Spider behaviors include oral sexual encounters

Matjaž Gregorič1, Klavdija Šuen1, Ren-Chung Cheng1, Simona Kralj-Fišer1 & Matjaž Kuntner1,2

Several clades of spiders whose females evolved giant sizes are known for extreme sexual behaviors such as sexual cannibalism, opportunistic mating, mate-binding, genital mutilation, plugging, and emasculation. However, these behaviors have only been tested in a handful of size dimorphic spiders. Here, we bring another lineage into the picture by reporting on sexual behavior of Darwin’s bark spider, *Caerostris darwini*. This sexually size dimorphic Madagascan species is known for extreme web gigantism and for producing the world’s toughest biomaterial. Our field and laboratory study uncovers a rich sexual repertoire that predictably involves cannibalism, genital mutilation, male preference for teneral females, and emasculation. Surprisingly, *C. darwini* males engage in oral sexual encounters, rarely reported outside mammals. Irrespective of female’s age or mating status males salivate onto female genitalia pre-, during, and post-copulation. While its adaptive significance is elusive, oral sexual contact in spiders may signal male quality or reduce sperm competition.

Sexual selection shapes numerous animal traits, notably their morphology, physiology, and behaviors. Since sex specific phenotypes likely result from differing selection regimes in each sex, studying the biology of extremely sexually dimorphic clades may be particularly revealing in this context1. Although sexual dimorphism measures encompass traits other than size2, it is the size difference between genders, or sexual size dimorphism (SSD), that is often symptomatic of sex-specific adaptations3 including coercive mating, sexual cannibalism, toxic seminal fluids, genital damage and severance1,4.

Among terrestrial animals, spiders exhibit the most extreme female-biased SSD5,6. In species with giant females small males are more abundant due to asynchronous development of the sexes7,8. In theory, skewed sex ratios early in the season lead to intense male-male competition, and to monogyny achieved by male adaptations to sperm competition9. As a result, spiders from such size-dimorphic clades perform extreme sexual behaviors such as sexual cannibalism, opportunistic mating, mate-binding, genital mutilation, plugging, and emasculation with remote copulation10–12. In spiders, at least two of these behaviors, emasculation and sexual cannibalism, show phylogenetic links with SSD13,14. However, whether or not other sexually conflicted behaviors are associated with extreme sexual size dimorphism is difficult to conclude due to lack of comparative studies.

Among highly sexually dimorphic orb-web spiders, only selected few clades have been extensively studied for sexual behavior, notably widows (*Latrodectus*), cross spiders (*Argiope*), and golden orb weavers (*Nephila*)15–17. Of the lesser known such clades, bark spiders (genus *Caerostris*) are becoming models in silk research, but remain rather poorly known behaviorally18,19. Here, we report on operational sex ratios and sexual behaviors of the recently discovered Darwin’s bark spider (*C. darwini* Kuntner & Árnason 2010), a species from Madagascar, whose giant web is made of the toughest known silk19–21. Our field and laboratory study uncovered a rich sexual repertoire in this species, including a behavior that involves oral sexual encounters.

**Results**

In the field transect, we detected a male biased operational sex ratio (1.41), and females were on average 14.0 times heavier and 2.3 times larger than males (for complete results, see Supporting information 1). The first encounter of a male with female webs was independent of female ontogenetic stage, but resident males guarded subadult females longer than adult females (Mann-Whitney $U = 162$, $p = 0.017$, $N = 51$). In field-encountered matings ($N = 5$), four males mated with freshly molted (teneral) females (Supporting video 1), and these males always inserted both palps. In comparison, during mating trials with older females in the laboratory, 58% of males used both palps and the insertions were shorter (albeit observations for teneral females were low, see Supporting information 1 for details). Furthermore, teneral females did not attack their mates, while 31% of older females cannibalized them (Supporting video 2). Males mating with an older female always bound her in silk, a behavior

1Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Slovenia.
2Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.
Correspondence and requests for materials should be addressed to M.G. (email: matjaz.gregoric@gmail.com)
known as mate binding22 (Fig. 1D, Supporting video 3), before copulation, between both palp insertions, and after
copulation. Conversely, males mating with teneral females never wrapped their mate.

During field-encountered and laboratory matings (N = 29), all males damaged their palps after the first inser-
tion. In females mating once, broken-off embolic palpal parts (genital plugs) were externally visible in 58.8%
(N = 17) cases. Remating data show that genital plugs were inefficient in preventing further female copulations,
and indicate plug removal by subsequent males (Supporting information 1). Within 24 hours after copulation,
82.4% (N = 17) of males self-amputated their disfigured palps by chewing off the entire palpal bulb (Figs 1B and
2A,B, Supporting video 4), a behavior known as post-mating emasculation10,13.

Invariably, males (N = 29) performed a behavior that involved oral sexual encounters. Typically, a performing
male first hooked one of his cheliceral fangs to female’s copulatory opening, turned his body perpendicular to the
female, then orally secreted fluids into the copulatory openings (Supporting video 5). Males salivated onto geni-
talia of teneral females (N = 4), older virgins (N = 18), and previously mated females (N = 9). The number of oral
sexual contacts was independent of whether females were virgin or mated (Table 1), and of whether the male had
previously copulated with one or both palps (χ² = 3.063, d.f. = 1, p = 0.216). Supporting information 1 provides
additional details of the C. darwini mating system and mate choice.

**Discussion**

In species with giant females, small males are more abundant and have high mortality rates while searching for
females7,8. In theory, this leads to increased sperm competition and to male adaptations to monopolize females9.
We show that, as predicted from biology of other extremely sexually size-dimorphic spiders, C. darwini males
avoid cannibalism by opportunistically mating with teneral females, and by mate-binding22,23 (see Supporting
Material 1 for review of the evidence in spiders). Furthermore, males engage in strategies to monopolize females
via mate guarding, genital mutilation and plugging, and emasculation23. However, C. darwini males also engage
in oral sexual contact with female genitalia, a hitherto poorly known behavior in spiders whose significance we
discuss below.

Female monopolization avoidance mechanisms in spiders include mate choice through precopulatory sexual
aggression and cannibalism24, post-copulatory cryptic female choice25, and genital morphologies that decrease
male plug effectiveness26. Male genital plugs in C. darwini are deemed inefficient because females readily remate
and genital plugs can be removed by subsequent males. Thus, C. darwini males are mono- or at most bigynous,
while females likely have a polyandrous mating strategy.

While several phenotypes uncovered by our study fit the predicted evolutionary correlates of female biased
sexual size dimorphism, we also found a surprising behavior. Our results suggest that oral sexual contact is an
obligate sexual repertoire performed before, between and after copulation with females of any adult stage and
condition. With the data at hand, it seems premature to establish a precise adaptive significance of oral sexual contact, but several possibilities are plausible.

Oral sexual contact may function as a cannibalism avoidance mechanism equivalent to mate binding, opportunistic mating with teneral females, and remote copulation\textsuperscript{12,22,27}. This seems an unlikely function of oral sexual contact because males perform it with all females regardless of their aggressiveness, including the defenseless teneral ones.

Alternatively, oral sexual contact may function as assessment or manipulation of preexisting mating plugs. An assessment function, however, appears to be unlikely because the behavior is not only performed prior to copulation but also in between bouts, and after copulation. A manipulation of plugs is also unlikely because males perform it regardless of females being plugged or not.

We find the following two explanations the most plausible. Oral sexual contact may signal male quality. This would imply the existence of cryptic female choice mechanisms, where females may bias paternity in favor of better quality males\textsuperscript{25}. Additionally, enzymes in the saliva could provide physiological advantage to the donor’s over rival’s sperm. This would be an adaptation for lowering sperm competition, and would function analogously to seminal toxins and aggressive sperm known in insects\textsuperscript{28,29}. These two possibilities are intriguing but they require testing that was outside of the scope of this report.

Sexual activities involving the contact of the mouth or mouth parts of one individual with genitils of another, is rare in the animal kingdom. While fellatio-like behaviors were observed in several mammal groups, e.g. macaques, lemurs, bonobos, hyenas, cheetahs, lions, dolphins and bats\textsuperscript{30,31}, cunnilingus-like behaviors are rarer still. In birds, dunnock males peck out rival male’s sperm from the female cloaca\textsuperscript{32} and among mammals, cunnilingus-like behaviors have been observed in bonobos and have been demonstrated to play an important role.

Figure 2. Genital mutilation and mate plugging in \textit{C. darwini}. (A) Palps of an intact male. (B) Palps of a eunuch male. (C) Epigynum in ventral view with an externally visible genital plug (arrow). (D) Epigynum in dorsal view with the male embolic part visible in the right spermatheca (arrow).

| Condition          | Virgin females | Mated females | Significance |
|--------------------|----------------|---------------|--------------|
| Before 1st insertion | 8.5 ± 4.5, N = 17 | 14.6 ± 11.7, N = 9 | MWU = 58, p = 0.316 |
| After 1st insertion | 7.8 ± 7.8, N = 16 | 9.1 ± 5.9, N = 7 | MWU = 44, p = 0.420 |
| After 2nd insertion | 27.7 ± 22.9, N = 7 | 24.8 ± 5.9, N = 5 | MWU = 14, p = 0.563 |

Table 1. The number of oral sexual contacts between a male and virgin and mated \textit{C. darwini} females, with medians ± interquartile ranges (Mann-Whitney U test).
role in mating of fruit bats\textsuperscript{31}. Males of fruit flies lick female genitals as part of the courtship\textsuperscript{34,35}, which does not influence paternity, but influences the duration of copulation\textsuperscript{36}.

The only other spiders known to exhibit oral sexual encounters are the size dimorphic widows (\textit{Latrodectus}), where nothing is known about the phenomenon apart from its occurrence, i.e. reports of oral contact and salivation\textsuperscript{37–39}. The evidence for precise adaptive function of oral sexual encounters in spiders is currently elusive, and therefore specifically designed experiments are to be employed for more precise future tests. Nevertheless, our discovery adds to a more general understanding of how spider sexual dimorphism relates to extreme sexual phenotypes, including male strategies to monopolize females\textsuperscript{10,40}.

**Methods**

During a two-week monitoring of a \textasciitilde100 m transect in Andasibe-Mantadia National Park, Madagascar, we assessed the SSD level and operational sex ratio in \textit{C. darwini}, documented interactions between individuals, and recorded mating details. In the laboratory, we subjected 17 virgin older (three to 10 days after maturation) females to mating trials, then subjected four of these to a total of nine remating trials. In the field and laboratory we quantified male courting duration, mate binding and oral sexual contacts, the duration of palpal insertions, palpal damage, and female aggressive behaviors and sexual cannibalism. Supporting information 1 contains detailed experimental and statistical procedures.

**References**

1. Fairbairn, D. J., Blanckenhorn, W. U. & Szekely, T. Sex, size, and gender roles: Evolutionary studies of sexual size dimorphism. (Oxford University Press, 2007).
2. Cheng, R.-C. & Knutner, M. Disentangling the size and shape components of sexual dimorphism. \textit{Evol Biol} 42, 223–234, doi: 10.1007/s11692-015-9313-z (2015).
3. Blanckenhorn, W. U. Behavioral causes and consequences of sexual size dimorphism. \textit{Ethology} 111, 977–1016, doi: 10.1111/j.1439-0310.2005.01147.x (2005).
4. Arnqvist, G. & Rowe, L. Sexual conflict. 330 (Princeton University Press, 2005).
5. Knutner, M., Zhang, S., Gregoric, M. & Li, D. \textit{Nephila} female giganticity attained through post-maturity molting. \textit{J Arachnol} 40, 345–347 (2012).
6. Foeldner, M. W. & Moya-Larazo, J. In Sex, size, and gender roles: Evolutionary studies of sexual size dimorphism (eds D. J. Fairbairn, W. U. Blanckenhorn, & T. Szekely) 71–81 (Oxford University Press, 2007).
7. Higgins, L., Coddington, J., Goodnight, C. & Knutner, M. Testing ecological and developmental hypotheses of mean and variation in adult size in nephilid orb-weaving spiders. \textit{Evol Ecol} 25, 1289–1306, doi: 10.1007/s10682-011-9475-9 (2011).
8. Robinson, M. H. & Robinson, B. Ecology and behavior of the giant wood spider \textit{Nephila maculata} (Fabricr.) in New Guinea. \textit{Smithson Contrib Zool} 149, 1–73 (1973).
9. Fromhage, L., Elgar, M. A. & Schneider, J. M. Faithful without care: The evolution of monogamy. \textit{Evolution} 59, 1400–1405 (2005).
10. Knutner, M. et al. Eunuch supremacy: evolution of post-mating spider emasculation. \textit{Behav Ecol Sociobiol} 69, 117–126, doi: 10.1007/s00265-014-1824-6 (2012).
11. Knutner, M., Agnarsson, I. & Li, D. Q. The eunuch phenomenon: adaptive evolution of genital emasculation in sexually dimorphic spiders. \textit{Bio Rev} 90, 279–296, doi: 10.1111/bre.12109 (2015).
12. Wilder, S. M., Rypstra, A. L. & Elgar, M. A. The importance of ecological and phylogenetic conditions for the occurrence and frequency of sexual cannibalism. \textit{Annu Rev Ecol Evol Syst} 40, 21–39, doi: 10.1146/annurev.ecolsys.110308.120238 (2009).
13. Eberhard, W. G. Female control: Sexual selection by cryptic female choice. 472 (Princeton University Press, 1996).
14. Wilder, S. M., Rypstra, A. L. & Elgar, M. A. The importance of ecological and phylogenetic conditions for the occurrence and frequency of sexual cannibalism. \textit{Annu Rev Ecol Evol Syst} 40, 21–39, doi: 10.1146/annurev.ecolsys.110308.120238 (2009).
15. Elgar, M. A., De Crespiigny, F. C. & Ramamurthy, S. Male copulation behaviour and the risk of sperm competition. \textit{Anim Behav} 66, 211–216, doi: 10.1016/anbeh.2003.2189 (2003).
16. Andreu, M. C. B. Sexual selection for male sacriﬁce in the Australian redback spider. \textit{Science} 271, 70–72, doi: 10.1126/science.271.5245.70 (1996).
17. Schneider, J. M., Uhl, G. & Herberstein, M. E. In \textit{Cryptic female choice in arthropods} (eds A. V. Peretti & A. Aisenberg) (Springer International Publishing, 2015).
18. Gregorich, M., Blackledge, T. A., Agnarsson, I. & Knutner, M. A molecular phylogeny of bark spiders reveals new species from Africa and Madagascar (Araneae: Caerostris). \textit{J Arachnol} 43, 293–312 (2015).
19. Gregorich, M., Agnarsson, I., Blackledge, T. A. & Knutner, M. Darwin’s bark spider: giant prey in giant orb webs (\textit{Caerostris darwini}, Araneae: Araneidae)? \textit{J Arachnol} 39, 287–295 (2011).
20. Agnarsson, I., Knutner, M. & Blackledge, T. A. Bioprospecting finds the toughest biological material: Extraordinary silk from a giant riverine orb spider. \textit{Plos One} 5, e11234, doi: e11234.1371/journal.pone.0011234 (2010).
21. Gregorich, M., Agnarsson, I., Blackledge, T. A. & Knutner, M. Phylogenetic position and composition of Zyggiellinae and Caerostridae, with new insight into orb-web evolution and gigantism. \textit{Zool J Linn Soc} 175, 225–243 (2015).
22. Zhang, S., Knutner, M. & Li, D. Q. Mate binding: male adaptation to sexual conflict in the golden orb-web spider (\textit{Nephila pilipes}). \textit{Anim Behav} 82, 1299–1304, doi: 10.1016/j.anbehav.2011.09.010 (2011).
23. Knutner, M., Coddington, J. A. & Schneider, J. M. Intersexual arms race? Genital coevolution in nephilid spiders (Araneae, Nephilidae). \textit{Evolution} 63, 1451–1463, doi: 10.1111/j.1558-5646.2009.00634.x (2009).
24. Elgar, M. A., Schneider, J. M. & Herberstein, M. E. Female control of paternity in the sexually cannibalistic spider \textit{Argiope keyserlingi}. \textit{Proc Roy Soc B} 267, 2439–2443 (2000).
25. Eberhard, W. G. Female control: Sexual selection by cryptic female choice. 472 (Princeton University Press, 1996).
26. Knutner, M., Kralj-Fišer, S., Schneider, J. M. & Li, D. Mate plugging via genital mutilation in nephilid spiders: an evolutionary hypothesis. \textit{J Zool} 277, 257–266, doi: 10.1111/j.1469-7998.2008.00533.x (2009).
27. Robinson, M. H. & Robinson, B. Comparative studies of the courtship and mating behavior of tropical araneid spiders. \textit{Pacific Insects} 36, 1–218 (1980).
28. Arnqvist, G. & Rowe, L. Antagonistic coevolution between the sexes in a group of insects. \textit{Nature} 415, 787–789 (2002).
29. Chapman, T., Liddle, L. E., Kallf, J. M., Wolfer, M. P. & Partridge, L. Cost of mating in \textit{Drosophila melanogaster} females is mediated by male accessory-gland products. \textit{Nature} 373, 241–244, doi: 10.1038/373241a0 (1995).
30. Maruthupandian, J. & Marimuthu, G. Cunnilingus apparently increases duration of copulation in the Indian flying fox, \textit{Pteropus giganteus}. \textit{Plos One} 8, doi: 10.1371/journal.pone.0059743 (2013).
31. Sergiel, A. et al. Fellatio in captive brown bears: Evidence of long-term effects of suckling deprivation? \textit{Zoo Biology} 33, 349–352, doi: 10.1002/zoo.21137 (2014).
32. Davies, N. B. Polyandry, cloaca-pecking and sperm competition in dunnocks. \textit{Nature} 302, 334–336, doi: 10.1038/302334a0 (1983).
33. Tan, M. et al. Fellatio by fruit bats prolongs copulation time. Plos One 4, doi: 10.1371/journal.pone.0007595 (2009).
34. Hall, J. C. Control of male reproductive-behavior by the central nervous-system of Drosophila - dissection of a courtship pathway by genetic mosaics. Genetics 92, 437–457 (1979).
35. Yamamoto, D. & Koganezawa, M. Genes and circuits of courtship behaviour in Drosophila males. Nat Rev Neuro 14, 681–692, doi: 10.1038/nrn3567 (2013).
36. Wellbergen, P. & Vandijken, F. R. Asymmetric response to directional selection for licking behavior of Drosophila melanogaster males. Behav Genet 22, 113–124, doi: 10.1007/bf01066796 (1992).
37. Forster, L. The behavioural ecology of Latrodictus hasselti (Thorell), the Australian redback spider (Araneae: Theridiidae): a review. Records West Aust Mus 52, 13–24 (1995).
38. Berendock, B. Reproductive strategies in Latrodictus revivensis (Araneae: Theridiidae): functional morphology and sexual cannibalism Philosophy doctor thesis, Heinrich-Heine-Universität Düsseldorf (2003).
39. Rivera, M. J. Apareamiento del Latrodictus formidabilis. Revista Chilena de historia natural 8, 161–163 (1901).
40. Kuntner, M. & Elgar, M. A. Evolution and maintenance of sexual size dimorphism: aligning phylogenetic and experimental evidence. Front Ecol Evol 2, 26, doi: 10.3389/fevo.2014.00026 (2014).

Acknowledgements
This work was funded by the Slovenian Research Agency and the Explorers Club. We thank the MICET crew in Antananarivo, S. L. Rahanitriniaina and H. Nono Rabarison for help in the field, and T. Celik and T. Lokovsek for analytical and lab help.

Author Contributions
Designed: M.G., S.K.F., R.C., M.K. and K.Š. Performed experiments and analyzed data: M.G., R.C., K.Š. and S.K.F. Wrote paper: M.G., M.K. and S.K.F.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Gregorič, M. et al. Spider behaviors include oral sexual encounters. Sci. Rep. 6, 25128; doi: 10.1038/srep25128 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/