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Aggression, but not testosterone, is associated to oxidative status in a free-living vertebrate

Caroline Isaksson[1,5)], **Geoffrey M. While**[2)], **Jo McEvoy**[2)], **Janske van de Crommenacker**[1)], **Mats Olsson**[3)], **Ton G. G. Groothuis**[4)], **Jan Komdeur**[1)] **& Erik Wapstra**[2)]

([1] University of Groningen, Animal Ecology Group, P.O. Box 11103, 9700 CC Groningen, The Netherlands; [2] University of Tasmania, School of Zoology, Hobart, 7001 Tasmania, Australia; [3] University of Wollongong, School of Biological Sciences, Wollongong, 2522 NSW, Australia; [4] University of Groningen, Behaviour Biology, P.O. Box 11103, 9700 CC Groningen, The Netherlands)

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Summary

Aggression often shows large inter-individual variation, but high intra-individual consistency. Although the physiological basis and direct costs of aggression are generally well known, less is known about the physiological costs such as increased oxidative stress (OS). This can occur via increased leakage of oxidants during high metabolic demands such as physical activity, or by hormones regulating both metabolism and aggression. Here we address this within a natural population of White's skinks, *Egernia whitii*; a species in which both sexes exhibit consistent aggressive phenotypes, and sex-specific associations between testosterone and aggression. The results reveal that males' aggressive phenotype, independent of testosterone, was positively associated with antioxidant capacity (OXY), while there was no significant association in females. Oxidative damage (ROM) and oxidative stress index (OI), were not influenced by aggressive phenotype or testosterone, but showed borderline positive associations with body size (i.e., age). The results failed to show that high testosterone increases OS. Instead, OS may be related to sex-specific behavioural patterns associated with aggressive phenotype such as territory and mate acquisition. Although experimental work is needed to identify the causal links for these patterns, the results highlight the need to consider proximate mechanisms to understand constraints on phenotypic variation.

Keywords: antioxidants, ectotherm, *Egernia whitii*, oxidative stress, personality.

[5)] Corresponding author's current address: University of Oxford, EGI, OX1 3PS, UK, e-mail: caroline.isaksson@zoo.ox.ac.uk

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1. Introduction

Genetic and phenotypic variation in individual-level aggression is widespread within animal populations. In many cases, this individual-level variation in aggression shows considerable consistency (i.e., individuals have a distinctive aggressive phenotype; Sinervo et al., 2000; Gosling, 2001; Sih et al., 2004; Duckworth, 2006a; While et al., 2009a). Such aggressive phenotypes can have important implications for individual fitness as recent work across a range of taxa has demonstrated that an individual's aggressive phenotype can play an important role in both its reproductive success and, to a lesser extent, its survival (Dingemanse & Reale, 2005; Duckworth, 2006a; Boon et al., 2007; Reale et al., 2007; Sinn et al., 2008; Smith & Blumstein, 2008). As a result, the presence of stable aggressive phenotypes has the potential to exert a significant influence on both large-scale population dynamics and important evolutionary processes (e.g., Duckworth, 2006b, 2009; Duckworth & Badyaev, 2007; see also Moss et al., 1994; Mougeot et al., 2003).

Despite evidence for fitness benefits of consistent inter-individual aggression, the relationship between behaviour and fitness is not clear cut. Indeed, consistency in aggression has the potential to generate trade-offs, such that levels of inherent aggressiveness may have fitness benefits in some functional contexts but costs in others. For example, aggressive individuals may be more successful in defending resources (such as food or territories) from conspecifics but these same individuals may also display inappropriate aggression towards potential mates (Sih et al., 2004; Johnson & Sih, 2005). Aggression can also generate physiological costs (e.g., Costantini et al., 2008). Specifically, aggression is a strong mediator of physical performance, which has been shown to increase oxidative metabolism (Marler et al., 1995). During oxidative metabolism the mitochondria leak out reactive oxygen species (ROS), which can cause oxidative damage to adjacent biomolecules if not detoxified by antioxidants or prevented from being generated in the first place by mitochondrial un-coupling proteins (Finkel & Holbrook, 2000). A higher oxidant generation to antioxidant protection is referred to as oxidative stress (OS) (Halliwell & Gutteridge, 2002). As the accumulation rate of oxidative damage is often found to increase with age, it has been suggested that activities that increase oxidative metabolism and thereby OS, such as aggression, may speed up the rate of senescence (Beckman & Ames, 1998).

Additionally, aggression is often regulated by steroids, such as testosterone and glucocorticoids (e.g., Woodley & Moore, 1999; Soma, 2006), which themselves can influence oxidative metabolism and increase whole body metabolic rate and physical activity independently of aggression (e.g., Gupta & Thapliyal, 1985; Al-Sadoon et al., 1990; Wikelski et al., 1999; Olsson et al., 2000; Ros et al., 2004; Soma, 2006). Therefore, if aggression comes with a physiological cost through dealing with increased ROS, understanding the links between an individual's inherent level of aggressiveness, its mediating hormones (e.g., testosterone) and OS will be crucial for fully understanding the consequences of consistent behavioural phenotypes and how interspecific variation in behaviour is maintained (Isaksson et al., 2011). Despite this, the covariation between behavioural phenotypes, such as aggression and OS, are yet to be explored in natural populations.

The main aim of this study is to examine links between aggression, circulating testosterone and OS parameters in a free-living vertebrate population. Specifically, we examined three OS parameters, measured in blood plasma, including antioxidant capacity (OXY), reactive oxygen metabolites (ROMs) and an estimated oxidative stress index (OI) based on the proportion of ROM to OXY (Vassalle, 2008). We used White's skink (*Egernia whitii*) as our model organism, as it exhibits high intra-individual consistency in conspecific aggression within (Sinn et al., 2008; While et al., 2009a) and between seasons (While et al., 2010). Furthermore, individuals exhibit temporal intra-individual consistency in their circulating testosterone concentrations, which is unrelated to aggression in females, and negatively related in males (While et al., 2010). Consequently, this system offers the potential to disentangle the direct (through aggression itself) and indirect (through testosterone) links between aggression and OS. In males we predict that, if OS status is positively associated with aggression, a negative relationship should be revealed between testosterone and OS, and *vice versa* if the relationship is reversed (see Figure 1). For females, the testosterone levels are significantly lower, and an effect of testosterone may, therefore, be less pronounced. Thus, we predict that aggression should have a greater influence on female OS physiology compared to testosterone. More specifically, a positive association of either aggression or testosterone with ROM indicates that more oxidants are produced than the antioxidant system (OXY) can deal with, leading to oxidative damage. The ROMs are, however, not only markers of cellular damage; they are also active as oxidants, which is the reason for also using the

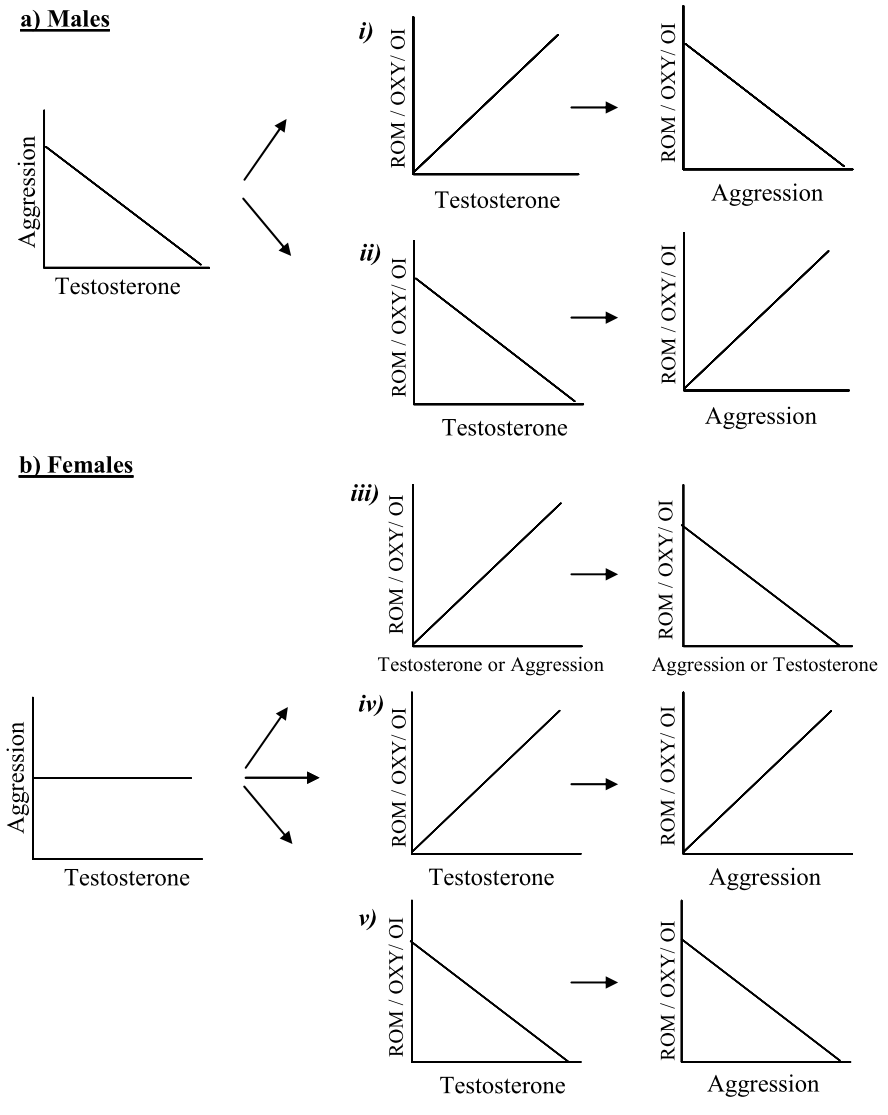

Figure 1. Schematic illustration of the predictions of relationships between OS and aggression, and OS and testosterone (T) given the negative association between aggression and T for (a) males, and lack of relationship for (b) female skinks (While et al., 2010). In males: if T is positively associated with OS parameters then a negative relationship is expected between aggression and OS (i) and vice versa (ii). In females, the prediction for T is independent of aggression; thus, four different scenarios are possible (summarized in iii, iv, v, i.e., two scenarios in iii).

oxidative stress index (OI, see below). High concentrations of ROMs have severe consequence for cellular functioning (Halliwell & Gutteridge, 2002). However, the lack of a relationship with ROMs, and instead a positive relationship with OXY, can indicate an up-regulated defence system that are able to combat the increased oxidative challenges (Costantini & Verhulst, 2009; Monaghan et al., 2009). Alternatively, high OXY in the plasma can be a result of phenotypic specific mobilization or intake of antioxidants. Short-term up-regulation of OXY is unlikely to have major costs, but to maintain high concentrations over a longer time period can generate conflicts with other resource demanding functions (Costantini & Verhulst, 2009; Dowling & Simmons, 2009; Monaghan et al., 2009; Isaksson, 2010). Finally, a positive association between aggression/testosterone and OI indicates that the effects can have long-term implications. A high OI, generated by high ROMs and low OXY, could possibly cause future costs in terms of increased susceptibility to oxidative stress and consequently in terms of fitness.

2. Materials and methods

2.1. Study species

Egernia whitii is a medium sized (up to 100 mm snout–vent length (SVL)) viviparous lizard found throughout a broad altitudinal (0–1600 m) and habitat (coastal heaths, grasslands, and forests) range in south-eastern Australia. Our study population occurs on the East coast of Tasmania, Australia (42°57′S, 147°88′E). Morphology and life history traits vary geographically in *Egernia* (Chapple, 2005), but in Tasmania, the species is sexually monomorphic, with individuals becoming reproductively mature at approximately 3 years, and displaying an overall lifespan of 9–10 years. Reproduction occurs annually, with mating occurring during the Austral spring (September–October) and gestation spanning 3–4 months (While et al., 2007).

2.2. Field protocol

All subject lizards were part of a larger life history study which took place across five subsequent reproductive seasons, 2004/2005 to 2008/2009 (see While et al., 2007, 2009a,b,c). All individuals in the population were, therefore, toe-clipped to allow for unique identification previous to the present

study. At the beginning of each field season, all individuals in the population were captured using a mealworm 'fishing' technique. This was achieved by attaching a mealworm to the end of a fishing line and placing it in front of the lizard and lifting the lizard into a collection bucket once it had grabbed the mealworm. Once captured, individuals were measured for weight (± 0.1 mg) and body size (SVL), ± 0.5 mm using digital calipers), and sex was determined via eversion of the hemipenes.

To collect blood samples for hormone and oxidative stress analyses, we caught a subset of the adult population at three further time periods during the 2007/2008 reproductive season. A drawback of working with this relatively small lizard is that the amount of blood that can be taken at a given time point is insufficient to measure all assays at the same time. Blood sampling for the OS assays was taken in late summer (February 18–March 6, 2008; $N = 29$ females and 25 males, $N = 54$) at the same time aggression assays were carried out (see below). Testosterone samples were collected at two time periods; the first proceeding the end of the mating season (Oct 31–Nov 13, 2007; $N = 25$ females and 18 males), and the second proceeding the end of (female) gestation (Jan 18, 2008; $N = 26$ females and 12 males). Of the 81 testosterone samples 27 individuals were assayed across both time periods, resulting in samples being collected for 54 unique individuals (full data set used in While et al., 2010). At each collection period, blood samples (90–110 μl, average 98.86 ± 0.47 μl) were taken within 1–6 min (average 3.44 ± 0.17 min) after capture by venipuncture of the *sinus angularis* (the corner of the mouth). Neither of the assays were significantly influenced by the time between capture and sampling, however, antioxidant activity showed a non-significant tendency to increase with time (testosterone: $N = 81$, $r = -0.18$, $p = 0.11$; OS assays: $N = 53$, ROM: $r = 0.09$, $p = 0.56$: OXY $r = 0.26$, $p = 0.06$). Blood samples were kept on ice until centrifugation (6000 rpm for 5 min) at the end of each field day. Plasma was then stored at -25° C until assayed.

2.3. *Behavioural assays*

A total of 64 *E. whitii* were assayed for conspecific aggression at a single time period in 2008 (February 18–March 6), comprising 25 males and 39 females (see also While et al., 2010). To assay aggression levels, individuals were captured in the field and then transported to the University of Tasmania

where they were housed individually in rectangular plastic terraria (30 × 60 × 40 cm), placed in temperature- and light-controlled rooms with lights set to ambient day lengths (Hobart, Tasmania, Australia). Housing terraria contained a basking rock and basking light at one end and a shelter at the opposite end. Food (Tenebrio larvae, crushed fruit) and water were available ad libitum. Basking lights were set on a timer to turn on 1 h after room lights and to turn off 1 h before room lights.

A detailed description of the methodology for the conspecific aggression assays is contained elsewhere (Sinn et al., 2008; While et al., 2009a, 2010) and only relevant details are reported below. During the test period, all individuals were subjected to two identical conspecific aggression tests given by a single experimenter (J.McE.) on two testing days, 24 and 48 h after capture. Behavioural tests were conducted between 14:00 and 17:00 h, allowing lizards to obtain preferred body temperatures before tests. The test order for individual lizards was randomized on each test day. Behavioural tests consisted of the experimenter touching the lizard with a realistic conspecific clay model attached at the end of a fishing rod (for similar approaches, see Lopez et al., 2005; Sinn & Moltschaniwskyj, 2005). Models were scented with male and female *Egernia* urine and faeces collected from unrelated laboratory animals. Lizards were touched on the center of the snout by the model up to 10 times or until they fled into or on top of the shelter. For each test, four behaviours were measured: the number of touches required before the lizard fled, the number of back arches (a display whereby the spine of the lizard is bent in a concave manner), the number of times the lizard displayed with an open mouth, and the number of times the subject actively bit the model. The four behaviours are highly inter-correlated, loading strongly on a single common PCA component (Sinn et al., 2008; While et al., 2009a, 2010) and closely resemble agonistic behaviours recorded within this and other species of *Egernia* (e.g., Langkilde & Shine, 2004, 2005, 2007; O'Connor & Shine, 2004; Langkilde et al., 2005). Behaviours in tests were recorded for the duration of stimulus presentation (i.e., the number of touches); thus, multiple frequencies of each behaviour were possible since lizards could perform behaviours anew after each touch with the model.

A unique scale score for each lizard was computed by summing the standardized frequencies of the observed variables in the two tests given within the collection period. Standardization was according to the grand mean of behaviours to mathematically allow for mean-level changes in aggregate scale

scores. Higher scale scores represented more aggressive overall responses (see Sinn et al., 2008; While et al., 2009a). We have previously shown that aggression scores exhibit strong repeatability both within (Sinn et al., 2008; While et al., 2009a) and between seasons (While et al., 2010), indicating a high degree of intra-individual stability in aggression for both males and females. Specifically, aggression scores calculated during this study were highly consistent with those calculated for the same lizards during previous studies (males, $\rho = 0.77$, $F_{10,20} = 4.49$, $p < 0.01$; females, $\rho = 0.62$, $F_{18,36} = 2.63$, $p < 0.01$) with no sex-specific differences in aggression. Aggression is unrelated to body size (While et al., 2009a).

2.4. Testosterone analysis

All plasma available was used during extraction (16.5–69 mg). In total all 81 plasma samples were assayed; however, here we only use data of those individuals from which we also scored aggression and measured OS. Thus, a subset of 36 individuals was used (see While et al., 2010 for presentation of full data set). We followed Kingma et al. (2009) with minor modifications, and a detailed description of the testosterone assays is presented in While et al. (2010). Briefly, to count the recovery of the assay, tritiated testosterone was added to the plasma samples. All samples were extracted twice by using diethyl-/petroleum ether (70:30 v/v), dried under nitrogen, and then reconstituted in 70% methanol and stored over night. The following day samples were spun down, decanted and dried. A competitive-binding radioimmunoassay (RIA) was used to determine the testosterone concentrations (Endocrine Science, Calabasas Hills, CA, USA). The average recovery rate for testosterone was 78%. Validation of the assay for the species was conducted by making dilution curves for four samples. All samples were analyzed within one assay and the intra-assay coefficient of variation (CV) was 3.8%. The concentration is presented as ng/ml plasma.

2.5. Oxidative stress assays (ROMs and OXY)

Oxidative damage and antioxidant capacity were measured by the d-ROM and OXY-Adsorbent test kits, respectively (Diacron, Grosseto, Italy). The d-ROM test measures the plasma concentration of hydroperoxides (ROOH), which is a group of reactive oxygen metabolites (ROMs). By being oxidants themselves, ROMs are directly related to reactive oxygen species (ROS), but

in contrast to ROS they are relatively stable. ROMs are generated by oxidation of a wide range of biomolecules such as lipids, amino acids, glycosides, proteins and nucleotides. Consequently, the d-ROM test provides a marker of current oxidative damage, but also potential future damages. Hydroperoxides increase with senescence, but an increase can also occur more rapidly for example, during physical exercise, disease or unhealthy living (e.g., Vassalle 2008; Vassalle et al., 2008; for ectotherms, see Costantini et al., 2009; Criscuolo et al., 2010). The OXY-adsorbent assay captures all non-enzymatic exogenously (e.g., carotenoids, flavonoids and tocopherols) and endogenously (e.g., glutathione, uric acid and bilirubin) synthesized antioxidants. The capacity of the circulating antioxidants is tested by adding a highly potent oxidant, hypochlorous acid (HClO), and thereby the OXY test provides a marker of the individuals' plasma antioxidant barrier to protect cells and tissues against free radical attack. Moreover, oxidative stress is defined as an imbalance between oxidants and antioxidants. The ROM and OXY data can be used to calculate an oxidative stress index (OI), which has been verified by Vassalle (2008). The OI was calculated as $\mathrm{OI} = \mathrm{SV}_{\mathrm{ROMs}} - \mathrm{SV}_{\mathrm{OXY}}$, in which SV is the measured value (V) minus the average value of the population (M) divided by the standard deviation (SD) of the population ($(V - M)/\mathrm{SD}$; see Vassalle, 2008). Consequently, a high ROM and low OXY will generate a high OI, which suggests that an OI is a powerful measure of oxidative stress because it reflects both oxidative and antioxidant components.

In total 54 blood samples were used, however, two blood samples were too small for us to be able to perform both assays and, therefore, the sample size for ROM was reduced to 52. The protocols provided with the kits were followed with minor modifications. Briefly, 15 μl (ROMs) and 5 μl (OXY) plasma was used. Incubation temperature was set to preferred body temperature (34°C) of *Egernia*. The d-ROMs are presented as mmol H_2O_2 equivalents/ml plasma, and OXY as mmol HClO neutralized/ml plasma, both at 546 nm. Before the assays, the colour of all plasma samples was scored. The scoring was conducted under standardized tungsten light, by the eye by the same observer (CI) and divided into one of five clearly defined colour categories: transparent, white, yellow, light red and red. These values were used to standardize the ROM and OXY values since it is known that in particular haemolysis of red blood cells can interfere with ROM and OXY (plasma colour, ANOVA, ROM: $p = 0.098$ and OXY: $p = 0.032$) due to leakage of

Table 1. Summary of the statistical analysis on aggression and OS.

Source	df	df model	Type III SS	*F*	*p*
Response variable ROM					
Sex	1	49	2.286	0.080	0.778
SVL	1	50	105.551	3.772	0.058
Sex × SVL	1	48	63.274	2.278	0.138
Aggression score	1	46	0.484	0.017	0.897
Sex × Aggr. score	1	45	0.275	0.010	0.923
Response variable OXY					
Sex	1	47	0.000	0.240	0.627
SVL	1	46	0.002	1.519	0.224
Sex × SVL	1	45	0.001	1.281	0.264
Aggression score	1	47	0.000	0.361	0.551
Sex × Aggr. score	1	47	0.006	6.253	0.016
Response variable OI					
Sex	1	48	0.000	0.000	0.990
SVL	1	48	1.572	1.464	0.232
Sex × SVL	1	48	4.069	3.788	0.058
Aggression score	1	46	1.553	1.404	0.242
Sex × Aggr. score	1	45	0.128	0.155	0.736

Results from three general linear models (GLM) with backward elimination: reactive oxidative metabolites (ROMs), antioxidant capacity (OXY) and oxidative stress index (OI). Results are shown prior to elimination; the order of elimination is indicated by the df of the model (the lowest value was eliminated first, and the highest df indicates final model). Sex was included as fixed factor, and the other variables as covariates.

intra-cellular antioxidants and metabolites. The results were standardized for plasma colour by using the residuals in the regression analysis. Although, the plasma colour did not show a significant influence on ROM in this dataset, we prefer to be conservative and standardize both ROM and OXY. Using the raw data as opposed to the standardized data of ROM did not change the interpretation of any of the results in Table 1. Unfortunately, insufficient plasma was available for assessing repeatability; however, other studies have found the assay to be highly repeatable. For example, in humans, intra-assay CV ranging between 1% and 3% and between-runs 5–9% (e.g., Vassalle, 2008) and for a lizard species (Galápagos land iguana *Conolophus subcristatus*) the correlation coefficient was $r = 0.98$, $p < 0.0001$ (Costantini et al., 2009).

2.6. Statistical analysis

Of the 64 individuals assayed for aggression, 54 were assayed for OS, and 36 were measured for both OS and testosterone. We have previously shown a seasonal decline in circulating testosterone ($p = 0.05$, While et al., 2010), however, this was not observed for the individuals used within this study (males: $N = 6$, paired t-test; $p = 0.449$, females: $N = 9$, $p = 0.194$); therefore, we pooled samples across both hormone sampling periods in order to increase statistical power. We ran general linear models (GLM) with backward elimination of non-significant factors to examine the effects of aggression and testosterone on ROS. In all models ROM, OXY and OI were included as response variables. We first ran full models, including aggression score, testosterone and SVL as covariates. We ran each of these models separately for males and females, due to the extreme sex differences in testosterone concentrations (While et al., 2010). As including testosterone as a predictor decreased our sample size considerably, we re-ran the above models while excluding testosterone to examine if the links between aggression and ROS held up with a greater sample size (Table 1). Sex was included as a fixed factor in these models. Sample sizes differ between models as data were not available for all target traits for some individuals. All model assumptions were fulfilled and the statistical analysis was analyzed in JMP 8 (SAS Institute, Cary, NC, USA).

3. Results

There was a significant interaction between aggressive phenotype and sex for OXY (Table 1). Post-hoc analyses revealed a positive correlation between aggression and OXY for males ($N = 25$, $r = 0.511$, $p = 0.009$) and no correlation for females ($N = 29$, $r = -0.242$, $p = 0.206$, Figure 2). These patterns were not significant for ROM or OI (Table 1). There was no significant sex difference in any of the OS parameters (Table 1, for mean concentrations for males and females see Table 2) or in body size (t-test: $p = 0.676$, Table 2). Body size showed a borderline non-significant positive association with ROMs ($p = 0.058$, Table 1). OI showed a borderline non-significant interaction between sex and body size ($p = 0.058$, Table 1). Similarly to above, males showed a positive correlation (now only approaching significance) between size and OI ($N = 25$, $r = 0.369$, $p = 0.070$), and

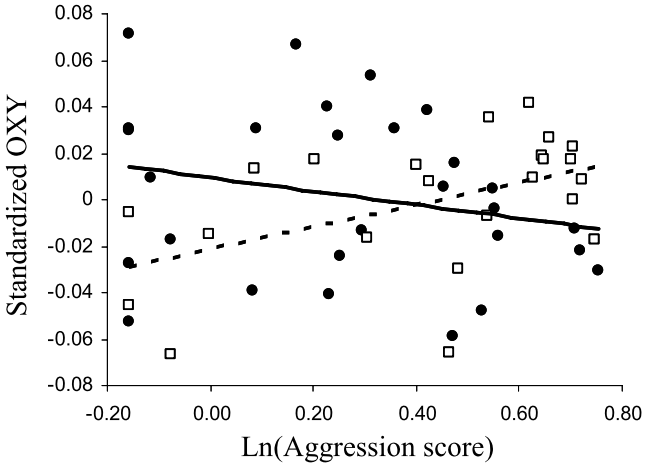

Figure 2. Aggression and antioxidant capacity in males and females. Significant interaction in the relationship between aggressive phenotype and antioxidant activity (OXY) between male and female skinks, *Egernia whitii* ($p = 0.016$, more details in section 3). Males with open square and open line (significant positive slope) and females are presented with filled circles and filled line. OXY was standardized for plasma colour (see section 2.5) and aggression was log-transformed (see section 2.3).

Table 2. Absolute mean ± SE values for males and female *E. whittii*.

	Females (mean ± SE)	Males (mean ± SE)
Body size (SVL, mm)	78.79 ± 0.65	79.16 ± 0.58
Testosterone (ng/ml)	1.76 ± 0.66	20.81 ± 2.36
OXY (mmol HCIO/ml plasma)	0.25 ± 0.01	0.25 ± 0.01
ROM (mmol H_2O_2/ml plasma)	16.60 ± 1.06	15.64 ± 1.25
OI	−0.002 ± 0.22	0.159 ± 0.24

Formula for OI presented in section 2.5.

for females there was no relationship ($N = 29$, $r = -0.122$, $p = 0.528$). Separate regression analyses of male and females revealed that testosterone was not significantly associated with ROM, OXY, or OI (all $p > 0.28$). In the models with males, OXY and aggression still revealed a significant positive relationship, although sample size was reduced when testosterone was included ($N = 13$, male aggressive phenotype: $F_{1,11} = 5.47$, $p = 0.039$; testosterone: $F_{1,11} = 1.31$, $p = 0.277$).

4. Discussion

It has been suggested that aggression-induced activity and testosterone elevate oxidative stress (e.g., Alonso-Alvarez et al., 2007; Dowling & Simmons, 2009), however, these links are yet to be examined in wild populations. We address this by exploring the relationship between aggression, testosterone and OS in a free-living reptile population. We show (1) different relationships between aggression and antioxidant capacity (OXY) for males and females, with a positive relationship between aggressive phenotype and OXY for males (independent of size) but no detectable relationship for females, and (2) no significant relationship between plasma testosterone and the OS parameters.

In *E. whitii*, there is large inter-individual variation in aggression in both males and females (Sinn et al., 2008; While et al., 2009a, 2010). The aggressive behaviour of *E. whitii* has been shown to have positive fitness consequences for females through increased offspring survival, potentially via protection of offspring against con-specific infanticide (Sinn et al., 2008; see also Lanham & Bull, 2000; O'Connor & Shine, 2004). Given the physical nature of aggressive interactions and the potential injuries in this genus (Chapple, 2003; Langkilde & Shine, 2004, 2005, 2007; O'Connor & Shine, 2004; Langkilde et al., 2005), we predicted that aggressiveness would increase oxidative metabolism and thereby influence parts of the OS system. In accordance to our prediction, there was a positive association between aggressive phenotype and OXY in males, but for females the association was lacking. Although these results still need to be verified experimentally, these results suggest that, at least in males, aggression may induce an increased oxidative challenge resulting in an elevation of antioxidant defence. This effect is as we predicted, independent of body size since there is no link between aggression and size in this species (While et al., 2009a). Alternatively, other traits correlated with aggressive phenotype (e.g., antioxidant mobilization or diet intake) could influence the antioxidant activity. However, the food quality (i.e., antioxidant content) would need to differ significantly between territories of aggressive versus less aggressive males in order to account for the variation induced by, for example, timing of last meal prior to sampling. The lack of association between aggressive phenotype and ROM and OI, suggests that the increased antioxidant defence is sufficient to combat accumulation of damages and oxidative stress in males. However, it could also

be the result of low statistical power preventing us from detecting any biologically significant differences. The only other study that explores the link between aggressive phenotype and OS showed that non-aggressive (long attack latency, LAL) selected strains of mice have higher antioxidant activity than aggressive (short attack latency, SAL) strains (Costantini et al., 2008). However, it is hard to make any valuable comparison between results from free-ranging lizards of different qualities and life-histories to those of selected strains of laboratory mice.

In contrast to males, we found no significant relationship between aggressive phenotype and antioxidants in females. There are a number of potential explanations for this difference between the sexes. Firstly, there may be sex differences in the frequency of aggressive interactions (as opposed to levels of aggression per se). Variation in the number of female partners is a key predictor of male reproductive success within this species (While et al., 2011), suggesting strong competition between males for access to females. Thus, the greater competition for resources (including females) between males may result in a male's aggressive phenotype being a greater predictor of physical activity than it is for females. Alternatively, the lack of relationship between aggressive phenotype and OXY in females could be a delayed consequence of pregnancy. For example, OXY could decrease in highly fecund females if they actively transfer antioxidants to yolk or embryo, or increase due to higher concentrations of circulating yolk proteins with antioxidant functions (Surai, 2002). Either way, it could mask any direct relationship between aggression and OXY. However, during pregnancy OXY could also be indirectly influenced by mild uncoupling of mitochondrial proteins reducing ROS generation during cellular respiration and thereby, masking the relationship (see Olsson et al., 2009). Further studies are required in order to be confident that the lack of association in females represents a sex-specific decoupling between aggressive phenotype and OXY.

We found no significant association between testosterone and any of the OS parameters measured in this study. Testosterone, and its metabolites serve multiple functions in vertebrates including direct increase of oxidative metabolism and regulation of aggressive behaviours (e.g., Al-Sadoon et al., 1990; Wikelski et al., 1999; Olsson et al., 2000; Ros et al., 2004; Soma, 2006; but see also Buttemer & Astheimer, 2000). However, unlike in many species, testosterone is negatively correlated with aggression in male *E. whitii* and unrelated to aggression in females (While et al., 2010; see also Rubenstein &

Wikelski, 2005). If the effect of testosterone on OS would have been mediated primarily through its effect on aggression (e.g., decreased testosterone results in increased aggression which increases oxidative metabolism) the functional decoupling of aggression and testosterone for females may explain the lack of an association. However, if the effect of testosterone on OS in males was primarily via aggression, a negative association between testosterone and OXY would have been revealed. Instead we were unable to detect a significant effect, which may suggest that testosterone is not associated with oxidative metabolism directly during breeding or indirectly via its effect on aggression. Unfortunately, testosterone assays require a substantial amount of plasma; thus, we were unable to quantify whether other hormones, such as estrogens or progesterone, which have been shown to also mediate aggression, influence oxidative metabolism (Gupta & Thapliyal, 1985; Silverin et al., 2004; Weiss & Moore, 2004). An alternative explanation is that the lack of a relationship between OS and testosterone is a function of our sampling regime, in which OS and testosterone samples were temporally separated (approximately 3 months). However, as plasma testosterone concentrations in *E. whitii* exhibit high levels of intra-individual repeatability (While et al., 2010), any relationship between testosterone and OS, at the time of OS sampling, should have been identified if present.

Although non-significant, the only predictor that showed a tendency to be associated with ROM was body size, with larger lizards having higher concentrations of oxidative metabolites. Male, but not female, body size also showed a positive tendency to associate with OI, suggesting that larger males may have an increased future susceptibility to OS. Since size and age are generally positively correlated in reptiles (e.g., Wapstra et al., 2001), the borderline non-significance may have reached significance if chronological age was known. Unfortunately, since this species is long-lived for a reptile (9–10 years), accurate data on chronological age are currently unavailable. Taken together, these results are intriguing and warrant further investigation, specifically in relation to sex differences in cellular degeneration with age (see free radical theory of ageing, e.g., Finkel & Holbrook, 2000), and the consequences it may have for male and female life-history.

In conclusion, the present study shows sex differences in the relationship between aggressive phenotype and OS within free-living White's skink population, with males showing a positive link between aggressive phenotype and antioxidant activity, and females no detectable relationship. While

our results are correlative, they do highlight the need to consider the oxidative costs of behavioural phenotypes when trying to understand their role in mediating evolutionary processes (e.g., Biro & Stamps, 2008; Dowling & Simmons, 2009). Such correlative patterns are crucial for generating hypotheses which can form the basis of ongoing experimental work aimed at identifying the causal mechanisms underlying these links (e.g., the manipulation of behavioural or endocrinological traits and examining their effects on the oxidative balance). In the long-term, this combination of correlative and experimental work will allow us to fully elucidate the mechanistic basis of social and aggressive behaviour within this species, and reveal whether this imposes unavoidable costs in relation to life history.

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