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SHORT COMMUNICATION

# Differential oxidative costs of locomotory and genital damage in an orb-weaving spider

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## ABSTRACT

In animals that regularly experience tissue loss, physiological responses may have evolved to overcome the related costs. Changes in oxidative status may reflect such self-maintenance mechanisms. Here, we investigated how markers of oxidative status vary in female orb-weaving spiders (*Larinia jeskovi*) by mimicking two distinct types of tissue loss they may naturally encounter: damage to their locomotory system and damage to their external genital structure (scapus), as inflicted by males during copulation (external female genital mutilation). Damage to the locomotory system resulted in a significant shift in oxidative status, reflecting investment in self-maintenance. In contrast, the loss of the scapus did not result in quantitative changes of oxidative markers. This lack of a physiological response suggests negligible physiological costs of genital mutilation for female spiders. However, not being able to remate with other males might be costly for females.

**KEY WORDS: Sexual conflict, Genital mutilation, Wound, Tissue loss, Oxidative status, *Larinia jeskovi***

## INTRODUCTION

In nature, tissue loss typically occurs because of sub-lethal predation, agonistic behaviours between conspecifics and abiotic physical damage (Bely and Nyberg, 2010). Tissue loss may be costly when it decreases the overall performance of the injured animals by limiting their ability to exploit resources (e.g. reduced locomotor or feeding ability) and/or by affecting their homeostasis (e.g. because of fluid loss or infection). By activating physiological processes such as healing and immune responses, injured animals may minimize these direct costs. However, investing in self-maintenance mechanisms may limit resources available for other fitness-related functions, such as reproduction (Stearns, 1989). This investment trade-off may be mediated by variation in oxidative status, with antioxidant defences neutralizing the action of oxidizing species on biomolecules, thereby limiting the generation of oxidative damage in tissues and increasing the survival probability of the organism (Monaghan et al., 2009). For instance, *Bicyclus anynana* butterflies solve the trade-off between longevity and fecundity under challenging conditions by increasing antioxidant defences, thereby prolonging lifespan but reducing fecundity (Beaulieu et al., 2015b). Alternatively, to facilitate healing and immune response following tissue loss, injured organisms may also locally reduce antioxidant defences, as oxidizing species may themselves enhance cell communication during tissue repair and eliminate pathogens (Wang et al., 2001; Schäfer and Werner, 2008). Moreover, in order to minimize oxidative damage on their tissues, injured animals may simultaneously reduce their physical activity, thereby reducing their overall production of oxidizing molecules (Beaulieu et al., 2015a). Hence, the optimal maintenance response of injured animals depends on the regulation of the balance between their production of oxidizing molecules and their antioxidant defences (Costantini, 2019).

Tissue loss occurs not only because of predation or agonistic behaviours between conspecifics but also during copulation. Indeed, in a broad range of species, males harm females while mating by inflicting physical damage inside or outside the female's genitalia (Reinhardt et al., 2015). Harmful mating may lead to sexual conflict when it alters the female's fitness, and results in sexually antagonistic coevolution (Arnqvist and Rowe, 2005). However, the evolutionary mechanisms of harmful mating are not clear as assessing the female costs due to physical damage may be confounded by other effects linked to mating (Morrow et al., 2003). For instance, sperm and seminal fluids transferred during mating can consist of nutritive resources or manipulative chemicals that affect the physiological response of inseminated females as well (Arnqvist and Rowe, 2005; Reinhardt et al., 2015). In several spider species, including the araneid spider *Larinia jeskovi*, males mutilate the outer structures of the female genitalia (Mouginot et al., 2015; Nakata, 2016). Experimental damage of external structures makes it possible to disentangle the physiological costs due to genital damage from other effects related to sperm or seminal fluids, which is not possible in cases of internal damage.

So far, the physiological response of females following internal genital damage has been assessed by mimicking genital damage by ablation of locomotory tissue (Morrow et al., 2003). However, locomotory damage may not elicit a similar physiological response to genital damage and may thus not help us to understand the underlying mechanisms and consequences of genital damage. Differences in oxidative status between sexes have been attributed to conflicting reproductive strategies in males and females in vertebrates (Costantini, 2018). Whether physical damage inflicted during copulation may contribute to sexual differences in other species, such as spiders, remains currently unknown. Indeed, males with higher antioxidant defences may have a competitive advantage over rival males in gaining access to females (Garratt and Brooks, 2012), while mutilated females may benefit from reducing antioxidant defences by enhancing cell communication and eliminating pathogens during tissue repair (Wang et al., 2001; Schäfer and Werner, 2008).

Here, we compared the physiological response to locomotory and genital damage in female spiders by measuring different markers of oxidative status. Oxidative markers have already been measured in

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spiders as a mediator of longevity and in response to exposure to heavy metals (Criscuolo et al., 2010; Aziz et al., 2020), but to our knowledge, this study is the first to examine variation in oxidative markers in response to tissue loss in invertebrates. To mimic locomotory and genital damage, we experimentally ablated one leg or the genital structure. If the coevolution of males and females resulted in a reduction of the physiological costs of genital mutilation (Morrow et al., 2003), we expected genital mutilation to trigger a weaker physiological response than locomotory tissue loss.

## MATERIALS AND METHODS

### Study animals

We collected sub-adult (one moulting stage from adulthood) females of the orb-weaving spider *Larinia jeskovi* (Marusik, 1986) (Araneidae) in August 2015 and 2016 in the Biebrza National Park, Poland (53°21′01.36″N, 22°34′37.45″E). In the laboratory, we housed females individually in 250 ml plastic cups at room temperature and under a natural light cycle (10 h:14 h, night: day). Females were fed with one fly (*Lucilia sericata*) every 3 days and provided with water daily. Sub-adult females were checked daily for moulting events. After their final moult, adult females were used for the experimental setup.

### Experimental setup

In order to assess the effect of tissue loss on the oxidative status of females, we amputated part of one of their forelegs (haphazardly right or left first leg) or ablated their external genital structure, the scapus (Fig. 1A,C) (Mouginot et al., 2015). To achieve this, females were first physically immobilized under a mesh and then wounded under a stereomicroscope using dissecting scissors for the legs and forceps for the scapi. A control group was left intact but was similarly handled. In the first experimental session (2015), 30 females were randomly assigned to three treatment groups by drawing numbers: control, amputated at mid-tibia, scapus amputated. Amputation at the mid-tibia is not meant to represent a natural phenomenon. Autotomy between the coxa and the trochanter (leg loss) has been shown to be an adaptation to injuries due to predators for several spider species, which may buffer the effect of the injury (Foelix, 2011). However, because we aimed at comparing the effect of physical damage to a non-genital structure with that to a genital structure, amputation at the mid-tibia seemed more appropriate in terms of expected physiological response. In the second experimental session (2016), we repeated the procedure with an additional amputation treatment in which we removed 200 µm of the tarsus (leg-tip amputated). The removal of only the tip of the leg assesses the effect of a wound comparable to the scapus mutilation in terms of the amount of tissue lost. Seventy-two females were randomly assigned to the four experimental amputation treatments; control, mid-tibia, leg-tip and scapus. In both experimental sessions, all females were cryofixed with liquid nitrogen 8 h after the treatment and stored at −80°C for later analysis. We chose this time interval between mutilation and physiological testing as the immune response of spiders may occur very rapidly (within minutes) (Fukuzawa et al., 2008; Kuhn-Nentwig and Nentwig, 2013). Age was calculated as the number of days between the final moult of the spider and the treatment. Spiders did not differ in body mass and age between treatments (Fig. S1).

### Oxidative stress markers

Before physiological measurements, all appendages (legs, pedipalps) were removed from the frozen specimens on dry ice, and the body of each female was weighed to the nearest 0.01 mg (LE225D, Sartorius AG, Göttingen, Germany) before being transferred to PBS buffer. Because it is difficult to predict which molecules among those involved in the regulation of the oxidative status will vary in response to amputation treatments, we measured different oxidative markers in the two experimental sessions to increase the probability of finding at least one marker affected by the treatment.

In the first experimental session, we measured two markers of oxidative status. We used the OXY-absorbent test (Diacron International, Grosseto, Italy), to measure spiders' total antioxidant capacity (in mmol of HOCl neutralized) as a quantitative marker of antioxidant defences (covering several classes of biomolecules with antioxidant properties), and the d-ROM test (Diacron International, Grosseto, Italy), to measure concentrations of hydroperoxides, a marker of oxidative damage deriving from the oxidation of fatty acids, proteins and nucleic acids and promoting cell death (expressed in mg $dl^{-1}$ $H_2O_2$ equivalents). For both tests, we followed the procedure described in Beaulieu et al. (2015b). Three individuals for which d-ROM values were below the detection threshold were excluded from the analyses. This resulted in a sample size of 9 control females, 10 tibia-amputated females and 8 scapus-amputated females.

In the second experimental session, we measured glutathione (GSH) levels (expressed in µmol GSH $mg^{-1}$ protein) as a marker of endogenous antioxidant defences, and malondialdehyde (MDA) levels to assess oxidative damage on lipids (expressed in mmol

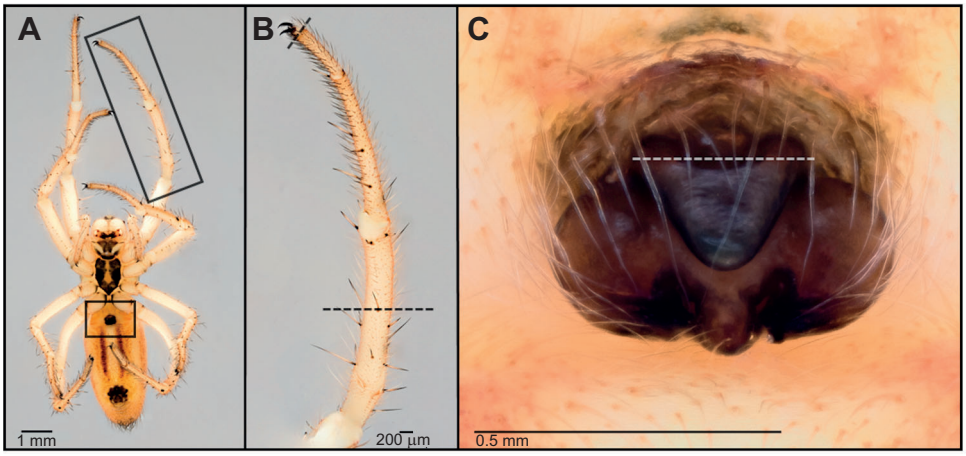


**Fig. 1. The study species, *Larinia jeskovi*.** (A) Female in ventral view. The two black rectangles indicate the leg and the external genital area where amputation was applied. (B) A close up picture of the leg with the experimental amputation treatments at the mid-tibia and leg-tip represented by the dashed lines. (C) A close up picture of the external female genitalia. The experimental amputation was applied at the base of the scapus (triangular protrusion) as indicated by the dashed lines.

Journal of Experimental Biology (2020) 223, jeb219758. doi:10.1242/jeb.219758

$mg^{-1}$ protein). The prosoma and the abdomen were homogenized together with Triton buffer (7.5 µl for each 1 mg sample) through high-speed shaking (3 times for 1 min; 24 shakes $s^{-1}$). The resulting homogenate was centrifuged (16,249 *g*, 30 min, 4°C) and the resulting supernatant transferred to a new tube and centrifuged again (16,249 *g*, 15 min, 4°C). The second supernatant was then used to analyse total protein (to correct for concentration differences between samples) and MDA concentration. MDA concentration was determined using the commercial kit MDA Microplate Assay Kit (CAK1011, Cohesion Bioscience; 532 and 600 nm). GSH levels were assessed with a spectrophotometric method, which involves oxidation of GSH by the sulfhydryl reagent 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB, also known as Ellman's reagent) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. Total protein concentration of all samples was determined using the Bradford protein assay at 595 nm. We initially treated 72 females, but two females died during the 8 h time interval following mutilation. Out of the 70 females left, we were able to measure MDA levels in 62 individuals (8 showing levels lower than the minimal detection threshold). Consequently, 62 females were used for GSH and MDA measurements (17 control females, 15 mid-tibia-amputated females, 16 leg-tip-amputated females and 14 scapus-amputated females).

### Statistical analyses

To test the effects of treatment on antioxidant capacity, hydroperoxide and MDA levels, we built linear models with antioxidant capacity, hydroperoxide or MDA level as dependent variables, and treatment, age, body mass and protein concentration as independent variables. To test the effect of treatment on GSH levels, we built a linear model with GSH corrected for body mass as a dependent variable (to reach normality) and treatment and age as independent variables. We only corrected GSH by body mass because applying the same correction to the other dependent variables led to a deviation from linear model assumptions. We checked linearity assumptions graphically as well as with a Bartlett test for homoscedasticity and Shapiro test for normality of the residuals. The model for antioxidant capacity required the exclusion of one outlier (from the control treatment) to match linearity assumptions. There was no significant correlation among variables (Table S1), leading to no collinearity among explanatory variables. In all models, quantitative variables were centred and standardized (Schielzeth, 2010).

For each model, we considered all plausible candidate models and ranked them according to their AICc value (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). To evaluate the contribution of each predictor to the model prediction, we calculated its sum of Akaike weights and used 'full model averaging' to calculate parameter estimates β (Symonds and Moussalli, 2011). Model selection tables are presented in Tables S2–S5. As the sum of weights may provide a poor evaluation of the predictor's importance (Galipaud et al., 2014), we calculated the 85% confidence interval for each parameter estimate (Arnold, 2010). Parameter estimates whose confidence interval did not include zero were considered as having a significant effect. The evaluation of a predictor's contribution results in parameter estimates for which the first level of a factor is set as a reference. Thus, the results of the amputation treatments are presented as mid-tibia, leg-tip and scapus amputation treatments compared with the control treatment as the reference. All analyses were performed in R software (https://www.r-project.org/). The MuMIn v1.40.4 (https://CRAN.R-project.org/package=MuMIn) and Plotrix 3.7-6 (Lemon, 2006) packages were used for procedures of model selection and model averaging and for calculating the standard errors of the means in Fig. 2, respectively.

## RESULTS AND DISCUSSION

Female spiders segregated in oxidative space depending on the treatment they experienced (Table 1, Fig. 2). Indeed, relative to control spiders, females amputated at the mid-tibia in the first experimental session showed higher antioxidant capacity, as revealed by the OXY-adsorbent test (Table 1, Fig. 2A). The opposite pattern was observed when measuring GSH levels as a marker of antioxidant defence in the second experiment, with mid-tibia- and leg-tip-amputated females showing a tendency towards or

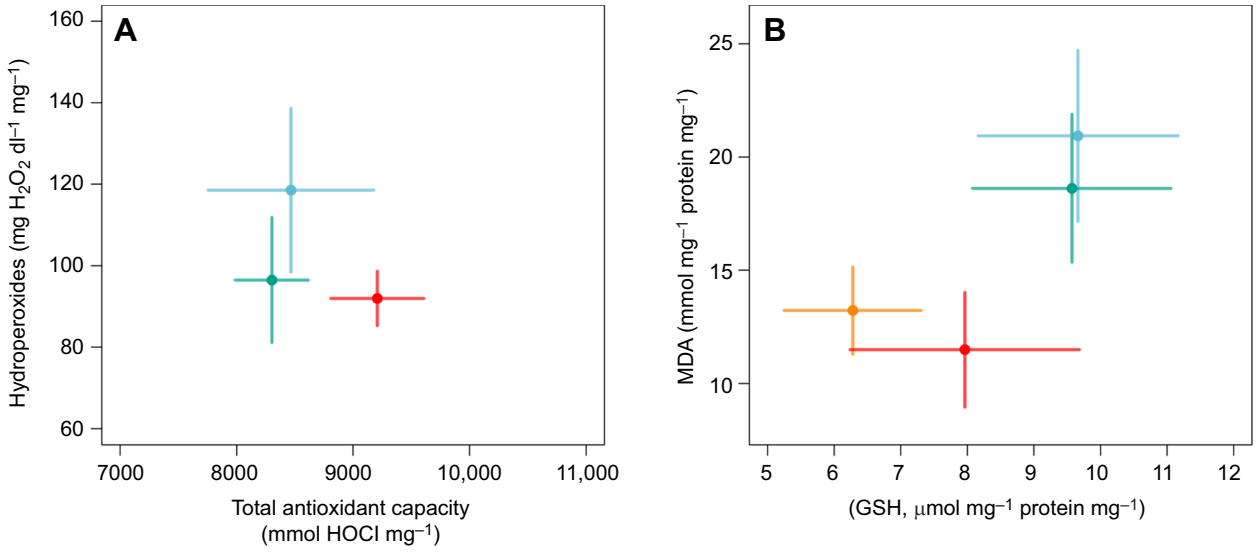


**Fig. 2. Oxidative stress markers.** Mean values (±s.e.m.) of (A) total antioxidant capacity (OXY-adsorbent test) and oxidative damage (hydroperoxide concentration, d-ROM test), and (B) endogenous antioxidant defence (glutathione concentration, GSH) and oxidative damage on lipids (malondialdehyde concentration, MDA) for each experimental amputation treatment: control (blue), mid-tibia (red), leg-tip (orange) and scapus (green) in the spider *L. jeskovi*. Markers of antioxidant defence are given on the *x*-axes and markers of oxidative damage on the *y*-axes. Levels of antioxidant defence and oxidative damage markers were corrected for body mass.

Journal of Experimental Biology (2020) 223, jeb219758. doi:10.1242/jeb.219758

**Table 1. Predictors' sum of weights ($\Sigma w_i$) and parameter significance with 85% confidence interval (CI) after full model averaging on the set of candidate models**

| Predictor | $\sum w_i$ | Parameter | Significance | 85% CI |
|---|---|---|---|---|
| **Antioxidant defence** | | | | |
| Total antioxidant capacity | | Intercept | + | 278.00; 297.49 |
| Body mass | 1 | Body mass | + | 19.04; 31.54 |
| Protein concentration | 0.30 | Protein concentration | – | −10.83; 1.81 |
| Treatment | 0.22 | Mid-tibia | + | 1.72; 30.29 |
| | | Scapus | NS | −0.59; 30.71 |
| Age | 0.20 | Age | NS | −4.52; 8.31 |
| Glutathione | | Intercept | + | 7.110; 10.076 |
| Treatment | 0.05 | Leg-tip | – | −6.306; −0.487 |
| | | Mid-tibia | NS | −4.738; 1.208 |
| | | Scapus | NS | −3.141; 2.894 |
| Age | 0.35 | Age | NS | −0.593; 1.568 |
| **Oxidative damage** | | | | |
| Hydroperoxides | | Intercept | + | 3.24; 4.38 |
| Age | 0.58 | Age | + | 0.09; 0.85 |
| Body mass | 1 | Body mass | + | 1.01; 1.78 |
| Protein concentration | 0.23 | Protein concentration | NS | −0.21; 0.61 |
| Treatment | 0.22 | Mid-tibia | – | −1.93; −0.17 |
| | | Scapus | NS | −1.68; 0.30 |
| Malondialdehyde | | Intercept | + | 0.395; 0.634 |
| Body mass | 0.24 | Body mass | NS | −0.066; 0.045 |
| Treatment | 0.83 | Leg-tip | – | −0.370; −0.086 |
| | | Mid-tibia | – | −0.419; −0.128 |
| | | Scapus | NS | −0.210; 0.084 |
| Protein concentration | 0.24 | Protein concentration | NS | −0.045; 0.065 |
| Age | 0.32 | Age | NS | −0.088; 0.019 |

Models assessed the effect of body mass, experimental amputation treatment, age and protein concentration on markers of antioxidant defence [estimated by the total antioxidant capacity (OXY-adsorbent test) and glutathione (GSH) levels] and oxidative damage [estimated by hydroperoxide (d-ROM test) and malondialdehyde (MDA test) levels] in the spider *Larinia jeskovi*. Note: parameter estimates after model averaging of treatment were compared with the reference level 'control'. An estimate whose 85% CI did not include zero was considered significant: NS, non-significant; +, positively significant; –, negatively significant.

significantly lower GSH levels than control females, respectively (Table 1, Fig. 2B).

In contrast to antioxidant defences, markers of oxidative damage consistently varied across experimental sessions, with all amputated females, irrespective of their amputation extent, showing lower levels of oxidative damage than control females, as revealed by the d-ROM and MDA tests (Table 1, Fig. 2).

In contrast to females experiencing locomotory damage, scapus-amputated females showed no alteration of their oxidative status (considering the markers of antioxidant defence and oxidative damage measured in our study) relative to control females in both experimental sessions (Table 1). Hence, scapus-amputated and control spiders segregated from mid-tibia and leg-tip amputated spiders in oxidative space (Fig. 2).

Leg amputation led to a shift in the oxidative status of female spiders irrespective of amputation extent. In contrast, scapus amputation did not affect their oxidative status. These results were consistent across different markers of oxidative damage measured in different individuals. Hence, our study suggests that physical damage inflicted on the locomotory system of female spiders affects their oxidative status, whereas damage to their external genitalia does not.

In agreement with our predictions, tissue loss due to leg amputation induced a shift in females' oxidative status, as amputated females showed a higher antioxidant capacity than intact females. In contrast, GSH levels tended to decrease in mid-tibia-amputated spiders and significantly decreased in leg-tip-amputated spiders. Even though the reason for this reduction in GSH levels in spiders with locomotory damage remains unclear, the higher antioxidant capacity of leg-amputated spiders suggests that they invested in self-maintenance mechanisms by upregulating their production of some antioxidant defences. This upregulation of antioxidant defences did not, however, involve GSH and possibly compensated for the reduction of this important endogenous antioxidant compound.

Changes in antioxidant defences were not associated with stable oxidative damage in amputated females but were linked to lower oxidative damage in all spiders with locomotory damage. Low oxidative damage in amputated females is probably related to a state of hypometabolism helping spiders to save resources that can be allocated to maintenance mechanisms, such as healing and antioxidant defences (Gorr, 2017). Fluid loss and lower physical activity, as expected in amputated spiders, may contribute to this suspected hypometabolism (Beaulieu et al., 2015a; França et al., 2007). Finally, the fact that both markers of oxidative damage decreased in amputated individuals can be explained by the biochemical proximity between the two markers, as MDA results from the decomposition of hydroperoxides (Beaulieu and Costantini, 2014).

In contrast to leg amputation, the removal of the external genital structure did not affect the females' oxidative status, which was comparable to that of control females. It might be argued that the extent of the harm inflicted was not sufficient to induce a detectable physiological response. However, the amputation of the leg tip, an injury comparable to scapus mutilation in terms of tissue loss, affected the females' oxidative status. This difference may result from the reduced physical activity of leg-tip-amputated females, which might not occur in scapus-amputated females. Fluid loss might also be substantially higher after leg loss compared with genital damage. We argue that ablating a locomotory tissue is

inadequate as a means to mimic the effects of genital damage (Morrow et al., 2003).

The absence of effects of scapus mutilation on the oxidative status of the females may also be due to the fact that our measurements captured only a snapshot (8 h after mutilation) of the systemic response of spiders, in contrast to a potential local response in tissues around the scapus. Given the rapidity of the immune response of spiders (Kuhn-Nentwig and Nentwig, 2013), it is indeed possible that this response was highly transient and localized. However, the absence of a systemic response, and hence the absence of a general reallocation of resources to self-maintenance mechanisms, suggests that the reproductive performance and survival of female spiders were probably not affected by genital mutilation.

External female genital mutilation by males is a feature of the mating system of several spider species that allows males to secure paternity (Mouginot et al., 2015; Nakata, 2016) and therefore may be subject to sexual conflict (Reinhardt et al., 2015). It has remained unclear whether females incur direct costs from genital damage (Morrow et al., 2003). Our findings suggest that physiological costs in terms of self-maintenance are probably negligible. Physiological adaptations reducing the effects of genital damage might have evolved in females to benefit not only them but also their manipulative males given that costs for females would affect the reproductive performance of both sexes (Morrow et al., 2003; Morrow and Arnqvist, 2003; Arnqvist and Rowe, 2005). We speculate that the possible main cost of genital mutilation for females might be the loss of further mating opportunities. In conclusion, our results are in agreement with the oxidative shielding hypothesis, postulating that oxidative damage in reproductive females is minimized to avoid deleterious effects on reproductive performance (Blount et al., 2016). Conversely, our study suggests that males benefit from mutilating female genitalia because this limits the subsequent mating of the female with other males but does not impair the female's physiology and probably also not her survival and fecundity, which sheds new light on our understanding of the evolution of external female genital mutilation in animals.

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#### Competing interests
The authors declare no competing or financial interests.

#### Author contributions
Conceptualization: P.M., G.U., M.B.; Methodology: P.M., N.T.; Validation: P.M., G.U., N.T., M.B.; Formal analysis: P.M., M.B.; Investigation: P.M., N.T.; Resources: P.M., G.U.; Data curation: P.M.; Writing - original draft: P.M., G.U., N.T., M.B.; Writing - review & editing: P.M., G.U., N.T., M.B.; Visualization: P.M.; Supervision: G.U.; Project administration: G.U.; Funding acquisition: G.U., M.B.

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#### Data availability
Data are available from GitHub: https://github.com/pierick-mouginot/ArticleDiffOxidCostSpiderEFGM.

#### Supplementary information
Supplementary information available online at
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#### References
**Arnold, T. W.** (2010). Uninformative parameters and model selection using Akaike's information criterion. *J. Wildl. Manag.* **74**, 1175-1178. doi:10.1111/j.1937-2817.2010.tb01236.x

**Arnqvist, G. and Rowe, L.** (2005). *Sexual Conflict*. Princeton, NJ: Princeton University Press.

**Aziz, N., Butt, A. and Elsheikha, H. M.** (2020). Antioxidant enzymes as biomarkers of Cu and Pb exposure in the ground spiders *Lycosa terrestris* and *Pardosa birmanica*. *Ecotox Environ Safe* **190**, 110054. doi:10.1016/j.ecoenv.2019.110054

**Beaulieu, M. and Costantini, D.** (2014). Biomarkers of oxidative status: missing tools in conservation physiology. *Conserv. Physiol.* **2**, cou14. doi:10.1093/conphys/cou014

**Beaulieu, M., Gillen, E., Hahn, S., Pape, J. M. and Fischer, K.** (2015a). Behavioural antioxidant strategies to cope with high temperatures: a study in a tropical butterfly. *Anim. Behav.* **109**, 89-99. doi:10.1016/j.anbehav.2015.08.010

**Beaulieu, M., Geiger, R. E., Reim, E., Zielke, L. and Fischer, K.** (2015b). Reproduction alters oxidative status when it is traded–off against longevity. *Evolution* **69**, 1786-1796. doi:10.1111/evo.12697

**Bely, A. E. and Nyberg, K. G.** (2010). Evolution of animal regeneration: re-emergence of a field. *Trends Ecol. Evol.* **25**, 161-170. doi:10.1016/j.tree.2009.08.005

**Blount, J. D., Vitikainen, E. I., Stott, I. and Cant, M. A.** (2016). Oxidative shielding and the cost of reproduction. *Biol. Rev.* **91**, 483-497. doi:10.1111/brv.12179

**Burnham, K. P. and Anderson, D. R.** (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. New York: Springer.

**Costantini, D.** (2018). Meta-analysis reveals that reproductive strategies are associated with sexual differences in oxidative balance across vertebrates. *Curr. Zool.* **64**, 1-11. doi:10.1093/cz/zox002

**Costantini, D.** (2019). Understanding diversity in oxidative status and oxidative stress: the opportunities and challenges ahead. *J. Exp. Biol.* **222**, jeb194688. doi:10.1242/jeb.194688

**Criscuolo, F., Font-Sala, C., Bouillaud, F., Poulin, N. and Trabalon, M.** (2010). Increased ROS production: a component of the longevity equation in the male mygalomorph, *Brachypelma albopilosa*. *PLoS ONE* **5**, e13104. doi:10.1371/journal.pone.0013104

**Foelix, R. F.** (2011). *Biology of Spiders*. New York, NY: Oxford University Press.

**França, M. B., Panek, A. D. and Eleutherio, E. C. A.** (2007). Oxidative stress and its effects during dehydration. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **146**, 621-631. doi:10.1016/j.cbpa.2006.02.030

**Fukuzawa, A. H., Vellutini, B. C., Lorenzini, D. M., Silva, P. I., , Jr, Mortara, R. A., da Silva, J. M. C. and Daffre, S.** (2008). The role of hemocytes in the immunity of the spider *Acanthoscurria gomesiana*. *Dev. Comp. Immunol.* **32**, 716-725. doi:10.1016/j.dci.2007.11.002

**Galipaud, M., Gillingham, M. A., David, M. and Dechaume-Moncharmont, F. X.** (2014). Ecologists overestimate the importance of predictor variables in model averaging: a plea for cautious interpretations. *Methods Ecol. Evol.* **5**, 983-991. doi:10.1111/2041-210X.12251

**Garratt, M. and Brooks, R. C.** (2012). Oxidative stress and condition-dependent sexual signals: more than just seeing red. *Proc. Royal Soc. B* **279**, 3121-3130. doi:10.1098/rspb.2012.0568

**Gorr, T. A.** (2017). Hypometabolism as the ultimate defence in stress response: how the comparative approach helps understanding of medically relevant questions. *Acta Physiol.* **219**, 409-440. doi:10.1111/apha.12747

**Kuhn-Nentwig, L. and Nentwig, W.** (2013). The immune system of spiders. In *Spider Ecophysiology* (ed. W. Nentwig), pp. 81-91. Springer-Verlag, Heidelberg.

**Lemon, J.** (2006). Plotrix: a package in the red light district of R. *R-News* **6**, 8-12.

**Marusik, Y. M.** (1986). The orb-weaver genus *Larinia* Simon in the USSR. *Spixiana* **9**, 245-254.

**Monaghan, P., Metcalfe, N. B. and Torres, R.** (2009). Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol. Lett.* **12**, 75-92. doi:10.1111/j.1461-0248.2008.01258.x

**Morrow, E. H. and Arnqvist, G.** (2003). Costly traumatic insemination and a female counter-adaptation in bed bugs. *Proc. Royal Soc. B* **270**, 2377-2381. doi:10.1098/rspb.2003.2514

**Morrow, E. H., Arnqvist, G. and Pitnick, S.** (2003). Adaptation versus pleiotropy: why do males harm their mates? *Behav. Ecol.* **14**, 802-806. doi:10.1093/beheco/arg073

**Mouginot, P., Prügel, J., Thom, U., Steinhoff, P. O. M., Kupryjanowicz, J. and Uhl, G.** (2015). Securing paternity by mutilating female genitalia in spiders. *Curr. Biol.* **25**, 2980-2984. doi:10.1016/j.cub.2015.09.074

**Nakata, K.** (2016). Female genital mutilation and monandry in an orb-web spider. *Biol. Lett.* **12**, 20150912. doi:10.1098/rsbl.2015.0912

**Reinhardt, K., Anthes, N. and Lange, R.** (2015). Copulatory wounding and traumatic insemination. *Cold Spring Harb Perspect Biol.* **7**, a017582. doi:10.1101/cshperspect.a017582

**Schäfer, M. and Werner, S.** (2008). Oxidative stress in normal and impaired wound repair. *Pharmacol. Res.* **58**, 165-171. doi:10.1016/j.phrs.2008.06.004

**Schielzeth, H.** (2010). Simple means to improve the interpretability of regression coefficients. *Methods Ecol. Evo.* **1**, 103-113. doi:10.1111/j.2041-210X.2010.00012.x

**Stearns, S. C.** (1989). Trade-offs in life-history evolution. *Funct. Ecol.* **3**, 259-268. doi:10.2307/2389364

**Symonds, M. R. and Moussalli, A.** (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav. Ecol. Sociobiol.* **65**, 13-21. doi:10.1007/s00265-010-1037-6

**Wang, Y., Oberley, L. W. and Murhammer, D. W.** (2001). Evidence of oxidative stress following the viral infection of two lepidopteran insect cell lines. *Free Radic. Biol. Med.* **31**, 1448-1455. doi:10.1016/S0891-5849(01)00728-6