6,7].

In spiders, male genital mutilation, the breakage of a male’s palp within the female genitals, is common in mono/bignous and highly sexually cannibalistic species, and is typically characterized by breakage of the tip of the male palp [6]. However, some spider species further exhibit the ‘eunuch phenomenon’, whereby males break off the entire palp [8–12] (figure 1). While this seems maladaptive because eunuchs are functionally sterile, several adaptive hypotheses have been suggested [10–12]. The broken male parts may effectively plug the female genitals (epigynum) leading to paternity protection—the mating-plug hypothesis [9,11,12]. Yet, entire emasculation is not necessary for effective plugg- ing, and so additional functions of emasculation have been suggested, including the better-fighter hypothesis; indeed, eunuchs are more aggressive and agile compared with intact males [12]. Finally, the remote copulation hypothesis postulates that eunuchs secure continuous sperm transfer through the severed palp attached to the epigynum [12,13], but to date this has remained untested. Here, we test for continual sperm transfer after genital severance in an extremely sexually dimorphic and cannibalistic nephilid orb-web spider, Nephilengys malabarensis (Araneae: Nephilidae).

2. MATERIAL AND METHODS

(a) Study subjects

Juvenile and subadult N. malabarensis were collected in the field, housed in controlled environmental conditions [11,12], and monitored until adulthood, so that their mating history and precise age (days elapsed from the final moult) were known. Only virgin spiders were used in trials.

(b) Mating trials

We staged each mating trial by introducing a virgin male onto a virgin female web (n = 25). All trials ended with copulation and genital damage, either through entire palp severance or only partial palp damage. We recorded the copulation duration as the time elapsed from palp insertion until breakage. To estimate the pattern of sperm transfer after copulation, the broken palp was allowed to remain in the epigynum (figure 1) for varying times (0–1255 s) before we experimentally removed it.

(c) Sperm quantification

Immediately after palp removal from the epigynum, we placed it in a 2 ml tube to prevent the loss of remaining sperm. The female’s genitals were dissected, and all reproductive parts placed in a separate tube.

The number of sperm in each of the reproductive parts was quantified separately using a modified sperm-counting procedure [14–17] (see the electronic supplementary material for details). Samples containing either the used palp or isolated female reproductive organs were extracted and placed on a haemocytometer. Sperm were counted under a compound microscope for each sample. Calculations were then performed to obtain the average number of sperm present in the used palp or female reproductive organ. Our sperm quantification was deemed reliable, judging by rare sperm clumping in the samples and high correlations between the sperm counts [14–17].

(d) Female- and male-initiated palp breakages

The inserted palps could be broken by either sex. In female-initiated palp breakages (n = 14), the female terminated copulation by cannibalization (figure 1) or by pulling the male away from her epigynum. In male-initiated palp breakages, the male deliberately severed his palp (n = 8). In all cases, copulation termination results in palp breakage and its lodging in the epigynum. Copulation durations of female- and male-initiated palp breakages were recorded.

3. RESULTS

All copulations (n = 25) led to palp damage either through entire palp severance (88%, n = 22) or partial severance leading only to palp damage (12%, n = 3). There was no significant difference in the occurrence of female versus male-initiated palp breakage frequencies (female-initiated: 63.6%; χ² = 1.636, p = 0.201, n = 22). However, female-initiated severance resulted in significantly shorter copulation durations than did male-initiated palp breakages (t20 = −2.746, n = 22, p = 0.012; figure 2a).
There was a mean number of 3309 (± 271) sperm (range: 1300–7400; n = 22) present originally in each copulating palp. Female-initiated palp breakages resulted in significantly fewer sperm transferred into spermatheca during copulation ($t_{20} = -3.031$, $n = 22$, $p = 0.007$; figure 2b). About 67–72% of sperm were still left in the broken palp lodged in the epigynum when the male and female separated. However, GLM results showed that sperm transfer continued after copulation termination: the longer the broken palp was left lodged in the female before its experimental removal, the more sperm were transferred (table 1 and figure 2c). Female-initiated palp breakage significantly increased the number of sperm transferred to female since the palp breakage (table 1), suggesting that female-initiated palp breakage induced faster sperm transfer in a given time. Moreover, a significant two-way interaction between the sex-initiating palp breakage and the time before experimental palp removal showed that, after female-initiated breakage, a significantly shorter time was needed to transfer equal sperm quantity from the emasculated palp to the female (before experimental palp removal) compared with male-initiated palp breakage (table 1). Other explorative variables (copulation duration, number of sperm remaining in the palp, male size) did not have significant effects on the number of sperm transferred (table 1).

Table 1. Results from a generalized linear model testing the effects of five explorative factors (the time interval since palp breakage until its experimental removal from the epigynum, the sex initiated palp breakage, copulation duration, male size (carapace width: CW), and the number of sperm remaining in the palp) on the number of sperm transferred to the female's spermatheca. The data for the dependent variable and all the continuous predictors were log-transformed. The maximal model that including all five factors and all possible interactions was fitted using normal with identity link and gamma with log link error. Akaike information criterion (AIC) was used to select the best model. Goodness of fit: AIC = 4.154; Omnibus test: $\chi^2 = 117.4$, d.f. = 8, $p < 0.0001$.

<table>
<thead>
<tr>
<th>explorative factors</th>
<th>Wald $\chi^2$</th>
<th>d.f.</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
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<td>time interval from palp breakage</td>
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<td>0.001</td>
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<tr>
<td>sex initiated palp breakage</td>
<td>11.406</td>
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<td>0.003</td>
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<td>copulation duration</td>
<td>0.188</td>
<td>1</td>
<td>0.665</td>
</tr>
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<td>number of sperm remaining in the emasculated palp</td>
<td>1.170</td>
<td>1</td>
<td>0.279</td>
</tr>
<tr>
<td>CW</td>
<td>1.805</td>
<td>1</td>
<td>0.179</td>
</tr>
<tr>
<td>sex initiated palp breakage × time from palp breakage</td>
<td>4.584</td>
<td>1</td>
<td>0.032</td>
</tr>
<tr>
<td>sex initiated palp breakage × copulation duration</td>
<td>2.669</td>
<td>1</td>
<td>0.102</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Our results clearly show that remote copulation enables continual sperm transfer from severed palp to female genitalia even after male detachment from copula. It not only directly prolongs the duration of potential sperm transfer, but also in fact increases the amount of transferred sperm. This may reduce sperm competition by increasing the eunuch’s fertilization success and paternity.

Remote copulation probably evolved as a male counter-adaptation to female aggression towards mates, female-enforced short copulation durations and, ultimately, the high prevalence of female sexual cannibalism (reaching 75%, [12]). Partial palp breakage, common in spiders (here only in 12% of copulations), functions as mate plugging [6]. However, internal plugs do not necessarily prevent female remating [10–12,18], and cannot
lead to continuous sperm transfer. Remote copulation is hence a function exclusive to complete palp emasculation, a taxonomically scattered phenomenon known in nephilids [8] and the theridiids [13,19]. A totally severed palp facilitates both plugging and continuous sperm transfer after the eunuch's detachment from copula.

Females may control copulation duration by sexual cannibalism: amounts of transferred sperm are thus seen as the ultimate manifestation of sexual conflict [20–21]. The frequency of palp breakage initiations did not differ between males and females; however, female-initiated cases resulted in shorter copulation durations and lower numbers of transferred sperm. This implies that copulation duration in N. malabaren sis, while already short (average 8.3 ± 1.1 s) compared with other spiders [22–25], is subject to female choice [20]. With only about 28–33% sperm transferred before palp severance, the male’s reproductive capacity is greatly reduced. However, sexual conflict commonly leads to sexually antagonistic coevolution, where male persistence strategies evolve to counter female resistance to monopolization [10,12]. Mate plugging to monopolize females is a common male response to sexual conflict [26], but our study suggests that remote copulation is an additional male adaptation to sexual cannibalism and to female control of copulation duration. Remote copulation enables the male to maximize his reproductive potential through (i) continuous sperm transfer after detachment from copula and (ii) prolonged female monopolization, during which the female is unavailable to rival males.

Another important finding is that the males transferred sperm even faster via broken palps after female-terminated copulations (table 1). This suggests that eunuch males may not, ultimately, suffer a paternity cost. However, it is unclear why the rates of sperm transfer differ between male- and female-terminated copulations. Presumably, there is an advantage of rapid ‘remote’ transfer after female-initiated breakages, but less so after male-initiated ones. However, there might have been a difference in the rate of sperm transfer if the duration of remote transfer had not been manipulated. Further studies are needed to test these hypotheses.

In sum, the adaptive significance of eunuchs seems to be justified judging from their evolutionary persistence [10]. Perhaps, there are other functions of palp severance in addition to plugging [9], remote copulation (this paper) and enhanced eunuch aggressiveness [12,26], which may further justify the high costs of male sterility.

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