

Article

Meta-analysis reveals that reproductive strategies are associated with sexual differences in oxidative balance across vertebrates

David COSTANTINI^{a,b,c,*}

^aUMR 7221, Muséum National d'Histoire Naturelle, 7 rue Cuvier 75231 Paris Cedex 05, France, ^bDepartment of Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, Berlin 10315, Germany, and ^cBehavioural Ecology & Ecophysiology Group, Department of Biology, University of Antwerp, Universiteitsplein 1, Wilrijk 2610, Belgium

*Address correspondence to David Costantini. E-mail: davidcostantini@libero.it.

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Abstract

Oxidative stress is a key physiological mechanism underlying life-history tradeoffs. Here, I use meta-analytic techniques to test whether sexual differences in oxidative balance are common in vertebrates and to identify which factors are associated with such differences. The dataset included 732 effect size estimates from 100 articles (82 species). Larger unsigned effect size (meaning larger sexual differences in a given marker) occurred in: reptiles and fish; those species that do not provide parental care; and oviparous species. Estimates of signed effect size (positive values meaning higher oxidative stress in males) indicated that females were less resistant to oxidative stress than males in: reptiles while males and females were similar in fish, birds, and mammals; those species that do not provide parental care; and oviparous species. There was no evidence for a significant sexual differentiation in oxidative balance in fish, birds, and mammals. Effect size was not associated with: the number of offspring; whether the experimental animals were reproducing or not; biomarker (oxidative damage, non-enzymatic, or enzymatic antioxidant), the species body mass; the strain (wild vs. domestic); or the study environment (wild vs. captivity). Oxidative stress tended to be higher in females than males across most of the tissues analyzed. Levels of residual heterogeneity were high in all models tested. The findings of this meta-analysis indicate that diversification of reproductive strategies might be associated with sexual differences in oxidative balance. This explorative meta-analysis offers a starting platform for future research to investigate the relationship between sex and oxidative balance further.

Key words: antioxidants, oviparity, oxidative damage, parental care, vertebrates, viviparity.

Males and females do not simply differ in how they look like, but differences greatly extend far beyond those of morphological traits. Sexually antagonistic selection has promoted different trait optima in males and females in many traits. For example, in many vertebrate species, the 2 sexes have conflicting reproductive strategies, particularly over the mode and frequency of mating (Parker 2006). Also the amount of parental care invested may greatly differ between males and females across species or even within species

(Alonso-Alvarez and Velando 2012; Balshine 2012). Because the expression of many of these traits is linked to physiological mechanisms, it might be expected that selection acting on the physiological traits would also differ between males and females, leading to different physiological phenotypes. Sexual differences are actually evident at physiological level, steroid hormones being a renowned example (Norris and Lopez 2010). Males and females may also differ in other traits, for example in their immunological responses to foreign and

self-antigens, and show distinctions in innate and adaptive immune responses (Klein and Flanagan 2016). Variation between sexes in basal metabolic rate has also been found and suggested to be due to sex linked nuclear genes (Boratyński et al. 2016).

In recent times, there has been growing interest in the role of oxidative stress as a mediator of life history evolution. Oxidative stress is the rate at which biomolecular oxidative damage is generated, which results from a complex interaction between compounds that oxidize (e.g., free radicals) and compounds that protect against oxidation (antioxidants) (Costantini and Verhulst 2009; Halliwell and Gutteridge 2015). Much recent work has shown that oxidative stress may be connected with life history traits like reproduction or growth (Costantini 2014). One common aspect of this recent work is that males and females have frequently been shown to differ in some aspects of the oxidative balance, be it generation of oxidative damage or up/down regulation of antioxidants (see references in the Supplementary Materials). The reasons for such sexual differences in oxidative balance are currently unknown. A reason might lie with the way sexes respond to selective pressures. For example, variation in the extent to which each sex contributes to parental care may influence the regulation of oxidative balance because of the metabolic demands required by parental investment. It might for example be expected that (i) differences in oxidative balance between males and females are attenuated in those species where both sexes contribute to parental care, (ii) females suffer more oxidative stress than males in those species where most of the parental work is on the female, or (iii) females of species that generate many offspring (e.g., number of eggs or pups) would suffer more oxidative stress than females of species that produce less offspring.

The aim of this study was to use meta-analytic techniques to test whether sexual differentiation in resistance to oxidative stress is ubiquitous across vertebrates and to review evidence for which factors might explain any differences between sexes in oxidative balance. A meta-analytical approach was used because it enables to estimate the size of a given difference. A diverse range of 4 taxonomic classes of vertebrates were considered in order to assess whether differences in oxidative balance between sexes are consistent across taxa with a different evolutionary history. Invertebrates were not considered in this meta-analysis given that they differ dramatically from vertebrates for many biological traits. The contribution of several factors that might be associated with sexual variation in oxidative balance was tested: if the species provides or does not provide parental care; if the species lays eggs (oviparous) or gives birth to fully formed offspring (viviparous); number of pups or eggs generated. The contribution of each factor was tested while taking into account some confounding factors that vary across studies, such as which markers of oxidative stress were measured and in what tissue. Sexual differences in tissue oxidative stress were also analyzed in order to test whether males and females differ in how they prioritize antioxidant protection of tissues.

Materials and Methods

Data collection

A comprehensive review of the literature was performed on the Web of Science using the combinations of the keywords “Fish”, “Amphibians”, “Reptiles”, “Birds”, or “Mammals” with “Oxidative stress”, “Oxidative damage”, or “Antioxidants”. I then searched for additional studies via cross-referencing from hits from this search. The authors of 56 articles were contacted to provide

data missing in the selected papers; data were obtained by 40 of them.

An article was selected if it contained a comparison of oxidative stress markers between adult males and females. The following exclusion criteria were applied: i) studies that measured expression of antioxidant genes because I were interested in the biochemical differences between sexes; ii) studies that used metrics of free radical generation as an index of oxidative stress, since they do not provide direct evidence of oxidative stress (reactive species might be mopped up before oxidative damage is generated); iii) studies where necessary information for calculating effect size was unavailable. Overall, the final dataset included 732 effect sizes from 100 articles (82 species: 7 fish, 5 reptiles, 44 birds, and 26 mammals) (Almroth et al. 2008; Alonso-Alvarez et al. 2004a, 2004b; Barrera-García et al. 2012; Beamonte-Barrientos and Verhulst 2013; Beaulieu and Schaefer 2014, Beaulieu et al. 2010, 2011, 2014; Bertrand et al. 2006; Bilham et al. 2013; Bize et al. 2008; Bonisoli-Alquati et al. 2010; Canovas et al. 2014; Casagrande et al. 2011, 2012a, 2012b; Cecere et al. 2016; Christensen et al. 2015; Christie et al. 2012; Cohen et al. 2008; Costantini and Bonadonna 2010; Costantini and Dell’omo 2015; Costantini et al. 2007, 2008, 2010, 2012a, 2012b, 2013, 2014, 2014a, 2014b; Costantini 2010; Cram et al. 2015a, 2015b; Depboylu et al. 2013; Ehrenbrink et al. 2006; Emaresi et al. 2016; Figueiredo-Fernandes et al. 2006b; Georgiev et al. 2015; Gomes et al. 2014; Grunst et al. 2014; Heiss and Schoech 2012; Herrera-Dueñas et al. 2014; Isaksson et al. 2009, 2011, 2013; Isaksson 2013; Jolly et al. 2012; Kamper et al. 2009; Kanerva et al. 2012; Kayali et al. 2007; Kurhalyuk et al. 2009; Langley-Evans and Sculley 2005; Leclaire et al. 2015; Lilley et al. 2014; Lopes et al. 2002; Lopez-Arrabé et al. 2014; Lopez-Cruz et al. 2010; Losdat et al. 2013; Lozano et al. 2013; Lucas and French 2012; Marasco et al. 2013; Mielnik et al. 2011; Montgomery et al. 2011, 2012; Norte et al. 2009; Ojeda et al. 2012; O’Keeffe 2013; Oropesa et al. 2013; Ouyang et al. 2016; Pap et al. 2014, 2015; Pike et al. 2007; Raja-Aho et al. 2012; Reichert et al. 2014; Romero-Haro et al. 2015, 2016; Rubolini et al. 2012; Schneeberger et al. 2013, 2014; Shao et al. 2012; Sharick et al. 2015; Stier et al. 2014a, 2014b; Tobler et al. 2013; van de Crommenacker et al. 2011; Vaugoyeau et al. 2015; Vazquez-Medina et al. 2007; Vitousek et al. 2016; Wegmann et al. 2015a, 2015b; and Wiersma et al. 2004).

Oxidative status metrics were categorized into the following groups: i) oxidative damage biomarkers including DNA damage (e.g., 8-oxo-dg), protein damage (e.g., protein carbonyls), lipid damage (e.g., lipid hydroperoxides, malondialdehyde-MDA, isoprostanes), and general damage (e.g., reactive oxygen metabolites-ROMs, total oxidant status-TOS, thiobarbituric acid reactive substances-TBARS); ii) non-enzymatic antioxidants including thiols (e.g., total thiols, glutathione) and non-enzymatic antioxidant capacity (e.g., KRL, OXY, ABTS); and iii) antioxidant enzymes (e.g., catalase, glutathione-S-transferase, glutathione peroxidase, superoxide dismutase). Oxidative status metrics were further categorized by assay (e.g., TBARS, MDA, Protein carbonyls, d-ROMs, KRL, GSH) and by tissue (e.g., blood, brain, liver, muscle).

Data on body mass were collected from online databases like <http://genomics.senescence.info/species/>, <http://animaldiversity.ummz.umich.edu/> and <http://www.fishbase.org/search.php>.

Effect size calculation

The compute.es package (Del Re 2013) in R (R Core Team 2013) was used to calculate the standardized effect size Hedges’ *g* from test statistics (e.g., *t*-values or *F*-ratios) or descriptive statistics (e.g., means,

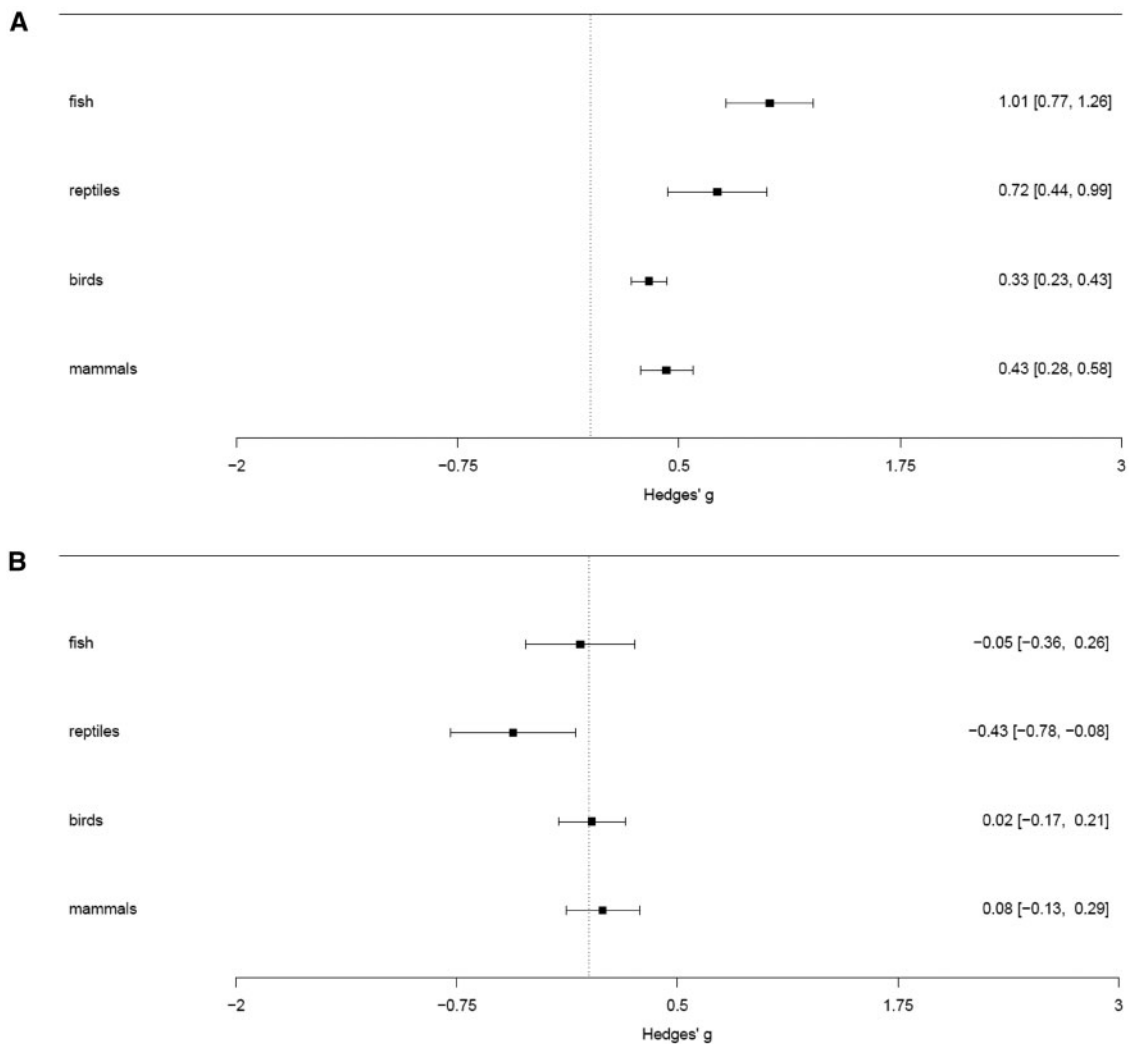


Figure 1. There was a significant association between taxonomic class and either (A) unsigned Hedges' *g* (higher values indicating larger differences between males and females) or (B) signed Hedges' *g* (positive values indicating higher oxidative stress in males than in females). Predicted effect sizes (mean and 95% confidence interval at right) are shown. When the confidence interval does not include zero, the effect size is significant.

standard deviations) and sample sizes that were reported in papers. For Hedges effect size estimate, the type I and II error rates can increase if the number of studies is very low (< 15) but the precision of the estimate increases with increasing number of studies (unlike other effect size measures; e.g., log response ratio) (Lajeunesse and Forbes 2003). Thus, given the large sample size of the current meta-analyses, Hedges was deemed an appropriate effect size estimate.

Moderators included and categorization

As the relationship between sex and oxidative balance might be explained by various factors, several explanatory variables (termed moderators in meta-analysis) were considered to be included in the analyses: taxonomic class; parental care (no parental care, female parental care, biparental care); mode of reproduction (oviparous and viviparous); family size (number of either eggs or pups); reproductive status (whether the experimental animals were reproducing or not when the biomarkers were measured); species body mass; strain (wild vs. domestic individuals); biomarker (oxidative damage, non-enzymatic antioxidant, enzymatic antioxidant); study environment (wild vs. captivity). Further moderators were included as random

effects: biological matrix where a given marker was analyzed (to accounting for variation in matrices analyzed across studies); laboratory assay (to accounting for variation in assays performed across studies); article (to accounting for the non-independence of effect sizes from the same study); species (to accounting for the non-independence of effect sizes from the same species); taxonomic class (to partly control for phylogeny, which is difficult to do as the dataset was rather unevenly distributed across 4 taxonomic classes).

Meta-analytic techniques

Meta-analytic multilevel mixed-effects models were implemented using the *rma.mv* function in the *metafor* package (Viechtbauer 2010) in R (R Core Team 2013). The extracted Hedges' *g* values were the response variables in the statistical models. Estimates were weighted according to the sampling variance to account for different sample sizes across studies. Each model output included the QE-test for residual heterogeneity, indicating whether the unexplained variance is greater than expected by chance. All the analyses were done using either unsigned or signed estimates of effect size. Unsigned values indicate the magnitude of the difference in a given marker between males and

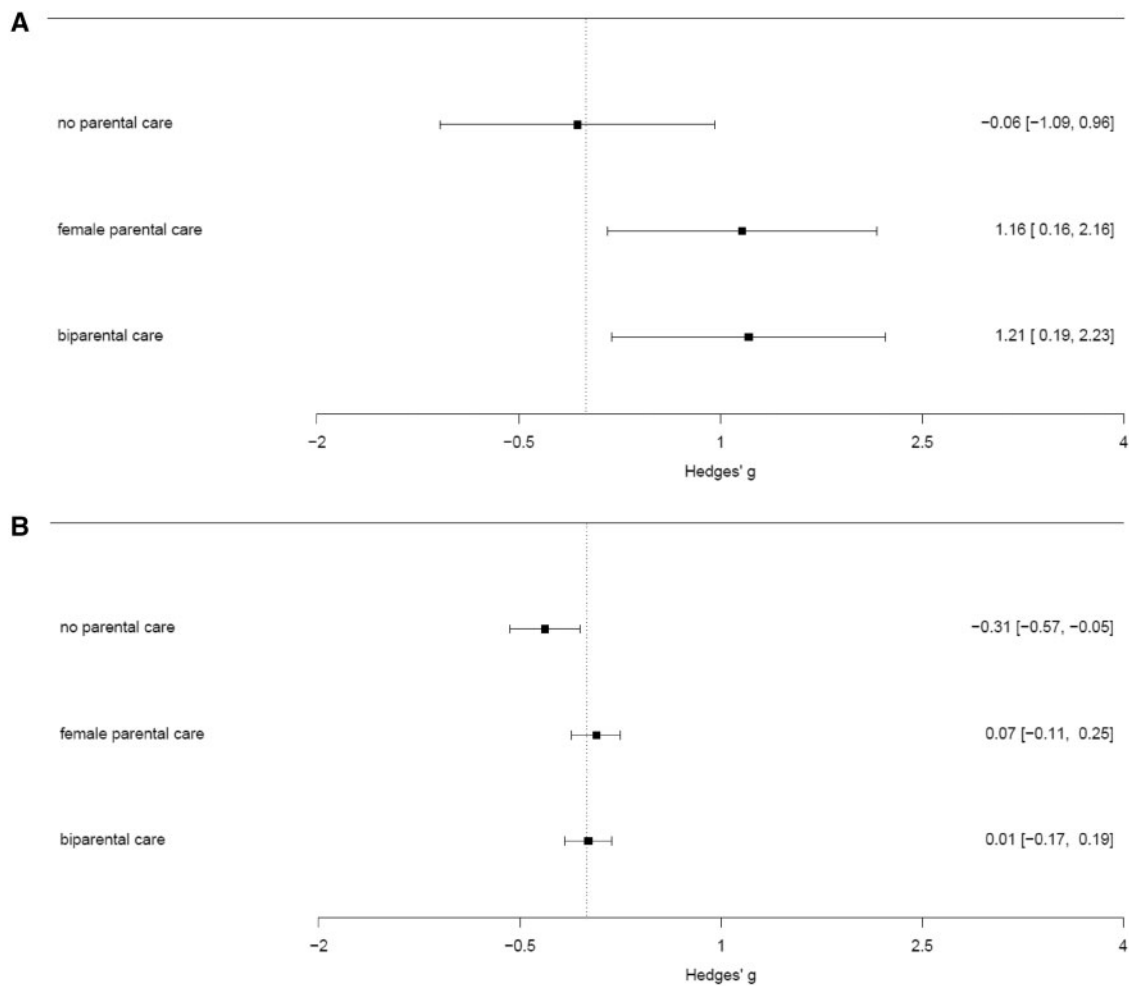


Figure 2. The meta-analysis showed that there was a significant association between occurrence of parental care and either (A) unsigned Hedges' *g* (higher values indicating larger differences between males and females) or (B) signed Hedges' *g* (positive values indicating higher oxidative stress in males than in females). Predicted effect sizes (mean and 95% confidence interval at right) are shown. When the confidence interval does not include zero, the effect size is significant.

females. Signed values indicate which sex suffered more oxidative stress: a positive effect size indicates that either oxidative damage is higher or a given antioxidant is lower in males than females, implying higher oxidative stress in males. Effect size estimates were considered significant only when they did not overlap zero. Between group comparisons for specific moderators were run only when effect size estimates of the 2 groups did not overlap zero. Between group comparisons are significant when there is no overlap in effect size estimates.

Publication bias

Publication bias was assessed by examining funnel plots of effect size against the log of sample size for each dataset (Møller and Jennions 2001). The plot should be in the shape of a "funnel" with larger variance in effect sizes at small sample sizes and a decreasing variance with increasing sample size. If only significant findings were published, one might expect there to be a "gap" in the lower left of the graph, where for small samples effect sizes must be relatively large to be statistically significant. The funnel plots in the present study indicate there was no publication bias. This is confirmed by the fact that sample size was not significantly associated with Hedges' *g* values ($Q_M = 1.77$, $df = 1$, $P = 0.18$ with article as random factor; $Q_M = 0.18$, $df = 1$, $P = 0.67$ with article and species as random factors).

Results

Preliminary analyses showed that the moderators biomarker (unsigned effect size: $Q_M = 2.75$, $df = 2$, $P = 0.25$; signed effect size: $Q_M = 0.77$, $df = 2$, $P = 0.68$), strain (signed effect size: $Q_M = 0.23$, $df = 1$, $P = 0.63$), study environment (signed effect size: $Q_M = 0.27$, $df = 1$, $P = 0.61$), reproductive status (unsigned effect size: $Q_M = 0.04$, $df = 1$, $P = 0.84$; signed effect size: $Q_M = 0.62$, $df = 1$, $P = 0.44$), or species body mass (unsigned effect size: $Q_M = 0.22$, $df = 1$, $P = 0.64$; signed effect size: $Q_M = 2.61$, $df = 1$, $P = 0.11$) were not significantly associated with estimates of effect size. Thus, these moderators were not further considered in the next analyses. Strain ($Q_M = 10.71$, $df = 1$, $P = 0.001$; mean, 95% lower and higher confidence interval: domestic, 0.84, 0.49, 1.19; wild, 0.56, 0.24, 0.89) and study environment ($Q_M = 8.57$, $df = 1$, $P = 0.003$; mean, 95% lower and higher confidence interval: captivity, 0.75, 0.43, 1.08; wild, 0.55, 0.24, 0.87) were, however, significantly associated only with unsigned effect size estimates. The inclusion of these 2 moderators in the following models for unsigned effect size did not affect substantially the outcomes, so they were not included in the final models (unless otherwise noted).

There was a significant association between unsigned effect size and taxonomic class ($Q_M = 29.46$, $df = 3$, $P < 0.001$); effect size

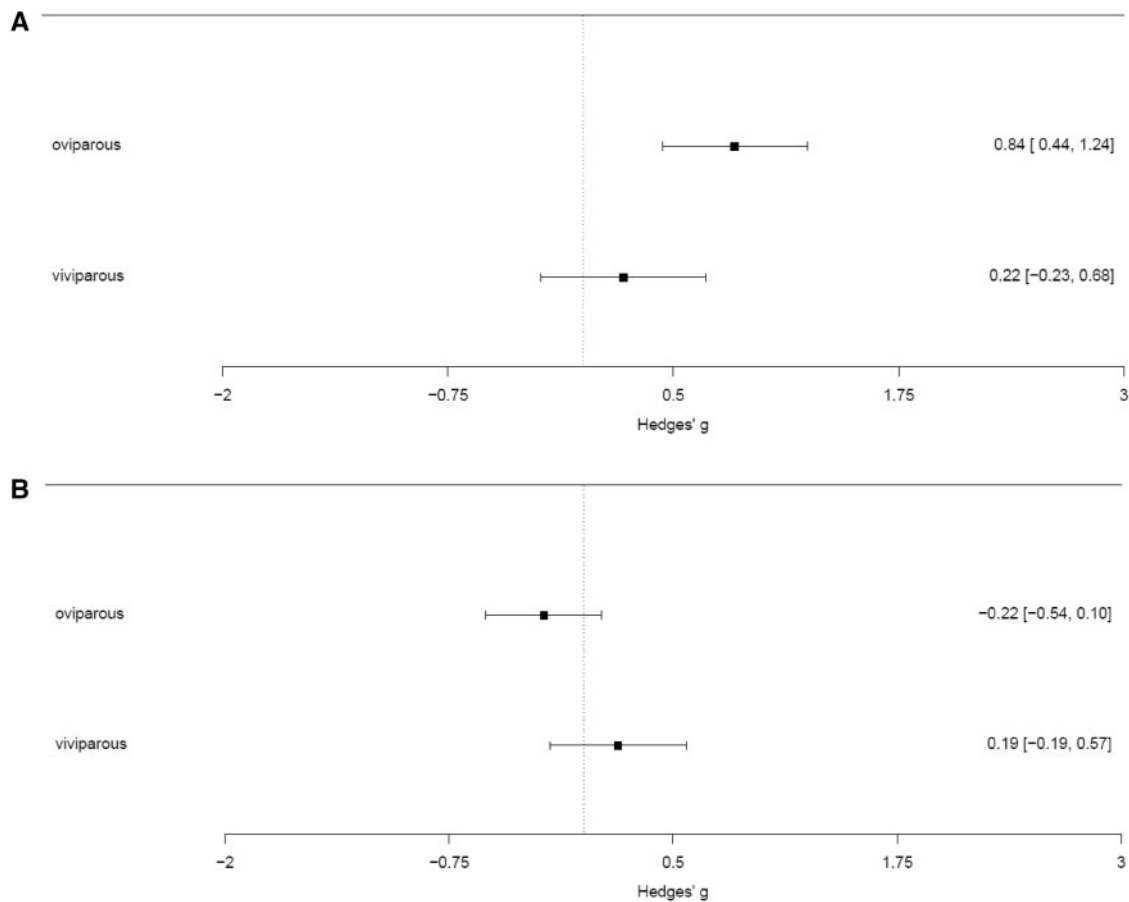


Figure 3. The association between mode of reproduction (oviparity vs. viviparity) was significant for either (A) unsigned Hedges' g (higher values indicating larger differences between males and females) or, although to a less extent, (B) signed Hedges' g (positive values indicating higher oxidative stress in males than in females). Predicted effect sizes (mean and 95% confidence interval at right) are shown. When the confidence interval does not include zero, the effect size is significant.

estimates were significantly larger in fish than birds and mammals (both $P < 0.001$) and in reptiles than birds ($P = 0.01$), while the difference between mammals and reptiles was close to significance ($P = 0.07$; $P = 0.04$ when both strain and study environment are included as moderators) (Figure 1A). There was also a significant association between unsigned effect size and parental care ($Q_M = 14.30$, $df = 2$, $P = 0.0008$), with only species that do not provide parental care producing effect size estimates that did not overlap zero (Figure 2A). The association between unsigned effect size and mode of reproduction was also significant ($Q_M = 9.09$, $df = 1$, $P = 0.0026$). The unsigned effect size was significantly larger than zero in oviparous species, while effect size estimates of viviparous species overlapped zero (Figure 3A). Family size was positively associated with unsigned effect size ($Q_M = 15.98$, $df = 1$, $P < 0.001$), but the association was no longer significant when 1 outlier *Salmo trutta* was removed from the model ($Q_M = 1.29$, $df = 1$, $P = 0.26$). All other moderators were not significantly associated with unsigned effect size. In a further model, species were categorized by mode of reproduction and parental behavior (5 categories in total, Figure 4A). This new predictor was significantly associated with unsigned effect size ($Q_M = 25.1$, $df = 4$, $P < 0.0001$). The confidence interval did not overlap zero only for oviparous species with biparental care or with female parental care, which did not differ from each other (Figure 4A).

There was a significant association between signed effect size and taxonomic class ($Q_M = 8.83$, $df = 3$, $P = 0.032$), with only reptiles

producing effect size estimates that did not overlap zero (Figure 1B). There was also a significant association between signed effect size and parental care ($Q_M = 9.04$, $df = 2$, $P = 0.011$), with only species that do not provide parental care producing effect size estimates that did not overlap zero (Figure 2B). The association between signed effect size and mode of reproduction was significant ($Q_M = 4.38$, $df = 1$, $P = 0.036$), however, the confidence interval overlapped zero for both viviparous and oviparous species (Figure 3B). Finally, there was a significant association between signed effect size and the tissue in which a given biomarker was measured ($Q_M = 16.06$, $df = 7$, $P = 0.025$) while controlling for article, species, assay, taxonomic class, and biomarker. The confidence interval of each analyzed tissue overlapped zero (Figure 5). All other tested moderators were not significant. As with unsigned effect size, in a further model, species were categorized by mode of reproduction and parental behavior (5 categories in total). This new predictor was significantly associated with signed effect size ($Q_M = 11.8$, $df = 4$, $P = 0.02$). The confidence interval did not overlap zero only for oviparous species (i.e., fish and reptiles) that do not provide any parental care (Figure 4B).

The QE-test revealed significant levels of residual heterogeneity in all models tested ($P < 0.0001$), implying that the variance not accounted for by the moderators was significantly greater than expected.

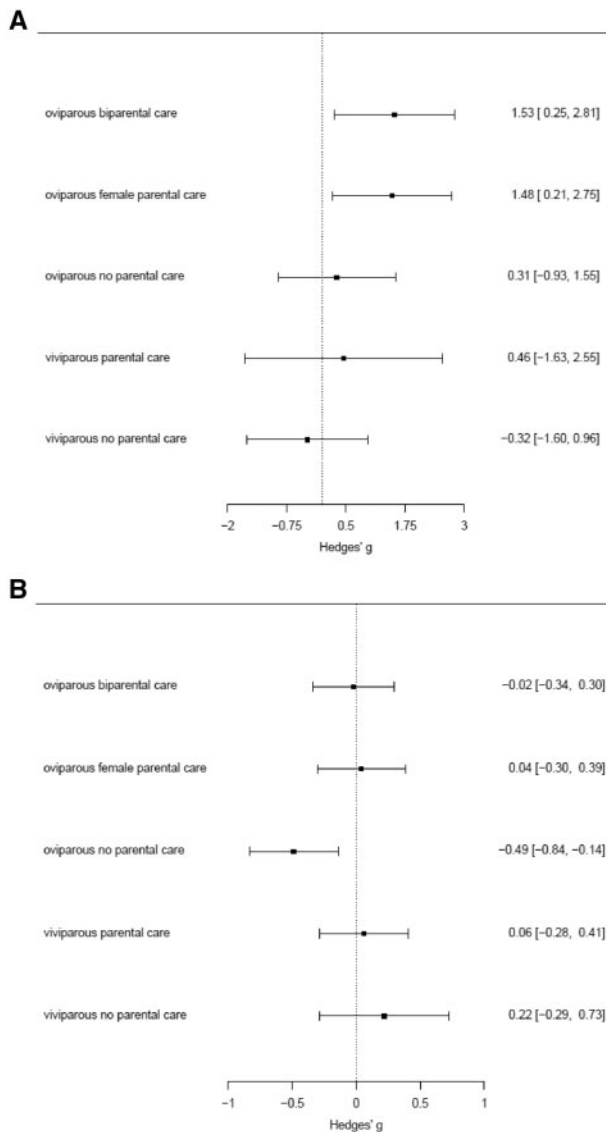


Figure 4. Species are categorized by mode of reproduction and parental behavior (5 categories in total). (A) The confidence interval for unsigned Hedges' g (higher values indicating larger differences between males and females) did not overlap zero only for oviparous species with biparental care or with female parental care; (B) the confidence interval for signed Hedges' g (positive values indicating higher oxidative stress in males than in females) did not overlap zero only for oviparous species (i.e., fish and reptiles) that do not provide any parental care. Predicted effect sizes (mean and 95% confidence interval at right) are shown.

Discussion

In using meta-analytical techniques to review available data on the relationship between sex and oxidative balance across vertebrates, I found that (i) sexual differences in oxidative balance are larger in fish and reptiles than birds and mammals, in oviparous than viviparous species and in those species that provide parental care; (ii) male reptiles suffer less oxidative stress than female reptiles; (iii) females suffer more oxidative stress than males in those species that do not provide any parental care; (iv) there was no difference between males and females in resistance to oxidative stress in fish, birds, and mammals; (v) the number of eggs or pups generated per

reproductive event is not associated with sexual differences in oxidative balance.

Although sexual differences in oxidative balance were particularly more pronounced in fish and reptiles, estimates of signed effect size showed that only in reptiles there was also a significant difference between males and females in terms of oxidative stress. The higher oxidative stress experienced by female reptiles should be taken cautiously because only 5 species were included in this meta-analysis, thus this result might be influenced by the nature of the selected papers. For example, in the *Crocodylus moreletii*, females provide parental care (Dzul-Caamal et al. 2016), which is widespread in crocodylians, with the females guarding nests and young (Ferguson 1985). In the *Conolophus subcristatus* the higher oxidative stress observed in females might have been due to the sampling that was mostly carried out during the reproductive season when females experience high metabolic costs for egg production and for nest excavation (Costantini et al. 2009). In the *Ctenophorus pictus*, Olsson et al. (2012) found that males have significantly higher antioxidant enzyme activity than females throughout the mating season, agreeing with a selection history for higher male activity levels due to long hours of patrolling territories at high temperatures in desert Australia and competing for mating opportunities. On the other hand, females had higher damage to DNA than males. The higher oxidative stress in female than in male reptiles might be explained by a high investment of female reptiles in the generation of offspring. A central paradigm of life history theory is that a high investment of resources into reproduction (e.g., number of offspring generated) would result in less resources available for self-maintenance (e.g., antioxidant protection). Fish species included in this meta-analysis invest massively in egg production, generating from approximately 14–1,285 eggs/offspring per reproductive event, while the number of either eggs or pups generated from the other classes of vertebrates range from 1 to 30. It is, therefore, unclear why female fish did not have more oxidative stress than male fish as was the case for reptiles.

The results of the meta-analysis also showed that sexual differences in oxidative balance were larger in those species that provide parental care when compared with those that do not provide parental care. Empirical research has shown that providing care benefits parents by increasing offspring survival and increasing their reproductive success (Alonso-Alvarez and Velando 2012; Balshine 2012). However, parental care also has potential costs, such as decreased survival and reproductive perspectives (Alonso-Alvarez and Velando 2012; Balshine 2012). Although sexual differences in oxidative balance were larger in those species that provide parental care, females suffered more oxidative stress than males only in those species that do not provide parental care. In those species that do not provide parental care, most of the reproductive cost is on the female, which has to invest in embryo development or in the production of multiple eggs. Thus, this result might indicate that generation of offspring is costly in terms of oxidative stress. It is, however, unclear why this oxidative cost for females did not also emerge in those species where it is only the female that provides parental care, rather the effect size was similar to that of species with biparental care. It might be that in these species mothers may be adapted to resist oxidative stress in order to not compromise their capability of providing parental care. This result raises the exciting hypothesis that evolution of parental care would have been associated with that of mechanisms governing the oxidative balance and that this coevolution might have differed between species with uni- or biparental care. Another reason for this result might lie with males of species

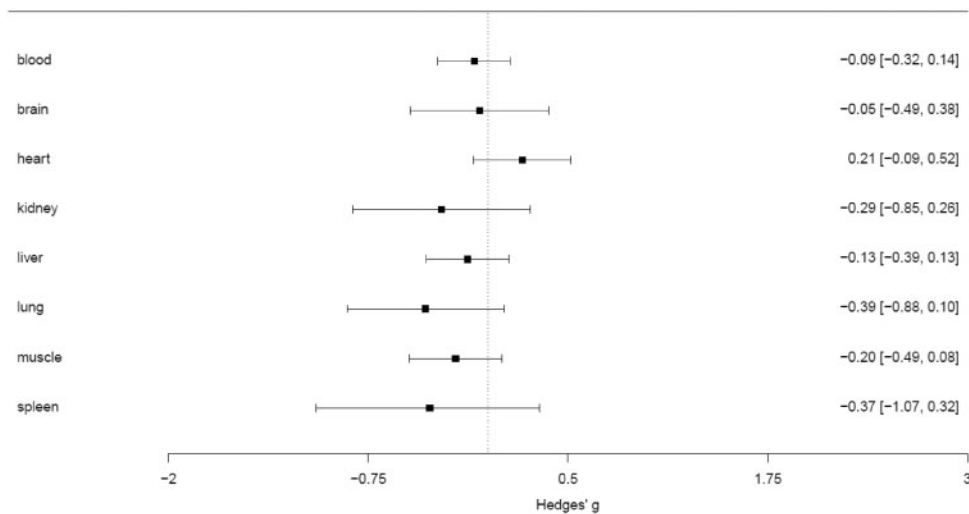


Figure 5. The meta-analysis showed a significant association between tissue in which a given marker of oxidative stress was measured and signed Hedges' g (positive values indicating higher oxidative stress in males than in females). The predicted effect sizes (mean and 95% confidence interval at right) of each analyzed tissue included zero, indicating that they were not statistically significant.

with biparental care experiencing high costs for male–male competition. In many vertebrate species, males typically compete intensely for mates (Alonso-Alvarez and Velando 2012; Balshine 2012). Thus, the oxidative costs of reproduction for males in species with intense male–male competition might be similar to those that females experience for care provisioning.

The reason for the lack of difference in oxidative stress between sexes in those species with biparental care might also lie with a high intra-species variation between mates in the amount of parental effort. Studies on passerine birds have shown that there is not a fixed amount of investment that a given sex puts into reproduction. For example, a member of the pair may increase its effort in order to compensate for a lower breeding effort of its mate who had previously stressful experiences (Spencer et al. 2010). Thus, these results suggest that the larger sexual differences in oxidative balance in species with biparental care as indicated by unsigned but not by signed effect size would indicate that only 1 of the 2 sexes is experiencing high oxidative stress.

In oviparous species, there was stronger evidence for sexual differences in oxidative balance, with a tendency for oviparous females to suffer more oxidative stress than viviparous females. One major problem in interspecific comparisons about variation in physiological costs between reproductive modes is that a number of physiological differences may complicate the ability to attribute differences in costs to reproductive mode only. There are a few species that can reproduce by either viviparity or oviparity that can provide excellent study models to test further the association between oxidative balance and mode of reproduction. For example, Foucart et al. (2014) compared oxygen consumption, as a reflection of energy costs, during reproduction between oviparous and viviparous females of the reproductively bimodal lizard *Zootoca vivipara*. Female oxygen consumption progressively increased over the course of reproduction, peaking just prior to laying/delivery when it was 46% (oviparous form) and 82% (viviparous form) higher than it was at the pre-reproductive stage. Conversely, post-ovulation total increase in oxygen consumption was more than 3 times higher in viviparous females, reflecting a dramatic increase in embryonic metabolism as well as maternal metabolic costs of pregnancy. It has therefore been suggested that selection for transition from oviparity

to viviparity should have provided benefits that outweigh the substantial energy costs that are incurred (Foucart et al. 2014). Given the results of this meta-analysis, it is tempting to speculate that selection for higher resistance to oxidative stress might have contributed to favor evolution of viviparity. For example, in viviparous species, a higher resistance of females to oxidative stress might protect offspring from the pathological consequences associated with accumulation of oxidative damage during embryogenesis (Vitikainen et al. 2016).

The results of this meta-analysis provided little support for sexual differences in tissue sensitivity to oxidative stress. Although confidence intervals overlapped zero for each of the tissues analyzed, in 7 out of 8 tissues the predicted effect size was negative (indicating higher oxidative stress in females). A previous meta-analysis suggested that females were more susceptible to oxidative stress when being exposed to an experimental increase of stress hormones (Costantini et al. 2011). What are the exact mechanisms via which females might be less resistant to oxidative stress, at least in some tissues, remains an open question. However, the results of the meta-analysis also showed that sexual differences in oxidative balance were not necessarily due to 1 of the 2 sexes always suffering more oxidative stress than the other. Although it cannot be excluded that regulation of the oxidative balance might differ to some extent between males and females, this differential regulation does not appear to translate in different oxidative statuses. The evolution of a given trait can be influenced by correlations between the effects of genes on male and female characters, and selection acting on 1 sex may produce a correlated response in the other sex (Lande and Arnold 1983). Many genes that regulate the resistance to oxidative stress have been identified (Allen and Tresini 2000; Rotblat et al. 2013), thus although selection on specific genes might differ between males and females, the overall selective effect on oxidative stress on 1 sex might produce a correlated response in the other.

In conclusion, this meta-analysis showed that phylogeny (class effect), parental behavior, and mode of reproduction contribute to explain sexual differences in either oxidative balance or resistance to oxidative stress. This work showed that males and females were generally similar in resistance to oxidative stress. Moreover, this work

did not provide strong support for role of reproductive investment in terms of the number of offspring generated in explaining sexual differences in oxidative balance. Because of the gaps in current literature, it was not always possible to disentangle the relative contributions of moderators. For example, females had higher oxidative stress than males in oviparous species that do not provide any parental care, which included only fish and reptile species in this dataset. In all the tested models, there was significant residual heterogeneity, implying that there are additional moderators not considered here that might be responsible for the residual variation. For example, previous work showed that hormonal differences between sexes may be associated with those in immunological traits and parasite burden (Klein 2000). Also sexual differences in the probability of extrinsic mortality (e.g., due to predation) might be important because investment in a phenotype resistant to oxidative stress is expected to decrease when chances of survival are low.

Overall, the results of this work emphasize that the need to manage oxidative stress in an optimal way may have contributed significantly to drive the evolution of reproductive strategies. The findings of this meta-analysis offer a starting platform for future research to investigate the reasons of and mechanisms driving sexual differences in oxidative balance further.

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Supplementary Material

Supplementary material can be found at <http://www.cz.oxfordjournals.org/>.

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