PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Romero-Haro AÁ, Pérez-Rodríguez L, Tschirren B. 2023 Increased male-induced harm in response to female-limited selection: interactive effects between intra- and interlocus sexual conflict? *Proc. R. Soc. B* **290**: 20230140.
https://doi.org/10.1098/rspb.2023.0140

Received: 16 June 2022
Accepted: 22 March 2023

Subject Category:
Evolution

Subject Areas:
ecology, evolution, physiology

Keywords:
mating costs, life-history trade-offs, oxidative stress, traumatic mating, maintenance of genetic variation, indirect costs of reproduction

Author for correspondence:
Ana Ángela Romero-Haro
e-mails: ana.romero@uclm.es, romeroharoa@gmail.com

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6533974.

THE ROYAL SOCIETY PUBLISHING

Increased male-induced harm in response to female-limited selection: interactive effects between intra- and interlocus sexual conflict?

Ana Ángela Romero-Haro[1,2], Lorenzo Pérez-Rodríguez[2] and Barbara Tschirren[1]

[1]Centre for Ecology and Conservation, University of Exeter, Penryn TR10 9FE, UK
[2]Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, Ronda de Toledo 12, 13005 Ciudad Real, Spain

AÁR-H, 0000-0002-7127-4733; LP-R, 0000-0002-5926-1438; BT, 0000-0003-4806-4102

Interlocus sexual conflict (IRSC) occurs because of shared interactions that have opposite effects on male and female fitness. Typically, it is assumed that loci involved in IRSC have sex-limited expression and are thus not directly affected by selective pressures acting on the other sex. However, if loci involved in IRSC have pleiotropic effects in the other sex, intersexual selection can shape the evolutionary dynamics of conflict escalation and resolution, as well as the evolution of reproductive traits linked to IRSC loci, and vice versa. Here we used an artificial selection approach in Japanese quail (*Coturnix japonica*) to test if female-limited selection on reproductive investment affects the amount of harm caused by males during mating. We found that males originating from lines selected for high female reproductive investment caused more oxidative damage in the female reproductive tract than males originating from lines selected for low female reproductive investment. This male-induced damage was specific to the oviduct and not found in other female tissues, suggesting that it was ejaculate-mediated. Our results suggest that intersexual selection shapes the evolution of IRSC and that male-induced harm may contribute to the maintenance of variation in female reproductive investment.

1. Introduction

Selection often acts in different ways on males and females, resulting in divergent sex-specific optima in morphology, behaviour, physiology and life history [1,2]. Such sex-specific optima can lead to sexual conflict when alleles at a shared locus have antagonistic effects on male and female fitness (intralocus sexual conflict, IASC), or when interests of males and females diverge during reproductive interactions (interlocus sexual conflict, IRSC) [1,3]. The latter can promote the evolution of sexually antagonistic male strategies, such as coercive behaviours or toxic ejaculates, which benefit male reproductive success to the detriment of their female partner. A classic example of such an antagonistic male strategy is seminal fluid proteins in fruit flies (*Drosophila melanogaster*), which increase female egg production and sperm storage, but come at the cost of higher female mortality [4,5]. IRSC can result in sexually antagonistic coevolution (i.e. 'sexual arms-race') of male and female adaptations and counteradaptations and thus accelerate evolutionary change, whereas IASC can constrain evolution because of competing male- and female-specific selective forces acting on jointly expressed genes [6–9] (but see [10]). Given the different modes of operation, and different evolutionary consequences, IASC and IRSC have traditionally been considered as separate forces. Indeed, it is typically assumed that loci involved in IRSC have sex-limited expression and are thus not directly affected by selective pressures acting on the other sex [3,11].

However, if loci involved in IRSC have pleiotropic effects in the other sex, IASC could influence the evolutionary dynamics of IRSC escalation and resolution, and vice versa [11]. However, to date the role of intralocus conflict in the evolution of IRSC has received little attention.

Costs of reproduction are a key tenet of life-history theory [12,13]. Traditionally, researchers have focused on *direct* costs of reproduction, such as reduced self-maintenance as a consequence of increased resource allocation to reproduction, or increased physiological damage generated by increased reproductive effort [14,15]. However, costs can also be *indirect*, for example, in the form of male-induced harm during mating. If, as outlined above, loci associated with such male-induced harm have pleiotropic effects on female reproductive function, such indirect costs of reproduction can shape the evolution of life histories.

Unlike in invertebrates where the conflict potential of ejaculate components is well documented, this is much less the case in vertebrates. Indeed, studies in humans and laboratory rodents have traditionally considered ejaculate-induced changes in female physiology as an adaptive process that promotes healthy offspring development that benefits both males and females [16–19], largely neglecting the conflict potential associated with such processes. Understanding the conflict potential of ejaculate-mediated effects, and their genetic links to other reproductive traits and functions, can thus contribute to an integrative understanding of the costs of reproduction and provide insights into the constraints that shape life-history evolution.

Here we use an artificial selection approach in a precocial bird, the Japanese quail (*Coturnix japonica*) [20], to test (i) if there is evidence for ejaculate-induced harm to female partners and (ii) if sex-limited selection on female reproductive investment shapes the severity of harm caused by males (i.e. pleiotropic effects of loci linked to female reproductive function on IRSC). We have previously shown that female-limited selection on reproductive investment has correlated effects on male reproductive success, with males from lines selected for high female reproductive investment (H-line) siring more offspring than males from lines selected for low female reproductive investment (L-line) [21]. This pattern was observed in both a competitive mating situation (i.e. including male–male competition and female mate choice) and a non-competitive mating situation (i.e. excluding male–male competition and female mate choice) [21], suggesting that the male reproductive advantage was ejaculate-mediated, rather than caused by increased dominance or attractiveness [21]. We predict that if increased female reproductive investment is genetically linked to increased male-induced harm (i.e. pleiotropic effects on IRSC [11]), levels of physiological damage in females mated to an H-line male will be higher than levels of damage in females mated to an L-line male. Furthermore, if this effect is ejaculate-mediated, we predict that increased damage will be specifically found in the female reproductive tract, but not in other tissues.

2. Methods

(a) Selection lines and breeding design

The study was conducted in a captive population of Japanese quail (*Coturnix japonica*) artificially selected for high (H-line) and low (L-line) maternal egg investment. Replicated selection lines were established as described in Pick *et al.* [20]. In brief, the top 25% of females producing the largest and smallest eggs relative to their body size, respectively, were selected for breeding in the first generation. In the subsequent generations, the 50% females producing the largest and smallest eggs were selected, respectively. In each generation, two sons and two daughters from each selected pair were used for the next breeding round. Breeding pairs were randomly allocated within line replicates and mating between relatives was avoided throughout the selection experiment. Note that unlike in experimental evolution experiments, this artificial selection approach does *not* provide potential for sexually antagonistic coevolution, and all correlated responses in males must thus be a direct consequence of female-limited selection on pleiotropic loci (see also Artificial selection approach in the electronic supplementary material).

After four generations of selective breeding, the H- and L-lines differed in egg size by more than 1 s.d., but they did not differ in the number of eggs laid [20]. We will refer to both females selected for low reproductive investment as well as males from the low female reproductive investment lines as L-line birds, and both females selected for high reproductive investment and males from the high female reproductive investment line as H-line birds.

For this experiment, both L-line and H-line females were randomly paired with either an L-line or H-line male (2 × 2 design; female / male pairs: $N = 11$ L / L, $N = 10$ L / H, $N = 10$ H / H, $N = 10$ H / L), and kept in breeding cages for two weeks (see [20] for a detailed description of the breeding and husbandry conditions). Eggs were collected every morning and weighed to the nearest 0.01 g. Three females (1 L / L, 1 H / H, 1 H / L) were not reproductively active (i.e. laid no eggs) and were thus removed from the analyses. There was no difference in the age of females between treatment groups (mean ± s.e.: L / L: 517 ± 105 days; L / H: 404 ± 58 days; H / H: 549 ± 111 days; H / L: 521 ± 107 days; all $p > 0.445$) or in the age of the males (L / L: 801 ± 60 days; L / H: 724 ± 60 days; H / H: 649 ± 45 days; H / L: 708 ± 67 days; all $p > 0.161$).

All females were blood sampled (approx. 100 µl) from the brachial vein using heparinized capillary tubes just before they were placed in the breeding cage. Blood samples were stored at 4°C until centrifugation (5 min at 20°C and 2000*g*) within 4 h. Plasma was then separated and frozen at −80°C until analysis. After breeding, all females were euthanized and tissue samples from the oviduct (isthmus), the liver and the spleen were snap frozen on dry ice and then stored at −80°C until analysis. All samples were obtained within a single session for all birds. All procedures were conducted under licenses provided by the Veterinary Office of the Canton of Zurich, Switzerland (permit numbers 195/2010; 14/2014; 156) and the ethical committee of the University of Exeter (permit eCORN002475).

(b) Quantification of lipid oxidative damage

Oxidative damage is the result of non-alleviated oxidative stress, and lipids are a main target of such damage [22,23]. To quantify lipid oxidative damage in the female reproductive tract (oviduct), we measured levels of malondialdehyde acid (MDA), one of the endpoint molecules in the lipid peroxidation cascade. MDA is a commonly used marker of oxidative damage [22,24–27]. It is an extremely toxic and mutagenic molecule with high reactivity, interacting with DNA and proteins and damaging them [22,28]. High MDA levels have been related to numerous illnesses in humans (review in [29]), as well as lower fitness in non-human animals [24–27].

MDA quantification was performed using HPLC following the protocol of [30] with modifications by [31] (described in detail in the electronic supplementary material and in [32]). In addition to MDA measures in the oviduct, we also

Table 1. Effects of male line, female line, their interaction and line replicate on oxidative damage (MDA) levels in the females' oviduct (µM).

	estimate	s.e.	*F*	d.f.	*p*
intercept	14.500	1.685			
male line (L)	−2.302	2.096	5.021	1, 31	*0.032*
female line (L)	3.449	2.044	3.178	1, 31	0.084
replicate (2)	1.805	1.441	1.569	1, 31	0.220
male line (L) × female line (L)	−1.747	2.886	0.366	1, 31	0.549

quantified MDA concentrations in the females' liver and spleen to verify that male-induced oxidative damage is specific to the reproductive tract, rather than systemic. Pre-breeding plasma MDA was quantified to test for potential differences in oxidative damage among females before the start of the experiment. One liver, one spleen and two oviduct samples were lost during handling, resulting in slightly lower sample sizes for some comparisons. All samples of a given tissue were analysed in a single session. Additional analyses of antioxidant activity in female tissues and plasma are presented in the electronic supplementary material.

(c) Statistical analyses

First, we used linear models to test for an effect of male line, female line, their interaction and line replicate on levels of oxidative damage (MDA) in the females' oviduct. To verify that these effects are specific to the reproductive tract rather than systemic, we ran the same analysis for female liver and spleen.

Second, we tested if male line origin affects female reproductive output. Quail are continuous layers and do not produce a traditional clutch. We therefore used the number of eggs a female laid over the 14-day breeding event (i.e. did/did not produce an egg on a given day) as a proxy for fecundity in a generalized linear model with a quasibinomial error structure. A quasibinomial model was used instead of a binomial model because of overdispersion. A linear model was used for mean egg mass. Male line, female line, their interaction and line replicate were included as fixed effects in the models. Significance of predictors was determined using the *car* package [33].

Normality of the residuals of linear models was confirmed by visual inspection and Shapiro–Wilk tests. All statistical analyses were performed in R version 4.1.2 [34]. Additional analyses of pre-breeding phenotypic traits, antioxidant activity in different tissues, effects of pre-breeding oxidative stress levels and body mass on post-breeding MDA levels in different tissues, effects of reproductive output on post-breeding MDA levels in different tissues, and correlations among oxidative stress markers within and across tissues are presented in the electronic supplementary material. Data and code are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.98sf7m0nn [35].

3. Results

(a) Oxidative damage in reproductive and non-reproductive tissues

Females experimentally paired with an H-line male had significantly higher levels of lipid oxidative damage (MDA) in their oviduct than females paired with a L-line male (mean ± s.e.: 17.2 ± 1.02 µM, $n = 18$ and 14.0 ± 1.01 µM, $n = 18$, respectively), independent of their own line origin (table 1, figure 1). This effect of male line was specific to the oviduct and was not observed in the females' liver or spleen (electronic supplementary material, table S1 and figure S1). The effect was robust to the removal of an extreme value from the analysis (electronic supplementary material, figure S2).

No differences in female pre-breeding body mass, pre-breeding plasma MDA or pre-breeding plasma total antioxidant status were observed with respect to male line (electronic supplementary material, table S2), and including these pre-breeding measures into the model did not change the male line effect on oxidative damage in the females' oviduct (electronic supplementary material, tables S3 and S4). No effect of male line on post-breeding measures of antioxidant activity in female reproductive or non-reproductive tissues was observed (electronic supplementary material, tables S5 and S6).

No effect of a female's own line on oxidative damage in the oviduct was observed (table 1, figure 1). However, L-line females had higher levels of MDA in the spleen than H-line females (mean ± s.e.: 88.4 ± 4.09 µM, $n = 19$, and 76.0 ± 4.20 µM, $n = 18$, respectively; electronic supplementary material, table S1 and figure S1b). There were no significant correlations among markers of oxidative status across tissues (electronic supplementary material, table S7).

(b) Reproductive output

The number of eggs laid by a female during the reproductive event was not affected by male line, female line or their interaction (table 2). Egg mass was not significantly affected by male line or the interaction between female and male line (table 2). As expected, L-line females laid significantly smaller eggs than H-line females (mean ± s.e.: 10.9 ± 0.145 g, $n = 20$, and 12.0 ± 0.153 g, $n = 18$, respectively; table 2). Neither the number of eggs laid during the reproductive event, nor mean egg mass were significantly associated with levels of oxidative damage in the females' oviduct or other tissues (electronic supplementary material, table S8).

4. Discussion

Our study provides experimental evidence for male-induced oxidative damage in the female reproductive tract in a precocial bird and shows that increased female reproductive investment is genetically linked to increased male-induced harm. Selection on increased female reproductive investment thus appears to generate a sexual conflict, by generating males that cause increased harm to females during mating.

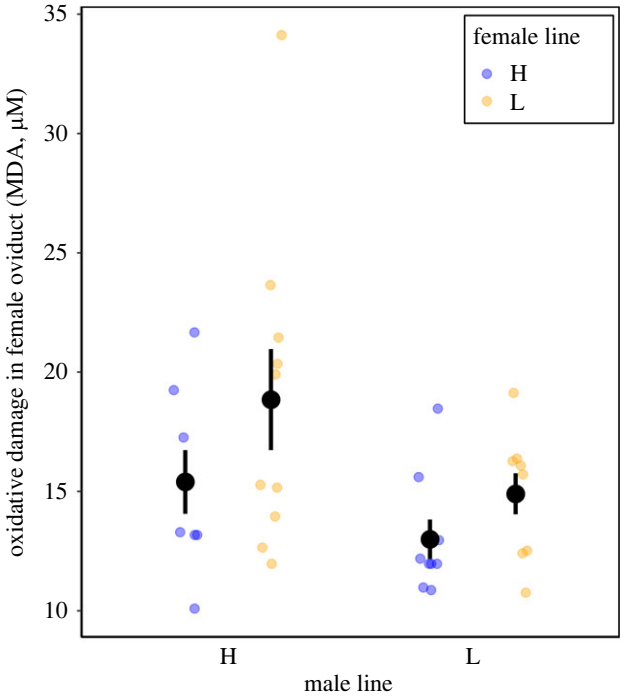


Figure 1. Effect of male line and female line on levels of oxidative damage to lipids (MDA) in the females' oviduct. Females mated to an H-line male had higher levels of damage in their oviduct than females mated to an L-line male. Means ± 1s.e. are shown. Dots represent raw data. H-line females are shown in light grey and L-line females in dark grey (online version in colour). Results did not change when removing the extreme value measured in an L-line female mated to an H-line male (electronic supplementary material, figure S1).

Such indirect costs of reproduction may constrain the evolution of female reproductive investment and contribute to the maintenance of variation in reproductive investment in populations.

We imposed selection on a female-limited trait (egg investment) in our experiment, and the study design prevented male–female coevolution. Correlated responses in males, including the level of harm caused to their partner during mating, must thus be the consequence of pleiotropic effects of genes selected in females. It suggests that IRSC and IASC may not be separate forces (as traditionally believed [3,11]), but may indeed be closely interlinked. Such interactions have important implications for conflict resolution and escalation, as well as the evolution of traits linked to sexually antagonistic male strategies.

The effect of male line origin on levels of oxidative damage in the female was restricted to the reproductive tract and not found in other tissues, suggesting that it was ejaculate-mediated. In laboratory mice, it has recently been shown that a male's diet affects seminal fluid immune-regulatory activity, leading to changes in inflammatory responses in the female reproductive tract [36]. If the amount and/or composition of the ejaculate of males from the divergent selection lines differ, either because they had more (H-line) or less (L-line) resources available during prenatal development or because ejaculate composition is genetically linked to female reproductive investment [21,37,38], then such effects could explain how selection on a female-limited trait can generate sexual conflict (see also [39]). Similarly, the foamy substance that is produced by the proctodeal gland of quail males and transferred to females during copulation has been shown to contain prostaglandins [40]. Prostaglandins induce contractions in the female reproductive tract that increase the mobility of sperm within the oviduct [40], but also stimulate inflammatory responses in the female reproductive tract (reviewed in [41]). If males from the divergent lines differ in the amount or composition of proctodeal gland foam they transfer, this could generate the observed effect [42,43]. Finally, oxidative damage generated through female reproductive tract–ejaculate interactions may underlie the observed patterns [44]. Sperm storage tubules (SST) in the female reproductive tract contain a complex antioxidant system to prevent and repair damage to spermatozoa caused by peroxidation (see [45] and references therein), and they secrete diverse metabolites, such as fatty acids, to maintain the functional integrity of stored sperm (see [46] and references therein). Recently, it has been demonstrated in chicken that these secreted lipids are highly vulnerable to peroxidation, and that females generating more fatty acids in their SST to protect stored sperm show higher levels of oxidative damage (MDA) in their oviducts [46]. Sperm of H-line males may be more susceptible to peroxidation and/or require a higher SST fatty acid production for maintenance, potentially explaining the higher levels of oxidative damage in the female's reproductive tract when mated to an H-line male. Indeed, a higher proportion of polyunsaturated fatty acids in the membrane of fowl spermatozoa has been related to an enhanced male fertility [47], but, at the same time, also to a higher susceptibility to oxidative damage (reviewed in [48,49]). Higher fertility is observed in H-line males compared to L-line males [21], making this a plausible scenario.

Irrespective of the exact mechanism underlying the observed male line effect, evidence from other studies suggests that increased levels of oxidative damage in the reproductive tract may have negative fitness consequences for the female. Indeed in humans, high levels of oxidative stress in the female reproductive tract are related to diverse disorders, such as infertility, miscarriages, preeclampsia, fetal growth restrictions and diverse embryopathies, leading to maternal and offspring morbidity and mortality [50–52]. Similarly, oxidative damage in the female reproductive tract negatively affects fecundity in laboratory rodents [53,54]. Increased MDA levels have also been associated with the ageing process [55] and the occurrence of certain diseases, such as cancer, Alzheimer's disease and diabetes (reviewed in [29]). Finally, negative associations between MDA levels and fitness have repeatedly been found in natural populations [24,26,27]. However, whereas this previous work provides evidence for a negative association between oxidative damage and fitness, we cannot directly demonstrate such fitness costs in our study because females had to be sacrificed for the quantification of physiological damage in the reproductive tract. Following a cohort of females throughout their life to quantify the long-term costs of male-induced harm for female reproductive success and lifespan would thus be a fruitful next step to directly quantify such fitness costs in our system.

Increased harm to females might directly increase male reproductive success, as for example observed in *Drosophila*, where accessory gland products increase female egg-laying rates at the expense of female survival [5]. However, we found no evidence that females mated to H-line males produced more or larger eggs (see also [56,57]), making this

Table 2. Effects of male line, female line, their interaction and line replicate on female reproductive output. Note that estimates for the number of eggs laid are presented on a logit scale.

	estimate	s.e.	*F*	d.f.	*p*
number eggs laid					
intercept	1.177	0.356			
male line (L)	0.477	0.484	0.045	1, 33	0.834
female line (L)	0.787	0.498	0.402	1, 33	0.531
replicate (2)	−0.218	0.341	0.407	1, 33	0.528
male line (L) × female line (L)	−1.113	0.692	2.642	1, 33	0.114
mean egg mass (g)					
intercept	11.944	0.238			
male line (L)	0.378	0.309	0.560	1, 33	0.460
female line (L)	−0.968	0.301	30.434	1, 33	*<0.001*
replicate (2)	−0.129	0.213	0.364	1, 33	0.550
male line (L) × female line (L)	−0.416	0.426	0.955	1, 33	0.336

an unlikely scenario. Alternatively, changes in ejaculate composition might directly or indirectly (via modifying physiological responses in the female reproductive tract, see above) benefit males in a sperm competition context, with increased harm in the female reproductive tract merely reflecting collateral damage [58,59]. Sperm competition experiments, and tests of sperm function when sperm is exposed to ejaculates from H- versus L-line competitors, would be interesting next steps to directly test these hypotheses.

Whereas we observed a strong male line effect, no effect of a female's own line origin on levels of oxidative damage in the reproductive tract was observed. Furthermore, oxidative damage in the female reproductive tract was not associated with the size or number of eggs a female laid. Interestingly, L-line females had significantly higher levels of oxidative damage in the spleen, an important immune organ [60], than H-line females. We have previously shown that the immune system of L-line females is upregulated compared to H-line females [61], and higher levels of oxidative damage might be a direct consequence of this upregulation [42,43]. Together, these findings suggest that increased oxidative damage is an indirect (i.e. male-induced) rather than direct cost of reproductive investment in our system.

Within a female, levels of oxidative damage and antioxidant activity were not, or only weakly correlated within and across tissues. This highlights that patterns of oxidative stress can be highly tissue specific, leading to within-body mosaics of damage accumulation. This not only has implications for our understanding of life-history evolution, but also questions the validity of using blood samples to quantify the overall oxidative status of an individual (e.g. [62]). Indeed, it is often assumed that oxidative stress in plasma accurately reflects oxidative stress in other tissues [63,64]. This would require oxidative stress levels in different parts of the body to be correlated (i.e. systemic oxidative stress). However, as we show here, this is not necessarily the case.

In conclusion, our study provides experimental evidence for a genetic link between female reproductive investment and male-induced damage in the female reproductive tract, suggesting that intralocus conflict may shape the evolutionary dynamics of IRSC, and that IRSC may constrain the evolution of reproductive investment. It suggests that the distinction between IASC and IRSC may not be as clear as traditionally thought [3,11], and that these two processes can indeed be interlinked.

Ethics. All procedures were conducted under licenses provided by the Veterinary Office of the Canton of Zurich, Switzerland (permit numbers 195/2010; 14/2014; 156) and the ethical committee of the University of Exeter (permit eCORN002475).

Data accessibility. Data and code are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.98sf7m0nn [35].

Additional data are provided in the electronic supplementary material [65].

Authors' contributions. A.Á.R.-H.: data curation, formal analysis, methodology, resources, validation, visualization, writing—original draft and writing—review and editing; L.P.-R.: funding acquisition, methodology, resources, software, validation and writing—review and editing; B.T.: conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. The study was financially supported by the Swiss National Science Foundation (grant nos. PP00P3_128386 and PP00P3_157455 to B.T.). Laboratory analyses were funded by the research project PGC2018-099596-B-I00, funded by MCIN/AEI/10.13039/501100011033 and ERDF 'A way of making Europe', awarded to L.P.-R. A.Á.R.-H. was supported by the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement 842085 and by a postdoctoral contract María Zambrano (University of Castilla-La Mancha) from the Program of Requalification of the Spanish University System (Spanish Ministry of Universities) financed by the European Union—NextGenerationEU.

Acknowledgements. We thank all the students and animal caretakers who contributed to quail breeding, data collection and husbandry, and Tanya Pennell and two anonymous reviewers for valuable comments on the manuscript.

References

1. Chapman T, Arnqvist G, Bangham J, Rowe L. 2003 Sexual conflict. *Trends Ecol. Evol.* **18**, 41–47. (doi:10.1016/s0169-5347(02)00004-6)
2. Parker GA. 1979 Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (eds MS Blum, NA Blum), pp. 123–166. New York, NY: Academic Press.
3. Schenkel MA, Pen I, Beukeboom LW, Billeter JC. 2018 Making sense of intralocus and interlocus sexual conflict. *Ecol. Evol.* **8**, 13 035–13 050. (doi:10.1002/ece3.4629)
4. Wigby S, Chapman T. 2005 Sex peptide causes mating costs in female *Drosophila melanogaster*. *Curr. Biol.* **15**, 316–321. (doi:10.1016/j.cub.2005.01.051)
5. Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L. 1995 Cost of mating in drosophila-melanogaster females is mediated by male accessory-gland products. *Nature* **373**, 241–244. (doi:10.1038/373241a0)
6. Arnqvist G, Rowe L. 2002 Antagonistic coevolution between the sexes in a group of insects. *Nature* **415**, 787–789. (doi:10.1038/415787a)
7. Rice WR. 1996 Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* **381**, 232–234. (doi:10.1038/381232a0)
8. Chippindale AK, Gibson JR, Rice WR. 2001 Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 1671–1675. (doi:10.1073/pnas.041378098)
9. Perry JC, Rowe L. 2015 The evolution of sexually antagonistic phenotypes. *Cold Spring Harb. Perspect. Biol.* **7**, a017558. (doi:10.1101/cshperspect.a017558)
10. Wright AE, Rogers TF, Fumagalli M, Cooney CR, Mank JE. 2019 Phenotypic sexual dimorphism is associated with genomic signatures of resolved sexual conflict. *Mol. Ecol.* **28**, 2860–2871. (doi:10.1111/mec.15115)
11. Pennell TM, de Haas FJH, Morrow EH, van Doorn GS. 2016 Contrasting effects of intralocus sexual conflict on sexually antagonistic coevolution. *Proc. Natl Acad. Sci. USA* **113**, E978–E986. (doi:10.1073/pnas.1514328113)
12. Stearns S. 1992 *The evolution of life histories*. Oxford, UK: Oxford University Press.
13. Kirkwood TBL, Rose MR. 1991 Evolution of senescence—late survival sacrificed for reproduction. *Phil. Trans. R. Soc. B* **332**, 15–24. (doi:10.1098/rstb.1991.0028)
14. Harshman LG, Zera AJ. 2007 The cost of reproduction: the devil in the details. *Trends Ecol. Evol.* **22**, 80–86. (doi:10.1016/j.tree.2006.10.008)
15. Zera AJ, Harshman LG. 2001 The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**, 95–126. (doi:10.1146/annurev.ecolsys.32.081501.114006)
16. Morgan HL, Watkins AJ. 2020 The influence of seminal plasma on offspring development and health. *Semin. Cell Dev. Biol.* **97**, 131–137. (doi:10.1016/j.semcdb.2019.06.008)
17. Sharkey DJ, Tremellen KP, Jasper MJ, Gemzell-Danielsson K, Robertson SA. 2012 Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus. *J. Immunol.* **188**, 2445–2454. (doi:10.4049/jimmunol.1102736)
18. Schjenken JE, Robertson SA. 2020 The female response to seminal fluid. *Physiol. Rev.* **100**, 1077–1117. (doi:10.1152/physrev.00013.2018)
19. Schjenken JE, Sharkey DJ, Green ES, Chan HY, Matias RA, Moldenhauer LM, Robertson SA. 2021 Sperm modulate uterine immune parameters relevant to embryo implantation and reproductive success in mice. *Commun. Biol.* **4**, 572. (doi:10.1038/s42003-021-02038-9)
20. Pick JL, Hutter P, Tschirren B. 2016 In search of genetic constraints limiting the evolution of egg size: direct and correlated responses to artificial selection on a prenatal maternal effector. *Heredity* **116**, 542–549. (doi:10.1038/hdy.2016.16)
21. Pick JL, Hutter P, Tschirren B. 2017 Divergent artificial selection for female reproductive investment has a sexually concordant effect on male reproductive success. *Evol. Lett.* **1**, 222–228. (doi:10.1002/evl3.21)
22. Del Rio D, Stewart AJ, Pellegrini N. 2005 A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrit. Metab. Cardiovasc. Dis.* **15**, 316–328. (doi:10.1016/j.numecd.2005.05.003)
23. Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA. 2007 Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol. Rev.* **87**, 1175–1213. (doi:10.1152/physrev.00047.2006)
24. Noguera JC, Kim SY, Velando A. 2012 Pre-fledgling oxidative damage predicts recruitment in a long-lived bird. *Biol. Lett.* **8**, 61–63. (doi:10.1098/rsbl.2011.0756)
25. Romero-Haro AA, Perez-Rodriguez L, Tschirren B. 2022 Intergenerational costs of oxidative stress: reduced fitness in daughters of mothers that experienced high levels of oxidative damage during reproduction. *Physiol. Biochem. Zool.* **95**, 1–14. (doi:10.1086/717614)
26. Vagasi CI, Vincze O, Patras L, Osvath G, Penzes J, Haussmann MF, Barta Z, Pap PL. 2019 Longevity and life history coevolve with oxidative stress in birds. *Funct. Ecol.* **33**, 152–161. (doi:10.1111/1365-2435.13228)
27. Vitikainen EIK *et al.* 2016 Evidence of oxidative shielding of offspring in a wild mammal. *Front. Ecol. Evol.* **4**, 58. (doi:10.3389/fevo.2016.00058)
28. Nair U, Bartsch H, Nair J. 2007 Lipid peroxidation-induced DNA damage in cancer-prone inflammatory diseases: a review of published adduct types and levels in humans. *Free Radical Biol. Med.* **43**, 1109–1120. (doi:10.1016/j.freeradbiomed.2007.07.012)
29. Ayala A, Munoz MF, Arguelles S. 2014 Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Long.* **2014**, 360438. (doi:10.1155/2014/360438)
30. Agarwal R, Chase SD. 2002 Rapid, fluorimetric-liquid chromatographic determination of malondialdehyde in biological samples. *J. Chromatogr. B* **775**, 121–126. (doi:10.1016/s1570-0232(02)00273-8)
31. Nussey DH, Pemberton JM, Pilkington JG, Blount JD. 2009 Life history correlates of oxidative damage in a free-living mammal population. *Funct. Ecol.* **23**, 809–817. (doi:10.1111/j.1365-2435.2009.01555.x)
32. Romero-Haro AA, Alonso-Alvarez C. 2014 Covariation in oxidative stress markers in the blood of nestling and adult birds. *Physiol. Biochem. Zool.* **87**, 353–362. (doi:10.1086/674432)
33. Fox J, Weisberg S. 2019 *An R companion to applied regression*, 3rd edn. Beverley Hills, CA: Sage.
34. R Core Team. 2021 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
35. Romero-Haro AA, Perez-Rodriguez L, Tschirren B. 2023 Data from: Increased male-induced harm in response to female-limited selection: interactive effects between intra- and interlocus sexual conflict? *Dryad Digital Repository*. (doi:10.5061/dryad.98sf7m0nn)
36. Schjenken JE, Moldenhauer LM, Sharkey DJ, Chan HY, Chin PY, Fullston T, McPherson NO, Robertson SA. 2021 High-fat diet alters male seminal plasma composition to impair female immune adaptation for pregnancy in mice. *Endocrinology* **162**, bqab123. (doi:10.1210/endocr/bqab123)
37. Charge R, Teplitsky C, Hingrat Y, Saint Jalme M, Lacroix F, Sorci G. 2013 Quantitative genetics of sexual display, ejaculate quality and size in a lekking species. *J. Anim. Ecol.* **82**, 399–407. (doi:10.1111/1365-2656.12023)
38. Fischer K, Zimmer K, Wedell N. 2009 Correlated responses to selection on female egg size in male reproductive traits in a butterfly. *Evol. Ecol.* **23**, 389–402. (doi:10.1007/s10682-007-9233-1)
39. Flintham EO, Savolainen V, Mullon C. 2023 Male harm offsets the demographic benefits of good genes. *Proc. Natl Acad. Sci. USA* **120**, e2211668120. (doi:10.1073/pnas.2211668120)
40. Sasanami T *et al.* 2015 A unique mechanism of successful fertilization in a domestic bird. *Sci. Rep.* **5**, 7700. (doi:10.1038/srep07700)
41. Adefuye AO, Adeola HA, Sales KJ, Katz AA. 2016 Seminal fluid-mediated inflammation in physiology and pathology of the female reproductive tract. *J. Immunol. Res.* **2016**, 9707252. (doi:10.1155/2016/9707252)
42. Costantini D, Moller AP. 2009 Does immune response cause oxidative stress in birds? A meta-analysis. *Comp. Biochem. Physiol. A* **153**, 339–344. (doi:10.1016/j.cbpa.2009.03.010)

43. Costantini D. 2022 A meta-analysis of impacts of immune response and infection on oxidative status in vertebrates. *Conserv. Physiol.* **10**, coac018. (doi:10.1093/conphys/coac018)
44. Friesen CR, Noble DWA, Olsson M. 2020 The role of oxidative stress in postcopulatory selection. *Phil. Trans. R. Soc. B* **375**, 20200065. (doi:10.1098/rstb.2020.0065)
45. Breque C, Surai P, Brillard JP. 2003 Roles of antioxidants on prolonged storage of avian spermatozoa *in vivo* and *in vitro*. *Mol. Reprod. Dev.* **66**, 314–323. (doi:10.1002/mrd.10347)
46. Wen CL, Mai CN, Wang B, Li JY, Sun CJ, Yang N. 2020 Detrimental effects of excessive fatty acid secretion on female sperm storage in chickens. *J. Anim. Sci. Biotechnol.* **11**, 26. (doi:10.1186/s40104-020-0432-8)
47. Blesbois E, Lessire M, Grasseau I, Hallouis JM, Hermier D. 1997 Effect of dietary fat on the fatty acid composition and fertilizing ability of fowl semen. *Biol. Reprod.* **56**, 1216–1220. (doi:10.1095/biolreprod56.5.1216)
48. Collodel G, Castellini C, Lee JCY, Signorini C. 2020 Relevance of fatty acids to sperm maturation and quality. *Oxid. Med. Cell. Long.* **2020**, 7038124. (doi:10.1155/2020/7038124)
49. Surai PF, Fujihara N, Speake BK, Brillard JP, Wishart GJ, Sparks NHC. 2001 Polyunsaturated fatty acids, lipid peroxidation and antioxidant protection in avian semen. *Asian-Austral. J. Anim. Sci.* **14**, 1024–1050. (doi:10.5713/ajas.2001.1024)
50. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. 2012 The effects of oxidative stress on female reproduction: a review. *Reprod. Biol. Endocrinol.* **10**, 49. (doi:10.1186/1477-7827-10-49)
51. Agarwal A, Gupta S, Sharma RK. 2005 Role of oxidative stress in female reproduction. *Reprod. Biol. Endocrinol.* **3**, 28. (doi:10.1186/1477-7827-3-28)
52. Jauniaux E, Poston L, Burton GJ. 2006 Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. *Hum. Reprod. Update* **12**, 747–755. (doi:10.1093/humupd/dml016)
53. Aiken CE, Tarry-Adkins JL, Ozanne SE. 2013 Suboptimal nutrition in utero causes DNA damage and accelerated aging of the female reproductive tract. *FASEB J.* **27**, 3959–3965. (doi:10.1096/fj.13-234484)
54. Aiken CE, Tarry-Adkins JL, Penfold NC, Dearden L, Ozanne SE. 2016 Decreased ovarian reserve, dysregulation of mitochondrial biogenesis, and increased lipid peroxidation in female mouse offspring exposed to an obesogenic maternal diet. *FASEB J.* **30**, 1548–1556. (doi:10.1096/fj.15-280800)
55. Martinez de Toda I, Vida C, Garrido A, De la Fuente M. 2020 Redox parameters as markers of the rate of aging and predictors of life span. *J. Gerontol. Ser. A* **75**, 613–620. (doi:10.1093/gerona/glz033)
56. Pick JL, Ebneter C, Hutter P, Tschirren B. 2016 Disentangling genetic and prenatal maternal effects on offspring size and survival. *Am. Nat.* **188**, 628–639. (doi:10.1086/688918)
57. Pick JL, Postma E, Tschirren B. 2019 The more you get, the more you give: positive cascading effects shape the evolutionary potential of prenatal maternal investment. *Evol. Lett.* **3**, 412–423. (doi:10.1002/evl3.125)
58. Edward DA, Stockley P, Hosken DJ. 2015 Sexual conflict and sperm competition. *Cold Spring Harb. Perspect. Biol.* **7**, a017707. (doi:10.1101/cshperspect.a017707)
59. Hotzy C, Arnqvist G. 2009 Sperm competition favors harmful males in seed beetles. *Curr. Biol.* **19**, 404–407. (doi:10.1016/j.cub.2009.01.045)
60. John JL. 1994 The avian spleen: a neglected organ. *Q. Rev. Biol.* **69**, 327–351. (doi:10.1086/418649)
61. Pick JL *et al.* 2020 Artificial selection reveals the role of transcriptional constraints in the maintenance of life history variation. *Evol. Lett.* **4**, 200–211. (doi:10.1002/evl3.166)
62. Stier A, Reichert S, Criscuolo F, Bize P. 2015 Red blood cells open promising avenues for longitudinal studies of ageing in laboratory, non-model and wild animals. *Exp. Gerontol.* **71**, 118–134. (doi:10.1016/j.exger.2015.09.001)
63. Margaritelis NV, Veskoukis AS, Paschalis V, Vrabas IS, Dipla K, Zafeiridis A, Kyparos A, Nikolaidis MG. 2015 Blood reflects tissue oxidative stress: a systematic review. *Biomarkers* **20**, 97–108. (doi:10.3109/1354750x.2014.1002807)
64. Veskoukis AS, Nikolaidis MG, Kyparos A, Kouretas D. 2009 Blood reflects tissue oxidative stress depending on biomarker and tissue studied. *Free Radical Biol. Med.* **47**, 1371–1374. (doi:10.1016/j.freeradbiomed.2009.07.014)
65. Romero-Haro AÁ, Pérez-Rodríguez L, Tschirren B. 2023 Increased male-induced harm in response to female-limited selection: interactive effects between intra- and interlocus sexual conflict? Figshare. (doi:10.6084/m9.figshare.c.6533974)

SUPPLEMENTARY MATERIALS FOR

Increased male-induced harm in response to female-limited selection: interactive effects between intra- and interlocus sexual conflict?

Proceedings of the Royal Society B: Biological Sciences

DOI: 10.1098/rspb.2023.0140

Ana Ángela Romero-Haro[1, 2*], Lorenzo Pérez-Rodríguez[2] & Barbara Tschirren[1]

[1] Centre for Ecology and Conservation, University of Exeter, Penryn TR10 9FE, UK

[2] Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM. Ronda de Toledo 12, 13005 Ciudad Real, Spain

*corresponding author: ana.romero@uclm.es and romeroharoa@gmail.com

1. Artificial selection approach

Readers might wonder if we invertedly selected for male exploitation rather than female reproductive investment in our selection experiment. Whereas it is theoretically possible that such indirect selection could occur, in particular in experimental evolution experiments, we can exclude this possibility in our study because:

a) We used relative egg investment (i.e. egg mass corrected for female body mass and size) – a female-limited trait – as the selection criterion. We demonstrate a response to selection, in line with evolutionary theory [1].
b) Because this was an artificial selection experiment, and not an experimental evolution experiment, there was no potential for male-female coevolution in our system.

c) We randomly assigned males to females, so it is not possible that we invertedly selected on a male trait in our experiment or that there was potential for male-driven differences in access to mating partners. Any correlated responses in males (including increased harm caused to the partner) must thus be due to pleiotropic effects of loci linked to the trait under direct selection (i.e. relative egg investment).

d) We do not find evidence that male line-origin influences a female's egg investment (this study and [2, 3]). This would be required, however, if male exploitation ability would have been selected.

e) Finally, females of the H-line also lay larger eggs without any contact to males, further demonstrating that the differences in maternal egg investment are not male-induced.

2. Selection of tissues

We quantified physiological damage in the female oviduct because this is where ejaculate-induced harm is predicted to occur. We specifically sampled the isthmus, a specific section of the oviduct, because it is clearly defined, making it possible to sample the same oviduct section across females. To show that male-induced harm is specific to the oviduct rather than systemic, we quantified physiological damage in two additional female tissues not in direct contact with the ejaculate: liver and spleen. To control for variation in pre-breeding oxidative status among females, plasma samples were taken before breeding.

3. Quantification of lipid oxidative damage

Malondialdehyde acid (MDA) quantification was performed using HPLC following the protocol of [4] with modifications by [5]. Tissue samples were diluted (1 : 5 w/v for oviduct and 1 : 10 w/v for spleen and liver samples) and homogenized in buffer (0.01 M phosphate-buffered saline and 0.02 M ethylenediaminetetraacetic acid), working on ice to avoid oxidation. A standard curve for calibration was prepared using a 1,1,3,3-tetraethoxypropane solution (5 µM in 40% ethanol), serially diluted using

40% ethanol. Fifty µL of a butylated hydroxytoluene (BHT) solution (0.05% w/v in 95% ethanol), 400 µL of a phosphoric acid solution (0.44 M), and 100 µL of a thiobarbituric acid (TBA) solution (42 mM) were added to the samples (plasma: 20 µL of plasma and 30µL of Mili-Q water, tissues: 50 µL of homogenized samples) and to 50 µL of standards. Samples and standards were then vortexed and heated at 100ºC for 1 h to allow for the formation of MDA-TBA adducts. The reaction was stopped by placing samples and standards on ice. 250 µL of *n*-butanol was then added to extract the MDA-TBA complex. Tubes were subsequently vortexed during one minute and centrifuged at 18 000 *g* for 3 min at 4ºC. One hundred µL of the upper (*n*-butanol) phase were then moved to HPLC vials, which were immediately saturated with N_2 to avoid oxidation (see also [6]). Samples were injected into an Agilent 1100 series HPLC system (Agilent, Waldbronn, Germany) fitted with a fluorescence detector set and a 5-µm ODS-2 C-18 4.0 x 250-mm column maintained at 37ºC. The mobile phase was MeOH : KH_2PO_4 (50 mM; 40 : 60 v/v), running isocratically for 10 min at a flow rate of 1 mL/min. Chromatograms were collected at 515 nm (excitation) and 553 nm (emission). All samples of the same tissue were analysed in the same lab session. Samples measured in duplicate revealed high repeatability of MDA quantification (oviduct: $R = 0.93$, $N = 11$, $P < 0.001$, liver: $R = 0.84$, $N = 7$, $P = 0.003$, spleen: $R = 0.90$, $N = 10$, $P < 0.001$, plasma: $R = 0.81$, $N = 7$, $P = 0.005$).

4. Quantification of antioxidant activity

To provide additional insights into the oxidative status of individuals, we complemented analyses of oxidative damage presented in the main text with analyses of antioxidant activity in different female tissues. Whereas levels of oxidative damage are a direct proxy for oxidative stress, because the former is a consequence of the latter [7-9], it is more difficult to infer levels of oxidative stress from antioxidant activity levels, as oxidative stress may be prevented because of high antioxidant activity or antioxidant activity may be upregulated in response to oxidative stress [7].

4.1. Quantification of Total Antioxidant Status (TAS) in pre-breeding plasma samples

This technique estimates the overall availability of non-enzymatic antioxidants in plasma [10]. TAS levels have been found to be positively related with fitness traits [11, 12], and negatively associated with MDA levels [12, 13], supporting the idea that TAS acts as a protector against lipid peroxidation. In barn swallow (*Hirundo rustica*), high levels of this marker positively predict long-term survival [11]. In pied flycatchers nestlings (*Ficedula hypoleuca*), there was a negative correlation between plasma TAS and MDA [13]. Finally, a interspecific comparative study of 88 free-living bird species showed that species with longer lifespan have higher levels of non-enzymatic antioxidant capacity and suffer less oxidative damage (MDA) than species with shorter lifespan, and that species with faster pace-of-life (i.e. prioritizing reproduction at the expense of self-maintenance) show lower non-enzymatic antioxidant capacity and higher levels of lipid oxidative damage [12].

To measure plasma TAS, we used the protocol of [10], with modifications by [14]. Briefly, a standard was made by dissolving a water-soluble α-tocopherol derivative, Trolox (Aldrich), in PBS buffer to 1 mM. Metmyoglobin was generated by mixing identical volumes of 400 µM myoglobin (from equine skeletal muscle; Sigma) and 740 µM potassium ferricyanide, then passing the mixture through a 0.45 µm nylon syringe filter (514-1265, VWR). The chromogen, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Sigma), was mixed in buffer to 153 µM. The assay was run in 96-well microplates on a Thermo Fisher Scientific reader (Multiskan GO, Thermo Fisher Scientific Oy, Vantaa, Finland), temperature maintained at 37 °C, and readings performed at 734 nm. Five µL of standard (Trolox), blank (PBS only) and samples were put separately into the wells. Next, 15 µl metmyoglobin and 250 µl ABTS were sequentially added to each well. A multi-channel pipette was used to simultaneously add 50 µl of 150 µM H_2O_2 to all the wells, starting the reaction. All the samples were measured twice showing high repeatability (R = 0.98, N = 38, P < 0.0001), and the mean was used in the statistical analyses.

Some plasma antioxidant capacity is explained by plasma uric acid levels [6, 15] (i.e. the main form of nitrogen excretion in birds). Uric acid is a potent antioxidant but whether uric acid plasma levels are

actively regulated in response to oxidative stress is unknown [6, 16]. For a clearer interpretation of TAS levels, uric acid levels were also measured in 5 µL of plasma using the uricase/peroxidase method (Kit from Biosystems, 11521) and a Thermo Fisher Scientific reader (Multiskan GO, Thermo Fisher Scientific Oy, Vantaa, Finland) fixed at 520 nm. Repeatability in 27 samples measured twice were high ($R = 0.98$, $P < 0.0001$).

Although this method was developed to measure total antioxidant status in body fluid, such as plasma and urine [10], we tried to also measure it in tissue samples, using the supernatant of tissue homogenates (see below SOD protocol) and different concentrations (from 4mM to 75µM) of H_2O_2. However, we were not able to obtain a clear delay in the start of the absorbance increase or a clear increase of absorbance, as the plasma samples did (see figure 1 in [14] for a common kinetic of a serum sample). Therefore, TAS could not be measured in tissue samples.

4.2. Quantification of superoxide dismutase in post-breeding tissue samples

Superoxide dismutase (SOD) is a frontline enzymatic antioxidant that catalyses the dismutation of superoxide radicals to oxygen and hydrogen peroxide [17, 18]. At cellular and tissue levels, an increase of SOD activity protects against the experimental generation of oxidative stress and damage [19-21]. The occurrence of certain illnesses in humans, such as atherosclerosis, Alzheimer's disease and cardiopathies, have been found to be related with the inability to increase SOD activity levels in response to oxidative stress and damage [22, 23]. Similarly, in laboratory mice, SOD-knockout individuals show several pathologies, such as anemia, neurodegeneration and hepatocarcinogenesis, and they do not survive more than a few weeks because high levels of oxidative damage in the heart, liver, brain and vertebral bone marrow are not alleviated by this antioxidant [24, 25]. Association between this antioxidant and fitness traits have also been documented in the ecological literature. In Soay sheep (*Ovis aries*), for example, lambs with higher levels of plasma SOD activity were more likely to survive their first winter [26], and in brown trout (*Salmo trutta*) high SOD activity protected individual from developing severe kidney disease [27].

Superoxide dismutase activity levels were determined spectrophotometrically (A25- Autoanalyzer, BioSystems) using the Ransod kit (SD 125, Randox Laboratories), based on the methods described by [28] and modifications of [29]. An aliquot of the homogenized tissue samples obtained for MDA analyses were centrifuged during 15 min at 10.000 *g* and 4ºC. The supernatant was diluted in Ransod diluent (SD124, Randox Laboratories), obtaining a final dilution of 1 : 100 for oviduct, 1 : 300 for liver and 1 : 200 for spleen samples. The subsequent steps were performed in an automated spectrophotometer (A25 autoanalyzer; Biosystems, Barcelona). A total of 170 µL of mixed substrate (containing 0.05 mmol/L xanthine and 0.025 mmol/L 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride) was mixed with 5 µL of diluted sample in a reaction cuvette at 37ºC, and 15 seconds later, 25 µL of 80 U/L of xanthine oxidase was added. The mixed substrate was prepared in 40 mmol/L of N-cyclohexyl-3-aminopropanesulfonic acid buffer, pH 10.2, with 0.94 mmol/L ethylenediamine tetraacetic acid. The absorbance was read at 505 nm between 45 and 225 seconds after sample addition and the kinetics of the enzyme activity was calculated based on a calibration curve performed with SOD standards at concentrations ranging from 0.186 to 4.48 U/mL in 0.01 mol/L phosphate buffer, pH 7. A reference control of bovine blood (307 ± 55 IU/mL) was analyzed after a 1:200 dilution and the obtained concentration was 349 IU/mL. All the samples were measured in duplicate, showing a high repeatability (oviduct: $R = 0.87$, $N = 36$, $P < 0.001$; liver: $R = 0.93$, $N = 37$, $P < 0.001$; spleen: $R = 0.88$, $N = 37$, $P < 0.001$). Average values of the two measures were used in the statistical analyses.

During homogenisation, all parts of the tissues were mixed, including cellular membranes as well as cytoplasmic content. Protein levels of a sample have been used as a proxy of the amount of cytoplasmic content released during homogenisation (e.g. [29]). Since this antioxidant is mostly active in the cytoplasm, some researchers have accounted for the protein levels of the samples when analysing SOD activity (e.g. [29, 30]). To control for variation in the amount of cytoplasmic content between samples, we measured the protein levels following the Bradford method [31] using a

commercial kit (B6916, Sigma) and following the manufacturer's instructions. Since mostly of the activity of this antioxidant is intracellular [17], SOD was not measured in plasma samples.

4.3. Quantification of Glutathione (GSH) in post-breeding tissue samples

Glutathione is a tripeptide thiol, which is highly conserved across taxa, and is often considered the most abundant and important intracellular antioxidant [32-34]. An experimental decrease of glutathione levels has been shown to constrain the expression of different fitness-related traits: decreased song rate (European starlings, *Sturnus vulgaris*, [35]), delayed laying date and reduced clutch size (Canaries, *Serinus canaria*, [36]), and reduced chick-rearing capacity (Zebra finches, *Taeniopygia guttata*, [37]), compared to control individuals. In all these studies, the specific decrease of glutathione also generated an increase of the levels of oxidative damage, including MDA [35, 36, 38, 39].

Glutathione was quantified following the protocol of [40], with modifications by [6], using homogenized tissue samples obtained for MDA analyses. Three working solutions were created in the same stock buffer as follows: 0.3 mM nicotinamide adenine dinucleotide phosphate (NADPH; solution 1), 6 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB; solution 2), and 50 U of GSH reductase/mL (solution 3). A mix between equal amounts of the homogenate of tissue samples and diluted trichloroacetic acid (10% in H2O) was vortexed for 5 s at the time of being mixed, and at 5 min and 10 min post-mixing. During the process, samples were protected from light and refrigerated to prevent oxidation. The mixture was then centrifuged (10.000 *g* for 10 min at 4ºC), and the supernatant was removed. Subsequent steps were performed in an automated spectrophotometer (A25 autoanalyzer; Biosystems, Barcelona). Solutions 1 and 2 were mixed at a ratio of 7 : 1 v/v, respectively, and 160 mL of this new mixture was automatically added to 40 mL of sample (i.e. supernatant) in a reaction cuvette. Then, 20 mL of solution 3 was added after 15 s, and absorbance at 405 nm was monitored after 30 and 60 s. The change in absorbance was used to determine GSH levels by comparing the output with the results from a standard curve generated by serial dilution of GSH from 0.5 to 0.016

mM. Results are given in millimoles per gram of pellet. Some samples were measured in duplicate, showing a high repeatability (liver: $R = 0.84$, $N = 25$, $P < 0.001$; spleen: $R = 0.97$, $N = 37$, $P < 0.001$). Preliminary analyses of GSH in oviduct samples revealed very low and non-repeatable levels, and GSH was thus not measured in oviduct samples of experimental females. Since most of the activity of glutathione is intracellular [34], it was not measured in plasma samples.

5. Additional statistical analyses

We used linear models to test for an effect of male line, female line, their interaction, and line replicate on pre-breeding female body mass, plasma MDA, plasma TAS, and on post-breeding SOD activity in the females' oviduct, liver, and spleen, and post-breeding GSH levels in the females' liver and spleen. Protein levels were included as an additional factor in alternative post-breeding SOD and GSH models to account for variation in the released intracellular content between samples during homogenization. To account for the possibility that variation in post-breeding oxidative status in tissues is influenced by variation in pre-breeding oxidative status, we included pre-breeding plasma MDA and uric acid-controlled TAS (UA-TAS) as additional covariates in the post-breeding MDA, SOD and GSH models. Uric acid-controlled TAS was defined as the residuals of a linear model with TAS levels as response variable and uric-acid level as predictor. Similarly, to account for potential effects of pre-breeding body mass on post-breeding oxidative status, we included body mass as an additional covariate in the post-breeding MDA, SOD and GSH models.

To test if higher reproductive investment is associated with higher oxidative damage in different tissues, we ran linear models with liver, spleen or oviduct MDA levels as dependent variables and the number of eggs produced and mean egg mass as explanatory variables.

Pairwise Pearson´s correlation coefficients were calculated to test for correlations among oxidative stress markers within and across tissues. Normality of the residuals of linear models was confirmed by visual inspection and Shapiro-Wilk tests. All statistical analyses were performed in *R* version 4.1.2 [41].

6. Supplementary results

Supplementary table S1: **Levels of post-breeding MDA in other female tissues**

Effects of male line, female line, their interaction, and line replicate on oxidative damage (MDA) levels in the females' liver and spleen after breeding (μM). No effect of male line on oxidative damage in female liver or spleen was observed.

	Estimate	**Std. Error**	**F**	**DF**	**P**
Tissue					
Liver					
Intercept	46.086	4.310			
Male line (L)	-4.959	5.373	0.093	1, 32	0.762
Female line (L)	-3.560	5.241	0.001	1, 32	0.978
Replicate (2)	0.420	3.639	0.013	1, 32	0.909
Male line (L) * Female line (L)	7.107	7.300	0.948	1, 32	0.338
Spleen					
Intercept	80.696	6.523			
Male line (L)	-2.688	8.443	0.201	1, 32	0.657
Female line (L)	12.941	8.235	4.823	1, 32	**0.035**
Replicate (2)	-7.526	5.915	1.619	1, 32	0.212
Male line (L) * Female line (L)	0.090	11.794	<0.001	1, 32	0.994

Supplementary table S2: Phenotypic measures of females before breeding

As expected, L-line females were lighter than H-line females. However, females paired to a H- or L-line male did not differ in body mass. Furthermore, there was no significant difference in plasma MDA or TAS levels before breeding among females with respect to the line of the partner. L-line females had higher levels of plasma TAS than H-line females. However, the effect of female line on spleen MDA (see table S1) was independent of a female's pre-breeding oxidative status (see table S3).

	Estimate	**Std. Error**	**F**	**DF**	**P**
Dependent variable					
Pre-breeding body mass					
Intercept	281.207	8.214			
Male line (L)	18.889	10.656	1.488	1, 33	0.231
Female line (L)	-33.989	10.394	34.867	1, 33	**<0.001**
Replicate (2)	5.884	7.356	0.640	1, 33	0.430
Male line (L) * Female line (L)	-18.889	14.689	1.654	1, 33	0.207
Pre-breeding plasma MDA					
Intercept	12.889	1.900			
Male line (L)	1.633	2.465	0.846	1, 33	0.365
Female line (L)	3.054	2.405	<0.001	1, 33	0.991
Replicate (2)	2.258	1.702	1.761	1, 33	0.194
Male line (L) * Female line (L)	-6.068	3.398	3.188	1, 33	0.083
Pre-breeding plasma TAS					
Intercept	0.012	0.064			
Male line (L)	0.035	0.058	0.089	1, 32	0.767
Female line (L)	0.123	0.057	6.207	1, 32	**0.018**
Replicate (2)	0.005	0.040	0.017	1, 32	0.896
Plasma uric acid	0.063	0.003	524.333	1, 32	**<0.001**
Male line (L) * Female line (L)	-0.043	0.081	0.290	1, 32	0.594

Supplementary table S3: Effect of pre-breeding oxidative status on post-breeding MDA levels in different female tissues

Effects of partner line, female line, the interaction between male and female line, and line replicate on levels of oxidative damage (MDA) in the females' oviduct, liver and spleen after breeding (µM). The effects of male and female line are very similar when including pre-breeding plasma MDA and pre-breeding plasma uric acid-controlled TAS levels (UA-TAS) as covariates in the models to account for variation in oxidative status among females before breeding. Since pre-breeding plasma MDA and UA-TAS levels are not correlated (see table S7), both variables were included together in the models.

	Estimate	**Std. Error**	**F**	**DF**	**P**
Tissue					
Oviduct					
Intercept	12.752	2.533			
Male line (L)	-2.536	2.163	4.098	1, 29	0.052
Female line (L)	3.047	2.301	2.609	1, 29	0.117
Replicate (2)	1.489	1.512	0.971	1, 29	0.333
Female plasma MDA	0.136	0.152	0.799	1, 29	0.379
Female uric acid-controlled TAS	-0.296	6.589	0.002	1, 29	0.964
Male line (L) * Female line (L)	-0.911	3.121	0.085	1, 29	0.772
Liver					
Intercept	50.003	6.221			
Male line (L)	-5.233	5.321	0.234	1, 30	0.632
Female line (L)	-5.788	5.660	0.297	1, 30	0.590
Replicate (2)	0.839	3.662	0.052	1, 30	0.820
Female plasma MDA	-0.188	0.374	0.253	1, 30	0.619
Female uric acid-controlled TAS	25.735	16.137	2.544	1, 30	0.121
Male line (L) * Female line (L)	6.737	7.566	0.793	1, 30	0.380
Spleen					
Intercept	77.197	10.113			
Male line (L)	-1.445	8.554	0.146	1, 30	0.706
Female line (L)	17.345	9.198	6.610	1, 30	**0.015**
Replicate (2)	-7.201	6.058	1.413	1, 30	0.244
Female plasma MDA	0.051	0.620	0.007	1, 30	0.936
Female uric acid-controlled TAS	-37.722	26.651	2.003	1, 30	0.167
Male line (L) * Female line (L)	-1.644	12.469	0.017	1, 30	0.896

Supplementary table S4: Effect of pre-breeding body mass on post-breeding MDA levels in different female tissues

Effects of partner line, female line, the interaction between male and female line, and line replicate on levels of oxidative damage (MDA) in the females' oviduct, liver and spleen after breeding (µM). The effects of male and female line remained unchanged when including female pre-breeding body mass as an additional covariate in the models.

	Estimate	**Std. Error**	**F**	**DF**	**P**
Tissue					
Oviduct					
Intercept	11.808	9.830			
Male line (L)	-2.462	2.204	5.013	1, 30	**0.033**
Female line (L)	3.789	2.409	2.280	1, 30	0.142
Replicate (2)	1.763	1.470	1.438	1, 30	0.240
Female pre-breeding body mass	0.009	0.034	0.077	1, 30	0.783
Male line (L) * Female line (L)	-1.599	2.978	0.288	1, 30	0.595
Liver					
Intercept	27.336	25.737			
Male line (L)	-6.648	5.874	0.162	1, 31	0.690
Female line (L)	-1.626	5.892	0.099	1, 31	0.756
Replicate (2)	-0.151	3.745	0.002	1, 31	0.968
Female pre-breeding body mass	0.068	0.092	0.546	1, 31	0.465
Male line (L) * Female line (L)	8.796	7.699	1.305	1, 31	0.262
Spleen					
Intercept	40.605	40.077			
Male line (L)	-5.376	8.845	0.453	1, 31	0.506
Female line (L)	17.766	9.508	5.139	1, 31	**0.031**
Replicate (2)	-8.168	5.947	1.887	1, 31	0.179
Female pre-breeding body mass	0.142	0.140	1.028	1, 31	0.319
Male line (L) * Female line (L)	2.409	12.009	0.040	1, 31	0.842

Supplementary table S5: Levels of post-breeding SOD in different female tissues

Effects of partner line, female line, the interaction between male and female line, and line replicate on SOD antioxidant levels in the females' oviduct, liver and spleen after breeding (IU/mL). Protein concentrations in homogenates were included as an additional covariate in alternative models to account for differences in protein concentration in the samples (i.e. differences in the released intracellular content between samples during homogenization).

	Estimate	**Std. Error**	**F**	**DF**	**P**
Tissue					
Oviduct					
Intercept	147.871	13.607			
Male line (L)	-9.653	16.965	0.618	1, 32	0.438
Female line (L)	-4.585	16.549	0.120	1, 32	0.731
Replicate (2)	-11.400	11.489	0.985	1, 32	0.329
Male line (L) * Female line (L)	1.150	23.049	0.003	1, 32	0.961
Oviduct					
Intercept	217.200	29.460			
Male line (L)	-5.732	15.690	0.094	1, 31	0.761
Female line (L)	-12.100	15.510	0.809	1, 31	0.375
Replicate (2)	-7.440	10.690	0.485	1, 31	0.491
Protein levels	-0.001	<0.001	6.761	1, 31	**0.014**
Male line (L) * Female line (L)	4.508	21.260	0.045	1, 31	0.833
Liver					
Intercept	1792.670	72.810			
Male line (L)	-170.890	90.780	0.490	1, 32	0.489
Female line (L)	-18.130	88.550	2.822	1, 32	0.103
Replicate (2)	-13.820	61.480	0.051	1, 32	0.824
Male line (L) * Female line (L)	236.010	123.330	3.662	1, 32	0.065
Liver					
Intercept	1529.000	313.400			
Male line (L)	-165.600	91.350	0.312	1, 31	0.581
Female line (L)	-20.930	88.960	2.857	1, 31	0.101
Replicate (2)	-11.340	61.790	0.034	1, 31	0.856
Protein levels	0.005	0.006	0.748	1, 31	0.394
Male line (L) * Female line (L)	244.00	124.200	3.862	1, 31	0.058
Spleen					
Intercept	528.930	29.530			
Male line (L)	-27.920	38.220	0.430	1, 32	0.517
Female line (L)	33.160	37.280	2.585	1, 32	0.118

Replicate (2)	-14.770	26.780	0.304	1, 32	0.585
Male line (L) * Female line (L)	20.330	53.390	0.145	1, 32	0.706
Spleen					
Intercept	501.300	78.330			
Male line (L)	-31.700	39.980	0.473	1, 31	0.497
Female line (L)	33.050	37.790	2.606	1, 31	0.117
Replicate (2)	-10.920	28.950	0.142	1, 31	0.709
Protein levels	<0.001	0.001	0.146	1, 31	0.705
Male line (L) * Female line (L)	24.300	55.110	0.195	1, 31	0.662

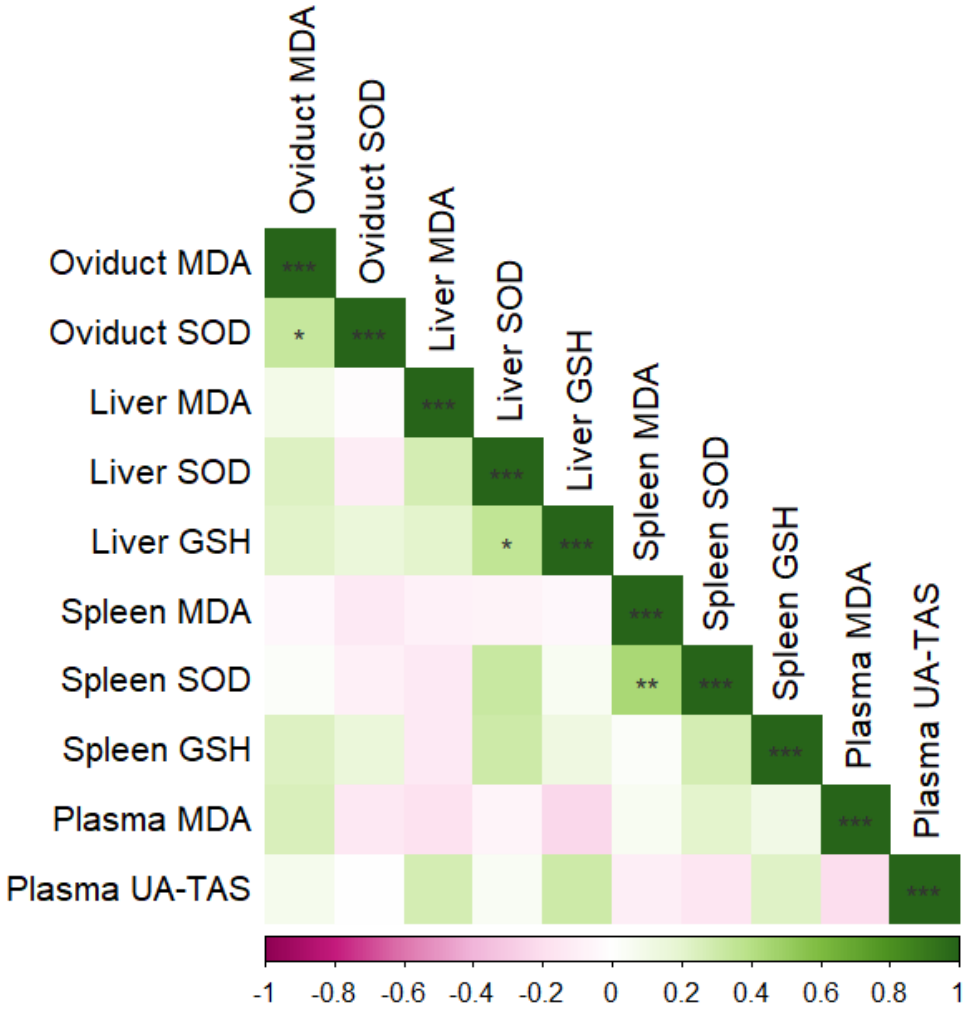
Supplementary table S6: Levels of post-breeding GSH in different female tissues

Effects of partner line, female line, the interaction between male and female line, and line replicate on GSH antioxidant levels in the females' liver and spleen after breeding (µmol/g). Protein concentrations in tissues were included as an additional covariate in alternative models to account for differences in protein concentration in the samples (i.e. differences in the released intracellular content between samples during homogenization).

	Estimate	**Std. Error**	**F**	**DF**	**P**
Tissue					
Liver					
Intercept	1.820	0.120			
Male line (L)	-0.208	0.150	0.030	1, 32	0.863
Female line (L)	-0.037	0.146	2.017	1, 32	0.165
Replicate (2)	0.002	0.101	0.001	1, 32	0.982
Male line (L) * Female line (L)	0.351	0.204	2.978	1, 32	0.094
Liver					
Intercept	1.968	5.229			
Male line (L)	-0.211	0.152	0.059	1, 31	0.809
Female line (L)	-0.035	0.148	1.930	1, 31	0.175
Replicate (2)	0.001	0.103	0.001	1, 31	0.993
Protein levels	<0.001	<0.001	0.085	1, 31	0.773
Male line (L) * Female line (L)	0.347	0.207	2.804	1, 31	0.104
Spleen					
Intercept	2.132	0.207			
Male line (L)	-0.205	0.268	2.400	1, 32	0.131
Female line (L)	0.450	0.262	3.868	1, 32	0.058
Replicate (2)	-0.356	0.188	3.600	1, 32	0.067
Male line (L) * Female line (L)	-0.167	0.374	0.198	1, 32	0.659
Spleen					
Intercept	1.700	5.442			
Male line (L)	-0.264	0.278	2.801	1, 31	0.104
Female line (L)	0.449	0.263	4.359	1, 31	**0.045**
Replicate (2)	-0.296	0.201	2.169	1, 31	0.151
Protein levels	<0.001	<0.001	0.737	1, 31	0.397
Male line (L) * Female line (L)	-0.105	0.383	0.075	1, 31	0.786

Supplementary table S7: **Correlations between oxidative stress markers within and across tissues**

Pearson's correlation coefficients (*r*) between oxidative stress-related markers in tissue and plasma samples. The significance levels are marked with * (* : p < 0.05, **: p < 0.01, ***: p < 0.001). Colours represent the strength and direction of the correlation coefficient *(r)*.



Supplementary table S8: Effects of female reproductive output on post-breeding MDA levels in different female tissues.

Effects of the number of eggs laid and the mean egg mass of those eggs on levels of MDA in the females' oviduct, liver and spleen (μM).

	Estimate	Std. Error	F	DF	P
Tissue					
Oviduct					
Intercept	27.496	10.730			
Number of eggs laid	0.124	0.325	0.146	1, 33	0.705
Mean egg mass	-1.154	0.905	1.627	1, 33	0.211
Liver					
Intercept	35.147	24.695			
Number eggs laid	-0.586	0.732	0.642	1, 34	0.428
Mean egg mass	1.328	2.075	0.410	1, 34	0.526
Spleen					
Intercept	77.440	41.622			
Number of eggs laid	1.749	1.259	1.929	1, 34	0.174
Mean egg mass	-1.280	3.494	0.134	1, 34	0.716

Figure S1: Effect of male line and female line on oxidative damage in female non-reproductive tissues.

Effect of male line and female line on levels of oxidative damage to lipids (MDA) in the females' liver (A) and spleen (B) after breeding. No effect of male line on MDA in female spleen or liver was observed (supplementary table S1). L-line females had higher levels of MDA in spleen than H-line females (supplementary table S1). No difference was found in liver (supplementary table S1). H-line females are represented in blue and L-line females in orange. Means ± 1SE are shown. Dots represent raw data.

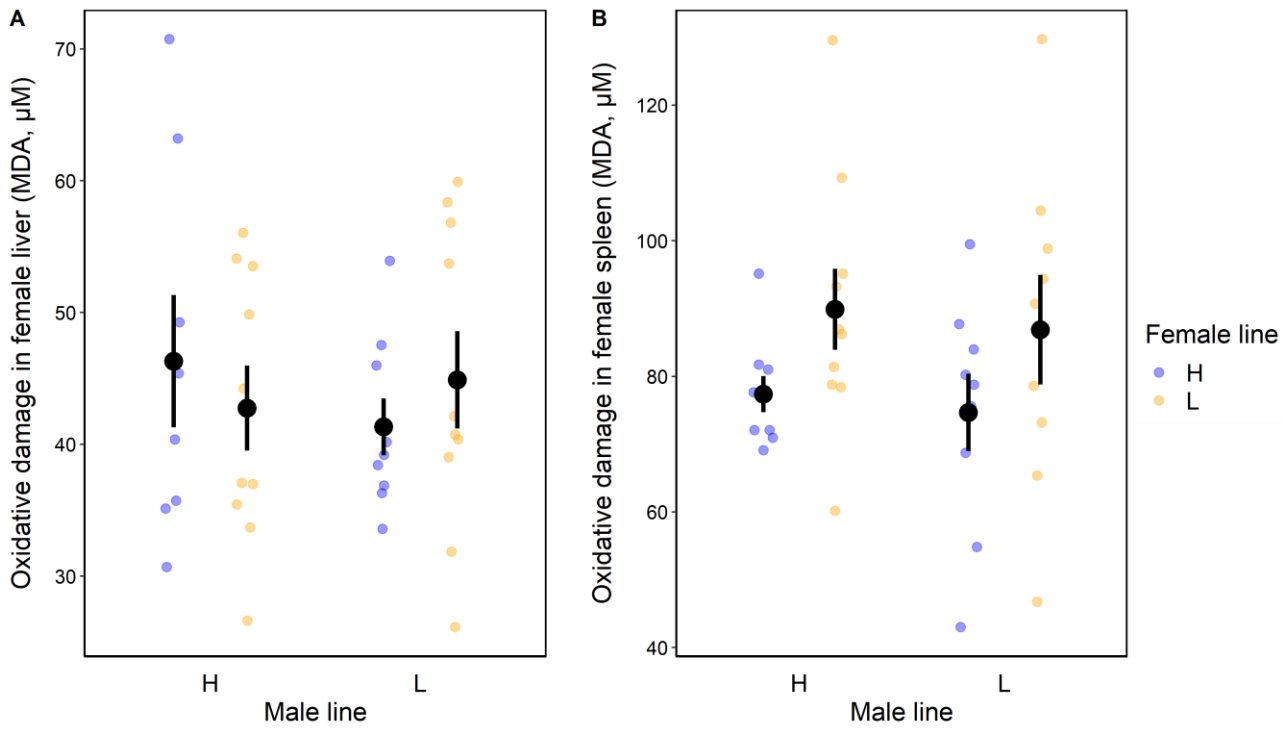
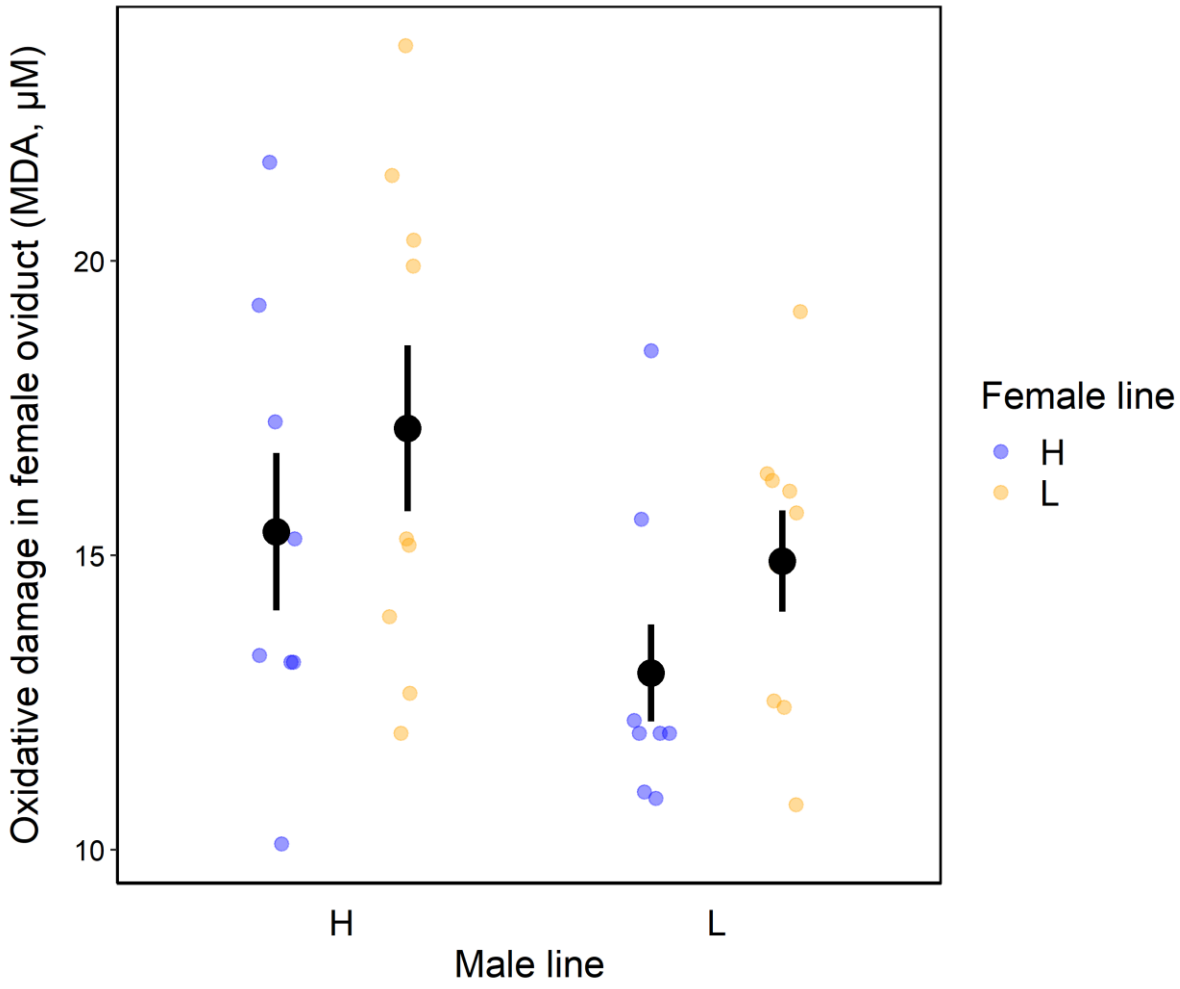


Figure S2: **Effect of male line and female line on female oviduct MDA when removing an extreme value**.

The effect of male line on levels of oxidative damage (MDA) in the female's oviduct remained significant when removing one extreme value (male line: $F_{1, 30}$ = 4.271, P = 0.048; female line: $F_{1, 30}$ = 2.511, P = 0.124; male line x female line: $F_{1, 30}$ < 0.001, P = 0.999). H-line females are represented in blue and L-line females in orange. Means ± 1SE are shown. Dots represent raw data.



References

[1] Pick, J.L., Hutter, P. & Tschirren, B. 2016 In search of genetic constraints limiting the evolution of egg size: direct and correlated responses to artificial selection on a prenatal maternal effector. *Heredity* **116**, 542-549. (doi:10.1038/hdy.2016.16).

[2] Pick, J.L., Ebneter, C., Hutter, P. & Tschirren, B. 2016 Disentangling Genetic and Prenatal Maternal Effects on Offspring Size and Survival. *American Naturalist* **188**, 628-639. (doi:10.1086/688918).

[3] Pick, J.L., Postma, E. & Tschirren, B. 2019 The more you get, the more you give: Positive cascading effects shape the evolutionary potential of prenatal maternal investment. *Evolution Letters* **3**, 412-423. (doi:10.1002/evl3.125).

[4] Agarwal, R. & Chase, S.D. 2002 Rapid, fluorimetric-liquid chromatographic determination of malondialdehyde in biological samples. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **775**, 121-126. (doi:10.1016/s1570-0232(02)00273-8).

[5] Nussey, D.H., Pemberton, J.M., Pilkington, J.G. & Blount, J.D. 2009 Life history correlates of oxidative damage in a free-living mammal population. *Functional Ecology* **23**, 809-817. (doi:10.1111/j.1365-2435.2009.01555.x).

[6] Romero-Haro, A.A. & Alonso-Alvarez, C. 2014 Covariation in oxidative stress markers in the blood of nestling and adult birds. *Physiol Biochem Zool* **87**, 353-362. (doi:10.1086/674432).

[7] Costantini, D. & Verhulst, S. 2009 Does high antioxidant capacity indicate low oxidative stress? *Functional Ecology* **23**, 506-509. (doi:10.1111/j.1365-2435.2009.01546.x).

[8] Monaghan, P., Metcalfe, N.B. & 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).

[9] Boonekamp, J.J., Mulder, E. & Verhulst, S. 2018 Canalisation in the wild: effects of developmental conditions on physiological traits are inversely linked to their association with fitness. *Ecol Lett* **21**, 857-864. (doi:10.1111/ele.12953).

[10] Miller, N.J., Riceevans, C., Davies, M.J., Gopinathan, V. & Milner, A. 1993 A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science* **84**, 407-412. (doi:10.1042/cs0840407).

[11] Saino, N., Caprioli, M., Romano, M., Boncoraglio, G., Rubolini, D., Ambrosini, R., Bonisoli-Alquati, A. & Romano, A. 2011 Antioxidant Defenses Predict Long-Term Survival in a Passerine Bird. *Plos One* **6**. (doi:10.1371/journal.pone.0019593).

[12] Vagasi, C.I., Vincze, O., Patras, L., Osvath, G., Penzes, J., Haussmann, M.F., Barta, Z. & Pap, P.L. 2019 Longevity and life history coevolve with oxidative stress in birds. *Functional Ecology* **33**, 152-161. (doi:10.1111/1365-2435.13228).

[13] Lopez-Arrabe, J., Cantarero, A., Perez-Rodriguez, L., Palma, A., Alonso-Alvarez, C., Gonzalez-Braojos, S. & Moreno, J. 2015 Nest-dwelling ectoparasites reduce antioxidant defences in females and nestlings of a passerine: a field experiment. *Oecologia* **179**, 29-41. (doi:10.1007/s00442-015-3321-7).

[14] Cohen, A., Klasing, K. & Ricklefs, R. 2007 Measuring circulating antioxidants in wild birds. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* **147**, 110-121. (doi:10.1016/j.cbpb.2006.12.015).

[15] Costantini, D. 2008 Oxidative stress in ecology and evolution: lessons from avian studies. *Ecology Letters* **11**, 1238-1251. (doi:10.1111/j.1461-0248.2008.01246.x).

[16] Costantini, D. 2011 On the measurement of circulating antioxidant capacity and the nightmare of uric acid. *Methods in Ecology and Evolution* **2**, 321-325. (doi:10.1111/j.2041-210X.2010.00080.x).

[17] Halliwell, B. & Gutteridge, J. 2007 *Free radicals in biology and medicine*. Oxford, UK, Oxford University Press.

[18] Zelko, I.N., Mariani, T.J. & Folz, R.J. 2002 Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radical Biology and Medicine* **33**, 337-349. (doi:10.1016/s0891-5849(02)00905-x).

[19] Bhattacharya, A., Hegazy, A.N., Deigendesch, N., Kosack, L., Cupovic, J., Kandasamy, R.K., Hildebrandt, A., Merkler, D., Kuhl, A.A., Vilagos, B., et al. 2015 Superoxide Dismutase 1 Protects

Hepatocytes from Type I Interferon-Driven Oxidative Damage. *Immunity* **43**, 974-986. (doi:10.1016/j.immuni.2015.10.013).

[20] Dong, A., Shen, J., Krause, M., Akiyama, H., Hackett, S.F., Lai, H. & Campochiaro, P.A. 2006 Superoxide dismutase 1 protects retinal cells from oxidative damage. *Journal of Cellular Physiology* **208**, 516-526. (doi:10.1002/jcp.20683).

[21] Shan, X.Y., Chi, L.Y., Ke, Y., Luo, C., Qian, S., Gozal, D. & Liu, R.G. 2007 Manganese superoxide dismutase protects mouse cortical neurons from chronic intermittent hypoxia-mediated oxidative damage. *Neurobiology of Disease* **28**, 206-215. (doi:10.1016/j.nbd.2007.07.013).

[22] Fukai, T. & Ushio-Fukai, M. 2011 Superoxide Dismutases: Role in Redox Signaling, Vascular Function, and Diseases. *Antioxidants & Redox Signaling* **15**, 1583-1606. (doi:10.1089/ars.2011.3999).

[23] Noor, R., Mittal, S. & Iqbal, J. 2002 Superoxide dismutase--applications and relevance to human diseases. *Med Sci Monit* **8**, Ra210-215.

[24] Elchuri, S., Oberley, T.D., Qi, W.B., Eisenstein, R.S., Roberts, L.J., Van Remmen, H., Jepstein, C.J. & Huang, T.T. 2005 CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene* **24**, 367-380. (doi:10.1038/sj.onc.1208207).

[25] Lebovitz, R.M., Zhang, H.J., Vogel, H., Cartwright, J., Dionne, L., Lu, N.F., Huang, S. & Matzuk, M.M. 1996 Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 9782-9787. (doi:10.1073/pnas.93.18.9782).

[26] Christensen, L.L., Selman, C., Blount, J.D., Pilkington, J.G., Watt, K.A., Pemberton, J.M., Reid, J.M. & Nussey, D.H. 2016 Marker-dependent associations among oxidative stress, growth and survival during early life in a wild mammal. *Proceedings of the Royal Society B-Biological Sciences* **283**. (doi:10.1098/rspb.2016.1407).

[27] Stauffer, J., Bruneaux, M., Panda, B., Visse, M., Vasemagi, A. & Ilmonen, P. 2017 Telomere length and antioxidant defense associate with parasite-induced retarded growth in wild brown trout. *Oecologia* **185**, 365-374. (doi:10.1007/s00442-017-3953-x).

[28] Woolliams, J.A., Wiener, G., Anderson, P.H. & McMurray, C.H. 1983 Variation in the activities of glutathione-peroxidase and superoxide-dismutase and in the concentration of copper in the blood in various breed crosses of sheep. *Research in Veterinary Science* **34**, 253-256. (doi:10.1016/s0034-5288(18)32219-7).

[29] Reglero, M.M., Taggart, M.A., Monsalve-Gonzalez, L. & Mateo, R. 2009 Heavy metal exposure in large game from a lead mining area: Effects on oxidative stress and fatty acid composition in liver. *Environmental Pollution* **157**, 1388-1395. (doi:10.1016/j.envpol.2008.11.036).

[30] Rodriguez-Estival, J., de la Lastra, J.M.P., Ortiz-Santaliestra, M.E., Vidal, D. & Mateo, R. 2013 Expression of immunoregulatory genes and its relationship to lead exposure and lead-mediated oxidative stress in wild ungulates from an abandoned mining area. *Environmental Toxicology and Chemistry* **32**, 876-883. (doi:10.1002/etc.2134).

[31] Bradford, M.M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248-254. (doi:10.1006/abio.1976.9999).

[32] Isaksson, C., Sheldon, B. & Uller, T. 2011 The Challenges of Integrating Oxidative Stress into Life-history Biology. *Bioscience* **61**, 194-202. (doi:10.1525/bio.2011.61.3.5).

[33] Jones, D.P. 2006 Redefining oxidative stress. *Antioxidants & Redox Signaling* **8**, 1865-1879. (doi:10.1089/ars.2006.8.1865).

[34] Lu, S.C. 2013 Glutathione synthesis. *Biochimica Et Biophysica Acta-General Subjects* **1830**, 3143-3153. (doi:10.1016/j.bbagen.2012.09.008).

[35] Messina, S., Eens, M., Casasole, G., AbdElgawad, H., Asard, H., Pinxten, R. & Costantini, D. 2017 Experimental inhibition of a key cellular antioxidant affects vocal communication. *Functional Ecology* **31**, 1101-1110. (doi:10.1111/1365-2435.12825).

[36] Costantini, D., Casasole, G., AbdElgawad, H., Asard, H. & Eens, M. 2016 Experimental evidence that oxidative stress influences reproductive decisions. *Functional Ecology* **30**, 1169-1174. (doi:10.1111/1365-2435.12608).

[37] Romero-Haro, A.A. & Alonso-Alvarez, C. 2020 Oxidative Stress Experienced during Early Development Influences the Offspring Phenotype. *Am Nat* **196**, 704-716. (doi:10.1086/711399).

[38] Romero-Haro, A.A. & Alonso-Alvarez, C. 2015 The level of an intracellular antioxidant during development determines the adult phenotype in a bird species: a potential organizer role for glutathione. *Am Nat* **185**, 390-405. (doi:10.1086/679613).

[39] Horak, P., Sild, E., Soomets, U., Sepp, T. & Kilk, K. 2010 Oxidative stress and information content of black and yellow plumage coloration: an experiment with greenfinches. *Journal of Experimental Biology* **213**, 2225-2233. (doi:10.1242/jeb.042085).

[40] Griffith, O.W. 1980 Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* **106**, 207-212. (doi:10.1016/0003-2697(80)90139-6).

[41] R Core Team. 2021 R: A Language and Environment for Statistical Computing. (Vienna, Austria, R Foundation for Statistical Computing.