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Securing Paternity by Mutilating Female Genitalia in Spiders

Highlights

- Spider males remove a coupling structure on the female external genitalia
- Mutilation of female genitalia prevents harmed females from remating
- External female genital mutilation is an adaptation for securing paternity
- Mutilation of external female genitalia is a widespread but overlooked phenomenon

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In Brief

There are manifold ways by which males secure paternity. Here, Mougnot et al. present an obvious but as yet unexplored means of securing paternity: males remove the structure of the female genitalia that is necessary for genital coupling. This study provides the first evidence of a direct adaptive function for the mutilation of female genitalia.



Securing Paternity by Mutilating Female Genitalia in Spiders

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SUMMARY

Competition between males and their sperm over access to females and their eggs [1–3] has resulted in manifold ways by which males try to secure paternity, ranging from physically guarding the female after mating to reducing her receptivity or her attractiveness to subsequent males by transferring manipulative substances [4, 5] or by mechanically sealing the female reproductive tract with a copulatory plug [1, 3, 6]. Copulations may also result in internal damage of the female genitalia [7–9]; however, this is not considered as a direct adaptation against sperm competition but as a collateral effect [9–14]. Here, we present a drastic and direct mechanism for securing paternity: the removal of coupling structures on female genitalia by males. In the orb-weaving spider *Larinia jeskovi* [15] males remove the scapus, a crucial coupling device on the female external genital region. Reconstruction of the coupling mechanism using micro-CT-scanned mating pairs revealed that several sclerites of the male genitalia interact to break off the scapus. Once it is removed, remating cannot occur due to mechanical coupling difficulties. In the field, male-inflicted genital damage is very prevalent since all female *L. jeskovi* were found to be mutilated at the end of the mating season. External genital mutilation is an overlooked but widely spread phenomenon since 80 additional spider species were found for which male genital manipulation can be suspected. Interlocking genitalia provide an evolutionary platform for the rapid evolution of this highly effective mechanism to secure paternity, and we suspect that other animal groups with interlocking genital structures might reveal similarly drastic male adaptations.

RESULTS AND DISCUSSION

Mutilation of Female Genitalia in the Field and Laboratory

We used the orb-weaving spider *Larinia jeskovi* Marusik, 1986 to investigate whether the damage to the female (Figure 1) is caused

by males during mating. In the field, at the beginning of the mating season, 57% (12 of 21) of females were found to be mature, and 83.3% (10 of 12) of these were already missing the scapus. At the end of the mating season, all adult females (26 females) were missing the scapus. In the laboratory, we staged 40 mating trials with virgin females, of which 72.5% mated (29 of 40). Pedipalp insertions were very short, lasting for 2.48 s on average (SD = 0.81, n = 29). A randomly chosen subset of mating pairs (n = 14) were allowed to mate undisturbed by the experimenter, resulting in a median of four successive insertions of the two pedipalps that serve as secondary sperm transferring organs in spiders (interquartile range [IQR] = 0, range = 1–5). Postmating, 93% (13 of 14) of females were found without the scapus (Figure 2). Another randomly chosen subset of 15 mating pairs was only allowed a single insertion. Single insertions resulted in significantly fewer females with a broken-off scapus (1 of 15) compared to undisturbed matings ($\chi^2 = 18.23$, degrees of freedom [df] = 1, $p < 0.001$; Figure 2). These data show that external female genital mutilation in *L. jeskovi* results from mating and occurs at a high frequency and that the probability of mutilation depends on the number of insertions of the male's copulatory organs.

Mechanism of Mutilation

We cryo-fixed mating pairs of *L. jeskovi* and reconstructed the copulatory mechanism by using X-ray micro-computed tomography (micro-CT) (Figure 3A). The structures involved in coupling were segmented to visualize the mutilation mechanism: the female epigynum, with its lateral lobes and central scapus, is interlocking with various sclerites of the paired male copulatory organs, the pedipalps (Figures 3B, 3C, and 3E). While the sperm-transferring structure of the active pedipalp, the embolus, is inserted into the copulatory duct (Figure 3B), the terminal apophysis is secured under the lateral lobes and the median apophysis is inserted medially deep into the funnel-like groove of the scapus. In addition, two further sclerites grasp the scapus from the side: the so-called conductor, which is secured in its outside position by a basal-tooth-like protrusion of the median apophysis, and the tegular apophysis, which is positioned underneath the scapus opposite of the conductor (Figures 3B, 3C, and 3E). The tegular apophysis cuts the scapus like a blade (Figure 3D). Scapus removal seems to require more than one insertion, since with a single insertion the male cuts only half of the scapus (Figure 3E). Consequently, the copulatory mechanism can explain why more than one insertion is generally necessary for removal of the scapus (Figure 2). An interactive 3D PDF can be found in Data S1.

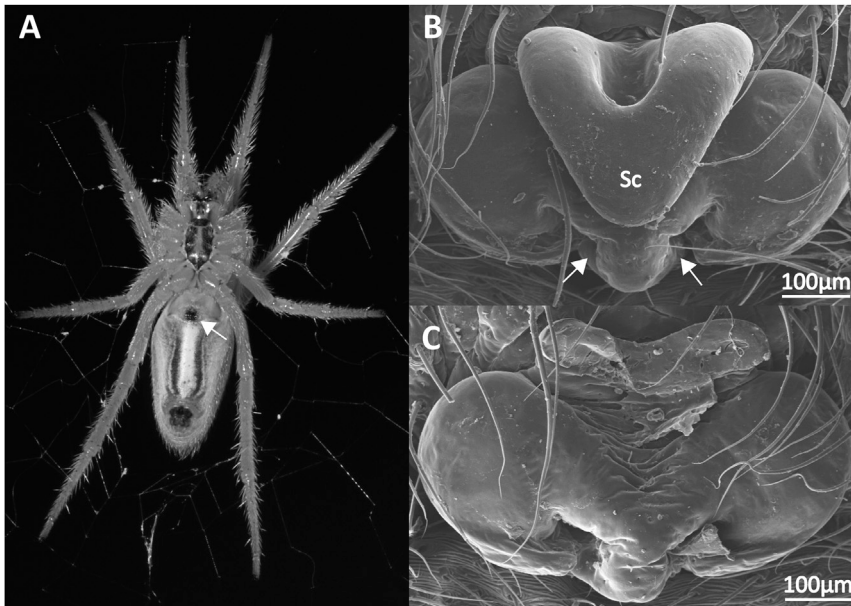


Figure 1. External Female Genital Mutilation

(A) Macro-photograph of female *Larinia jeskovi* in her web in ventral view. Arrow points to external genitalia.

(B and C) Scanning electron microscopic photographs of the external genitalia in ventral view with (B) an intact scapus (Sc) or (C) the scapus missing. Arrows point to copulatory openings. Scale bars, 100 μ m.

See also [Table S1](#) for 80 additional spider species with suspected female genital damage.

Remating Probability of Mutilated Females

For investigation of the effect of mutilation of the female genitalia on female remating probability, females that lost the scapus in the undisturbed mating trials ($n = 13$) and females that remained intact after the single-insertion mating trials ($n = 14$) received a second male. The proportion of pairs that performed mating attempts in second matings (22 of 27) did not differ from that of first matings (29 of 40; $\chi^2 = 0.31$, $df = 1$, $p = 0.58$). Mating attempts entail courtship by the male in the female orb web, the female approaching the male and entering a specific mating posture, and insertion attempts by the male. Remating trials with previously mutilated and non-mutilated females did not differ in the proportion of pairs that performed mating attempts (12 of 13 with a mutilated female and 10 of 14 with an intact female; Fisher's exact test, $p = 0.33$), suggesting that neither female receptivity nor female attractiveness to males was reduced by mutilation. However, none of the females with a broken-off scapus remated (0 of 12), in contrast to all females with an intact scapus (10 of 10; Fisher's exact test, $p < 0.001$; [Figure 4](#)). To control for the effect of mating experience on female receptivity, male courtship probability, and mating success, we experimentally mutilated another 14 virgin females. Mating attempts occurred in 78.6% (11 of 14) of the mating trials in the experimentally mutilated group, which is not significantly different from the overall 72.5% (29 of 40) of the two other groups with intact virgin females (Fisher's exact test, $p = 0.74$). There was only one successful mating with the experimentally mutilated females (1 of 11). Mating success of the experimentally mutilated females did not significantly differ from rematings of females mutilated during a previous mating (0 of 12; Fisher's exact test, $p = 1$; [Figure 3](#)). Our data demonstrate that willingness to mate does not decline for females postmating and that mutilated females are equally willing to remate as are intact females. Likewise, males court both virgin and mated females and do not distinguish in courtship activity between mutilated and intact females. Consequently, mating probability seems to be determined only by the presence of the scapus as the crucial interlocking structure.

The copulatory mechanisms in orb-weaving spiders generally entail a complex grasping mechanism of the scapus by several palpal sclerites that are hooked into its grooves and are pressed against it [16, 17]. For any given species with such a tight interlocking mechanism, slight changes in male behavior, i.e., twisting behavior or slight mechanical changes through larger pressure

implied on the scapus, can result in scapus damage and removal. The resultant high fertilization success for the manipulating male would lead to selection for twisting, and the mechanism would evolve to fixation.

Costs of Mutilation

The costs for the monopolizing male entailed in this strategy seem negligible compared to guarding the female, producing accessory seminal substances that alter female receptivity or attractiveness [18], or using body parts and secretory substances as mating plugs [6, 19, 20], all of which may severely reduce the male's future mating success [21, 22]. The transfer of mating plugs, be they broken male genitalia or secretory mating plugs, can easily lead to an arms race between males resulting in, for example, the evolution of means to remove the material when encountering an already mated female [23, 24]. However, the potential for the evolution of countermeasures in rival males is highly limited in the case of external mutilation of female genitalia. A mating mechanism that does not require the missing coupling structure, for example through traumatic insemination [25, 26], would be the only conceivable alternative.

Females may incur several types of costs from genital mutilation [2, 3]. As was shown for species that exhibit internal damage, it seems likely that external genital damage can reduce female survival and longevity or lifetime reproductive success by causing infections [27]. If *L. jeskovi* females benefit from polyandry, sexual conflict over mating rates should be strong [10, 28]. Under this scenario, females should exhibit marked mate choice and/or allow only one insertion to reduce the probability of becoming mutilated. In the laboratory, virgin female *L. jeskovi* were highly receptive and allowed an average of four insertions that lead to near 100% mutilation probability. In the field, however, female mating behavior may strongly depend on perceived availability of males that could alter female mating behavior [29]. Finally, costs for the female could be balanced by

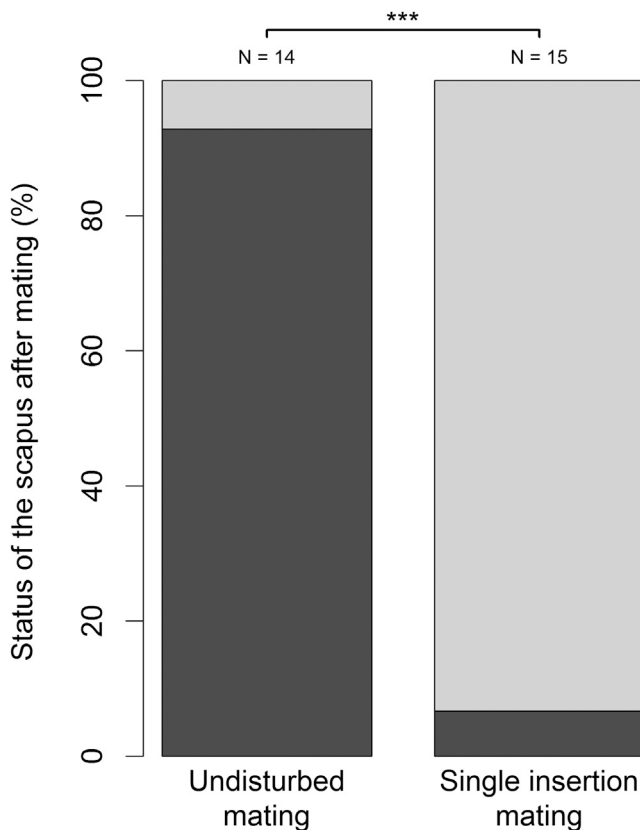


Figure 2. Mutilation Results from Copulation

Percentages of females with intact scapus (light gray) or broken off scapus (dark gray) after a mating trial that was undisturbed by the experimenter (median of four insertions) or a mating trial that was terminated by the experimenter after a single insertion.

the indirect benefit of producing sons that inherit the highly successful manipulative traits of their fathers [30].

Conclusions

Our study demonstrates that mutilation of external female genitalia is a highly effective means of securing paternity in *L. jeskovi* and represents the first evidence for the existence of male alteration of the female genital structure as a direct counter-adaptation to sperm competition. Species in which male and female structures interlock during copulation are pre-adapted for mutilation since slight changes in male behavior can result in substantial functional consequences that impact on the mating system. In fact, external female genital mutilation very likely occurs in many more spider species from diverse families, with and without scapus structures (see Table S1) and is likely to occur in other taxa with interlocking structures, for example in Odonata [31]. The costs and benefits for females and the consequential degree of sexual conflict remain to be explored.

EXPERIMENTAL PROCEDURES

Field Observations

Females of *Larinia jeskovi* were collected in August 2013 from a swamp close to Gugny in the Biebrza National Park, Poland (53°21'1.36" N, 22°34'37.45" E). Twenty-one individuals were collected at the beginning (August 15, 2013) and

26 at the end (August 27, 2013) of the mating season, and the status of the scapus was determined under a stereo microscope (Zeiss Discovery V20 Stereo Microscope, Carl Zeiss Microimaging).

Genital Morphology

In entelegyne spiders, such as *L. jeskovi*, females possess two insemination ducts, each leading sperm to a storage site from which sperm is ultimately released via a separate fertilization duct to meet the eggs during oviposition. Eggs are then laid through the oviduct opening. The insemination ducts are situated within the so-called epigynal plate, whereas the oviduct opening marks the posterior end of the epigynum [32]. In most entelegyne spiders, males inseminate the female by successively inserting parts of their paired sperm transfer organs, the pedipalps, into the copulatory ducts. In many entelegynes, and particularly within araneid spiders, the pedipalp consists of several sclerites and membranes. Prior to insertion, the pedipalp expands and twists, thereby moving the sclerites into specific positions. The sclerites play an essential role in coupling to structures of the epigynum [16, 17, 32–34].

Scanning Electron Microscopic Micrographs

Females with and without scapus were dehydrated in a graded ethanol series and were critical-point dried with a BAL-TEC CPD 030. The specimens were sputter coated with gold using a Polaron SC 7640 sputter coater and were investigated with a Zeiss DSM 940A scanning electron microscope.

Mutilation Mechanism: Cryofixation of Mating Pairs and Micro-CT

To explore the interlocking mechanism of male and female genitalia, we fixed several couples in copula by cryofixation. We staged a mating trial with a virgin female as described in the Mating Experiments section and fixed the couples during genital coupling by pouring liquid nitrogen (−196°C) over them. The couples were transferred to cold 80% ethanol at −40°C for several weeks to insure stable fixation [17]. The fixed couples were warmed up slowly and dehydrated in a graded ethanol series (80%, 90%, 96%, and three times in 99% ethanol for 24 hr each). The samples were then transferred to 1% iodine solution (iodine, Carl Roth) in 99.8% ethanol for two nights to enhance tissue contrast.

For micro-CT, the samples were either scanned in 90% ethanol or critical-point dried (Leica EM CPD300) and mounted on an aluminum rod with super glue. The scans were performed with an XRadia Micro XCT-200 (Carl Zeiss X-ray Microscopy). For the reconstruction depicted in Figure 3 we used a 4× and 10× object lens unit, at 40 kV and 8 W, with a pixel size of 4.65 μm and 2.25 μm, respectively. Tomography projections were reconstructed using the software provided by XRadia. For image segmentation, the software platform Amira 5.6.0 (FEI, Visualization Science Group) was used.

Mating Experiments

In the laboratory, double mating experiments were staged to assess the mutilation probability with virgin females and the remating success of mutilated females. To this aim, males and subadult females of *Larinia jeskovi* were collected in August 2014 from Biebrza National Park, Poland. Individuals were kept in individual 250 ml plastic cups, watered on a daily basis, and fed 2–3 days per week with one *Musca domestica*. The mating status of males was unknown as they were collected as adults. After their last molt, virgin females were transferred individually to plastic hexagonal boxes (18 × 18 × 6 cm) in which they built a web. We staged mating trials with 54 females. Females were randomly assigned to one of three treatment groups: (1) The female was mated with a first male in a mating trial undisturbed by the experimenter (n = 20). (2) The female was mated with a first male in a mating trial terminated by the experimenter after a single insertion (n = 20). A second male was introduced to the web of the females after 2 days to explore differential remating behavior. (3) Females were experimentally mutilated by immobilization under a net and removal of the scapus with forceps (n = 14); the mating trial was staged 2 days after the ablation of the scapus.

Mating experiments lasted for 1 hr and were started by placement of a male in an upper corner of the web. If contact between male and female began near the end of the observation period, observations were prolonged for an

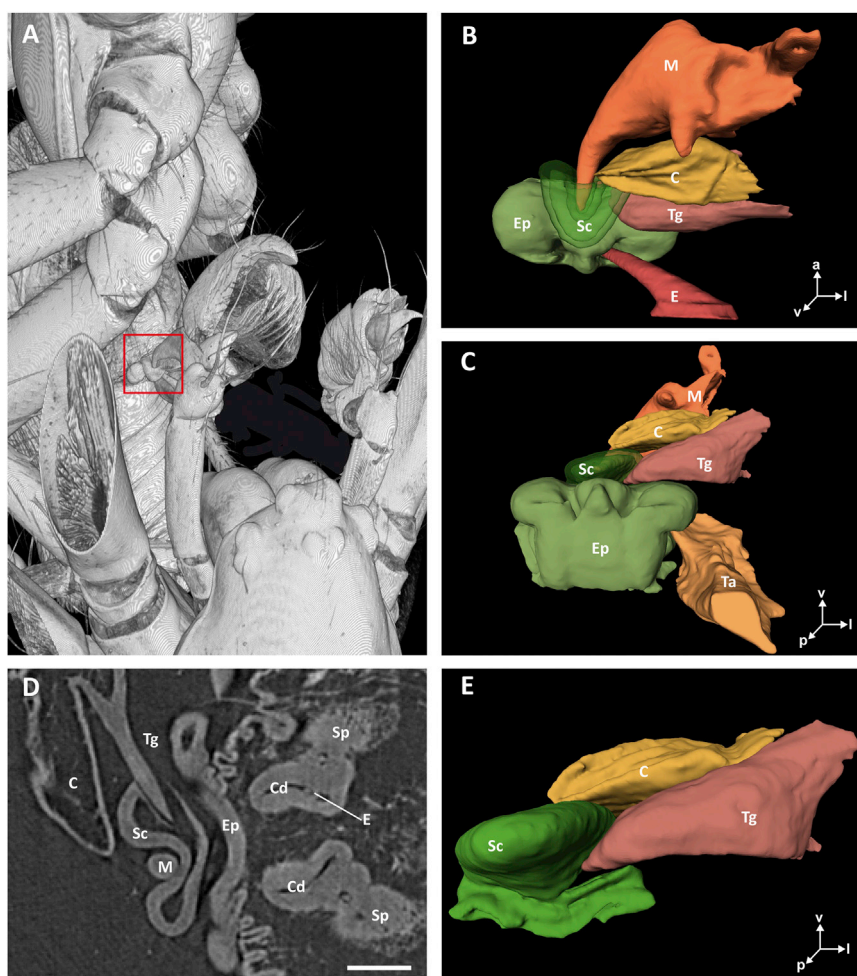


Figure 3. Mechanism of Mutilation of Female Genitalia in *Larinia jeskovi*, Obtained by High-Resolution X-Ray Tomography.

(A) Volume rendering of a *L. jeskovi* pair in copula (left, female; right, male). The male copulates with the left pedipalp.

(B) 3D reconstruction of the male and female genital structures engaged in mutilation. The median apophysis (M) of the male inserts into the groove of the females scapus (Sc). The conductor (C) and the tegular apophysis (Tg) are securing the scapus (Sc) similar to a precision grip; the tegular apophysis (Tg) works as a chock and cuts the scapus halfway. The actual sperm-transferring structure, the embolus (E), is inserted into one of the genital openings on the epigyne (Ep).

(C) Posterior view, showing also the terminal apophysis (Ta) interlocking with the lateral protrusions of the epigyne (Ep).

(D) Virtual horizontal section (obtained by X-ray tomography) through the female scapus region of the cryo-fixed pair. The tegular apophysis slashes the base of the scapus. Cd, copulatory duct; Sp, spermatheca. Scale bar, 100 μ m.

(E) 3D reconstruction demonstrating that the base of the scapus is mutilated by the tegular apophysis.

See also [Data S1](#) for an interactive 3D PDF.

additional 10 min. The number of insertion attempts was recorded for all groups as the number of times the male copulatory organ reached the female genitalia without coupling to the female's genital opening. Except for the group in which only one insertion was allowed, the number of successive insertions with alternating pedipalps was recorded. After copulation, the status of the scapus was determined under the stereo microscope.

Data Analysis

All the tests were performed in R [35]. The number of insertions was non-normally distributed and is given as median, IQR, and range. Differences in proportions were tested using chi-square or Fisher's exact test. The copulation success of mutilated females from undisturbed mating trials, intact females from single-insertion matings, and experimentally mutilated females were compared using pairwise Fisher's exact test with Bonferroni correction with the package *fmsb* [36].

Literature Survey

In order to assess the prevalence of external female genital mutilation in spiders, we scrutinized the spider taxonomic literature for notes, descriptions, and drawings of potential cases of genital mutilation. This amounted to a conservative estimate of 80 additional species with external mutilation of female genitalia.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and an interactive 3D PDF and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.09.074>.

AUTHOR CONTRIBUTIONS

G.U. designed the study and compiled the information on potential spider species with genital mutilation given in [Table S1](#). P.M., J.P., and U.T. conducted the field study. P.M. conducted the laboratory study and analyzed the data. P.M., G.U., and P.S. performed the cryo-fixations and micro-CT reconstruction. P.M. and G.U. wrote the manuscript. J.K. provided essential logistic support.

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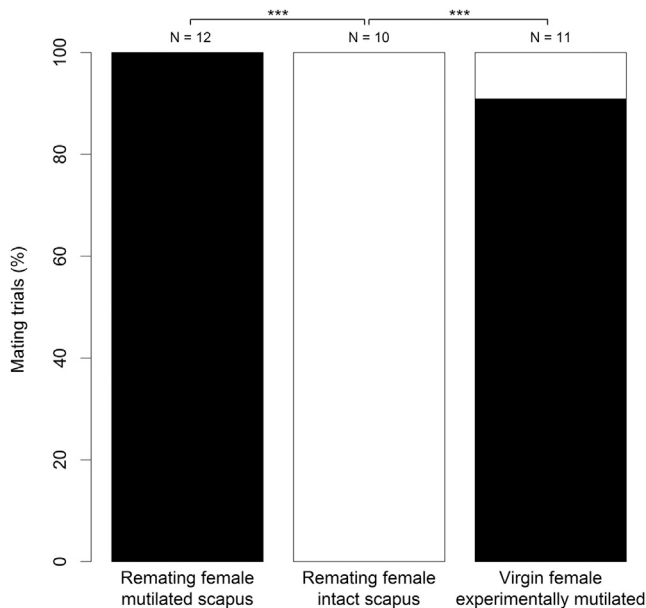


Figure 4. Mutilation as a Means to Impede Subsequent Copulations by Rival Males

Percentages of successful mating (white) and unsuccessful mating (black) of females remating with mutilated or intact scapus from previous matings and virgin females whose scapus was experimentally mutilated (** $p < 0.001$, pairwise Fisher's exact test with Bonferroni correction).

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