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Oxidative status and social dominance in a wild cooperative breeder

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Summary

1. Oxidative stress has been proposed as a key mediator of life-history trade-offs, yet the social factors that affect patterns of oxidative status amongst individuals in animal societies remain virtually unexplored.

2. This is important, as rank-related differences in reproductive effort in many social species have the potential to generate, or indeed arise from, differences in oxidative status across dominance classes.

3. Here, we examine rank-related variation in oxidative status before and after a lengthy breeding season in a wild cooperatively breeding bird with high reproductive skew, in the semi-arid zone of Southern Africa; the white-browed sparrow weaver (*Plocepasser mahali*).

4. Our findings reveal that prior to breeding, neither sex showed rank-related differences in markers of oxidative damage or antioxidant protection, suggesting that dominants' reproductive monopolies do not arise from superior pre-breeding oxidative status.

5. After breeding, however, females (who provision young at higher rates than males) suffered elevated oxidative damage, and dominant females (the only birds to lay and incubate eggs, and the primary nestling provisioners) experienced differential declines in antioxidant protection.

6. While males also showed reduced antioxidant capacity after breeding, this decline was not dependent on rank and not associated with elevated oxidative damage.

7. Our findings suggest that divisions of labour in animal societies can leave the hardest-working classes differentially exposed to oxidative stress, raising the possibility of hitherto unexplored impacts on health and ageing in social species.

Key-words: animal societies, antioxidants, cooperative breeding, oxidative status, oxidative stress, *Plocepasser mahali*, reproductive skew, social dominance, social rank

Introduction

Reactive oxygen species (ROS) are produced as a by-product of metabolism and can cause damage to proteins, lipids and DNA (Halliwell & Gutteridge 2007). Such damage is usually limited by the body's antioxidant system, but when the generation of ROS overpowers this protection, as may occur when the body is working hard or its antioxidant defences are depleted, extensive damage to biomolecules can occur (Finkel & Holbrook 2000). Recognition that cumulative exposure to such 'oxidative stress' can result in diverse pathological effects at the organismal level, including compromised reproduction (Bize *et al*. 2008; Heiss & Schoech 2012) and survival (Saino *et al*. 2011), and accelerated ageing (Beckman & Ames 1998; Monaghan *et al*. 2008), has led to a surge of interest among evolutionary, behavioural and medical biologists in understanding the causes and consequences of individual variation in oxidative status (Lin & Beal 2006; Costantini 2008; Franco *et al*. 2008; Dowling & Simmons 2009; Monaghan, Metcalfe & Torres 2009; Metcalfe & Alonso-Alvarez 2010; Selman *et al*. 2012). However, in vertebrates, research examining oxidative status has so far focussed almost exclusively on solitary or pair-breeding species, leaving the causes of variation in oxidative status in social species largely unknown (van de Crommenacker, Komdeur & Richardson 2011; van de Crommenacker *et al*. 2012; Vitousek, Stewart & Safran 2013).

In many vertebrate societies, socially dominant individuals experience differential access to resources and breed at substantially higher rates than subordinates (Keller & Reeve 1994; Magrath, Johnstone & Heinsohn 2004; Hodge

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2009; Koenig *et al.* 2009). The endocrine correlates of social dominance and their fitness implications have long been a focus of research effort in ecology and biomedicine (Wasser & Barash 1983; Creel 2001; Sapolsky 2005; Young *et al.* 2006; Young & Monfort 2009). By contrast, the possibility that social vertebrates also exhibit rank-related differences in oxidative status, which could underpin or arise from such differences in competitive ability and reproductive effort, remains largely unexplored, despite potential implications for the patterns of health, ageing and reproductive success in animal societies.

Dominant individuals could differ from subordinates in their oxidative status *prior* to reproductive episodes, as rank-related differences in intrinsic quality or access to key dietary resources could leave them with stronger antioxidant defences (Catoni, Peters & Martin Schaefer 2008; Cohen, McGraw & Robinson 2009; van de Crommenacker *et al.* 2011). As superior antioxidant defences (and/or lower levels of oxidative damage) can promote investment in reproduction (Bertrand *et al.* 2006; Pike *et al.* 2007; Heiss & Schoech 2012), and delay senescence (Saino *et al.* 2011), stronger defences among dominants might conceivably underpin their higher reproductive success than subordinates (Keller & Reeve 1994) and evidence of longer lifespans among dominants (Carey 2001; Dammann & Burda 2006). Indeed, in some eusocial insects, highly reproductive queens exhibit stronger resistance to oxidative damage than their non-reproductive, shorter-lived workers (honeybee *Apis mellifera*: Haddad, Kelbert & Hulbert 2007; Aamodt 2009). Barn swallows (*Hirundo rustica erythrogaster*) experimentally given elevated social status by plumage darkening subsequently exhibited lower blood concentrations of reactive oxygen metabolites (ROMs – a measure of intermediate oxidative damage products), potentially as a result of altered social interactions with their conspecifics (Vitousek, Stewart & Safran 2013). Similarly, prior to nesting, dominant female Seychelles warblers, *Acrocephalus sechellensis,* who subsequently reproduced also experienced lower ROMs than subordinate non-breeders (van de Crommenacker, Komdeur & Richardson 2011). While such patterns are broadly consistent with dominants exhibiting lower oxidative damage and stronger antioxidant defences, they might alternatively reflect a differential *need* for dominants to upregulate their antioxidant defences (Beaulieu *et al.* 2011), given the elevated exposure to oxidative stress that may accompany their higher reproductive rates (van de Crommenacker, Komdeur & Richardson 2011; Heiss & Schoech 2012; Olsson *et al.* 2012; Stier *et al.* 2012; Fletcher *et al.* 2013).

Dominant individuals may indeed be differentially at risk of oxidative stress, as dominance tenures frequently entail physiological challenges rarely faced by subordinates (Creel 2001; Sapolsky 2005). Dominants typically breed at substantially higher rates than subordinates (Keller & Reeve 1994; Magrath, Johnstone & Heinsohn 2004), are frequently involved in aggressive encounters with conspecifics (Clutton-Brock *et al.* 2006) and may invest more in mate and territory defence (Mass, Heistermann & Kappeler 2009). Both investment in reproduction (whether due to gamete production, mate attraction or parental investment) and elevated activity during aggressive interactions can promote the production of ROS and/or deplete antioxidant reserves (Blount, Houston & Møller 2000; Wiersma *et al.* 2004; Costantini *et al.* 2008; Radak *et al.* 2008; Rammal, Bouayed & Soulimani 2010; Stier *et al.* 2012). Indeed, a study of Seychelles warblers found evidence that male breeders suffered increased oxidative damage when guarding their nest and mate, relative to the pre-nesting phase (van de Crommenacker, Komdeur & Richardson 2011). That this result was not observed in non-breeding subordinate males highlights the potential for the activities of dominants to yield rank-specific oxidative burdens. Whether such effects are widespread, and whether dominants can avoid them by pre-emptively upregulating antioxidant defences before breeding episodes, remains unclear.

Here, we address this gap in our understanding by investigating whether dominants and subordinates differ in oxidative status before and after a lengthy breeding season in a wild cooperative breeder with high reproductive skew, the white-browed sparrow weaver (*Plocepasser mahali,* Fig. 1). We investigate whether rank-related differences in reproductive effort (i) reflect dominants starting the breeding season with stronger oxidative status (lower oxidative damage and/or higher antioxidant protection) and better body condition than their same-sex subordinates; and (ii) are associated with differential declines in oxidative status and body condition among dominants relative to subordinates over the course of the breeding season. We also investigate whether either sex shows improved oxidative status when living in larger groups; a potentially general but unexplored benefit of group-living.

As oxidative status is a complex, multifaceted physiological condition (Hõrak & Cohen 2010), we characterize a suite of relevant metrics: plasma levels of malondialdehyde (MDA; a marker of lipid peroxidation), plasma 'Trolox-equivalent antioxidant capacity' (TEAC; which reflects non-enzymatic antioxidant activity); and erythrocyte levels of superoxide dismutase (SOD; a key intracellular

Fig. 1. Male white-browed sparrow weaver (*Plocepasser mahali)*. Copyright Dominic Cram.

antioxidant enzyme). Recent work has highlighted the strong contribution of the nitrogen waste product uric acid towards measurements of TEAC in birds (Cohen, Klasing & Ricklefs 2007); we therefore statistically control for these potentially confounding effects. As oxidative status can also show considerable interindividual variation due to factors such as early life conditions (Blount *et al*. 2003), diet (de Ayala, Martinelli & Saino 2006) and territory quality (van de Crommenacker *et al*. 2011), we employ a longitudinal within-individual paired-sampling approach, sampling every focal bird (41 dominants and 52 subordinates across 32 wild social groups) both before and after the breeding season.

Materials and methods

STUDY SPECIES AND POPULATION

Data were collected in the context of a long-term study monitoring 40 cooperative groups of white-browed sparrow weavers, in the semi-arid Kalahari desert of Southern Africa, at Tswalu Kalahari Reserve, South Africa (27°16′S, 22°25′E). All birds were fitted with a metal ring and three colour rings for identification (SAFRING licence 1444); males and females were distinguished by beak colour (Leitner, Mundy & Voigt 2009). White-browed sparrow weavers live in year-round territorial groups of 2–12 birds, comprising a dominant pair and subordinates of both sexes that have typically delayed dispersal from their natal groups (Harrison *et al*. 2013b). Within-group reproduction is completely monopolized by a single dominant male and female (while *c*. 12% of young are sired by extra-group males, the vast majority of extra-group sires are also dominant males; Harrison *et al*. 2013a,b). The dominant female is the sole egg producer and incubator [mean clutch size ± standard deviation (SD): 2·11 ± 0·52 eggs] as well as the primary provisioner of nestlings, but is assisted with provisioning by all group members (Lewis 1982; Harrison *et al*. 2013b). The dominant male sings dawn song throughout the breeding season (Voigt, Leitner & Gahr 2006; York, Young & Radford 2014); although subordinate males do occasionally produce dawn song, they are invariably out-sung by their dominants (York 2012). Group size was assigned as the number of birds consistently seen foraging together and roosting in their territory's central tree(s).

CAPTURE AND BLOOD SAMPLING

Blood sampling was conducted during two phases, corresponding to immediately before and after the breeding season ('pre phase' – September 2011; 'post phase' – April 2012). Non-breeding status was confirmed for all groups by nest searches conducted every other day. All birds were sampled during both the pre- and post-season phases. The pre-season phase took place before any eggs had been laid. The post-season phase took place at least 2 months after egg-laying finished; groups were therefore not provisioning nestlings or young fledglings. Blood-sampled birds included 44 females (20 dominants and 24 subordinates) and 49 males (21 dominants and 28 subordinates; four of these subordinates became dominant during the breeding season) from 32 social groups. Although reproductive effort data were not available for all groups, of the 24 groups for which data were available, all produced eggs and provisioned nestlings during the breeding season. Sample sizes for the three markers of oxidative status varied slightly according to the availability of the required samples.

All captures, blood sampling and measurements were conducted by one person (DC). Birds were captured individually at night, by flushing them from their individual roost chambers into a custom capture bag. A small blood sample (*c*. 300 μL; 6·5% of total blood volume, assuming the latter is 10% of body mass w/v) was immediately collected from the brachial vein, using a 26-g needle and heparinized capillary tubes. The lag between bird capture and completion of blood sampling was minimized, but recorded to examine potential effects of capture stress on oxidative status. Recent evidence highlights circadian rhythms in oxidative status (Hardeland, Coto-Montes & Poeggeler 2003; van de Crommenacker *et al*. 2011; Patel, Velingkaar & Kondratov 2014). We therefore also measured the period of time between sunset (when sparrow weavers go to roost) and sample collection for each bird. Body mass was recorded to the nearest 0·01 g (Durascale 100; MyWeigh, Phoenix, AZ, USA) and tarsus length measured ±0·1 mm using callipers. Birds were then returned to their roosts to pass the remainder of the night.

BLOOD PROCESSING AND OXIDATIVE STATUS METRIC DETERMINATIONS

After collection, blood was immediately separated by centrifugation (12 000 ***g*** for 3 min, Haematospin 1400; Hawksley Medical and Laboratory Equipment, Lancing, UK). Erythrocytes drawn from the cellular phase of the separated whole blood were lysed in four times their volume of ice-cold distilled water. This solution was mixed, placed on ice for 5 min and centrifuged for 3 min (12 000 ***g***). The supernatant (erythrocyte lysate) was then drawn off. Plasma from the separated whole blood (for the determination of MDA, TEAC and uric acid levels) and lysed erythrocytes (for the determination of SOD activities) was stored on ice until they could be transferred to liquid nitrogen (mean ± SD time lag from processing to storage on liquid nitrogen: 131 ± 60 min). Samples were transported from the field site to the United Kingdom on dry ice where they were stored at −80 °C until analysis. All samples were analysed within 7 months of the end of the sampling period.

Oxidative damage to lipids

Plasma concentrations of MDA were determined by high-performance liquid chromatography, following Nussey *et al*. (2009). A subset of plasma samples run in duplicate showed high repeatability ($F_{66,67}$ = 15·92, r = 0·88, P < 0·001, see Lessells & Boag 1987).

Superoxide dismutase antioxidant protection

Superoxide dismutase forms part of the first line of defence against oxidative damage (Parkes *et al*. 1998). SOD activity of erythrocytes was determined using a colorimetric assay (Cayman Chemicals, Ann Arbor, MI, USA) and a spectrophotometer (Spectramax M2; Molecular Devices, Sunnyvale, CA, USA). One unit is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical; enzyme activities are reported as units per mL. A subset of samples were analysed in duplicate on separate plates; SOD activities were highly repeatable between plates ($F_{37,38}$ = 6·07, r = 0·72, P < 0·001).

Total Antioxidant Capacity

We estimated non-enzymatic TEAC by measuring the ability of a plasma sample to quench a standardized free radical challenge. Plasma TEAC was determined using a colorimetric assay kit (Cayman Chemicals) and spectrophotometer (Spectramax M2; Molecular Devices). Plasma TEAC values are expressed as concentrations of Trolox (a vitamin E analogue). A subset of plasma samples

were run in duplicate on two plates; TEAC values were highly repeatable between plates ($F_{41,42} = 8{\cdot}20$, $r = 0{\cdot}78$, $P < 0{\cdot}001$).

It has recently been highlighted that up to 90% of the variation in avian plasma TEAC may be due to the 'incidental' antioxidant effects of uric acid, a nitrogen waste product in birds (Cohen, Klasing & Ricklefs 2007) which increases during periods of low food availability (Alonso-Alvarez & Ferrer 2001). The importance of uric acid as an antioxidant *in vivo* remains unclear (Cohen, Klasing & Ricklefs 2007; Kilgas *et al.* 2010) and uric acid production can itself generate ROS (Dröge 2002), leaving avian plasma TEAC values potentially confounded by the incidental activity of uric acid (Cohen, Klasing & Ricklefs 2007; Cohen, Hau & Wikelski 2008; Cohen & McGraw 2009). We therefore used model residuals to statistically control for the effects of uric acid levels on TEAC, following Cohen, Klasing & Ricklefs (2007). First, a linear mixed model was used to confirm uric acid's strong predictive power of TEAC [$\chi^2_1 = 114{\cdot}76$, $P < 0{\cdot}001$, $n = 162$ samples; model estimate: $0{\cdot}39 \pm 0{\cdot}030$, Conditional $R^2 = 0{\cdot}56$, see Nakagawa & Schielzeth (2013)]. Bird identity was the random factor (as two measures were taken from each bird). Secondly, a separate linear model was used, with TEAC as the response and uric acid as the only predictor, to generate residuals (hereafter 'residual TEAC') which yield a measure of plasma antioxidant capacity excluding that arising from uric acid, but retaining any effects of individual identity (as no random factors were included in this model).

Uric acid

Plasma concentrations of uric acid were determined using a fluorescence assay kit (Cayman Chemical) and spectrophotometer (Spectramax M2; Molecular Devices). A subset of plasma samples were run in duplicate on separate plates; uric acid concentrations were highly repeatable between plates ($F_{39,40} = 8{\cdot}35$, $r = 0{\cdot}79$, $P < 0{\cdot}001$).

Scaled body mass

We calculated the Scaled Mass Index (SMI) to compare body condition among dominant and subordinate individuals (Peig & Green 2009). The SMI scales each individual's body mass to the value expected if all birds were of identical skeletal size, using the power relationship between mass and size modelled from the data. We used body mass and tarsus length measures from 186 capture records (before and after the breeding season) of the 93 focal birds. All body mass values were scaled to the mean tarsus length (24·6 mm), using a Secondary Major Axis slope of 2·64 (Peig & Green 2009). The resulting SMI is hereafter referred to as 'scaled body mass'.

STATISTICAL ANALYSES

Statistical analyses were carried out in R (R Development Core Team 2013), using a stepwise model simplification approach (Crawley 2007). Initially, all fixed terms of interest were fitted, followed by the stepwise deletion of terms whose removal from the model resulted in a non-significant change in deviance (using a likelihood ratio test for model comparison, alpha set at 0·05), until the minimal adequate model (MAM) was obtained, in which only significant terms remained. Dropped terms were then added back into the MAM to confirm their non-significance and were retained in the MAM when found to be significant in this context. The homoscedasticity and normality of residuals were inspected visually, and no transformations were necessary.

We focus on intrasexual rank-related differences in oxidative status and scaled body mass and therefore analysed males and females in separate models. For each response term (the oxidative status metrics and scaled body mass), a linear mixed effects model was fitted with dominance status (dominant or subordinate) and 'season phase' (before or after the breeding season), as well as their interaction, as the major predictors of interest. Group size, the number of hours after sunset that the bird was caught and blood sampled and the time lag between bird capture and blood sampling ('capture-to-bleed' lag in seconds) were also fitted as fixed effect predictors (capture-to-bleed lag was not included in models of scaled body mass). Bird ID was nested within Social Group ID with both fitted as random factors, to account for the sampling of multiple individuals within a group and the sampling of each bird twice (before and after the breeding season).

Initially, the association between age and the markers of oxidative status and scaled body mass was investigated using a larger data set containing all known-age individuals captured in either the pre- or post-season phase (84 males and 80 females from a total of 29 groups; mean age in years ± SD: dominants: $3{\cdot}32 \pm 0{\cdot}82$, subordinates $1{\cdot}70 \pm 0{\cdot}88$). The above models were used, with the addition of age in days as a fixed effect. Birds younger than 5 months were excluded from all analyses. Age in days did not significantly predict any of the response terms (MDA, SOD, residual TEAC or scaled body mass), in either males or females, when included in the MAMs or when fitted in isolation (all $P > 0{\cdot}18$). The data sets were therefore restricted to comprise only individuals captured in both pre- and post-seasons, including those whose exact age was not known. Models were then fitted with a two-level age term (younger or older than 2 years); these are the models that are presented in the results section.

Results

SOCIAL DOMINANCE AND OXIDATIVE STATUS AMONG FEMALES

Oxidative damage

Plasma levels of MDA did not differ significantly between dominant and subordinate females ($\chi^2_1 = 1{\cdot}34$, $P = 0{\cdot}25$, $n = 88$ captures of 44 females), and this relationship was not dependent on season phase (Fig. 2a, dominance × season phase interaction: $\chi^2_1 = 0{\cdot}31$, $P = 0{\cdot}58$). However, female MDA levels were significantly higher in the post-season phase ($\chi^2_1 = 8{\cdot}40$, $P = 0{\cdot}004$). Female MDA levels were not significantly related to social group size, age, capture-to-bleed lag or time since sunset (all $\chi^2_1 < 0{\cdot}38$, $P > 0{\cdot}54$).

Enzymatic and non-enzymatic antioxidant protection

The erythrocyte SOD activities of females were not significantly associated with dominance status either as a single term ($\chi^2_1 = 0{\cdot}06$, $P = 0{\cdot}80$, $n = 54$ captures of 27 females) or as the interaction with season phase (Fig. 2b, $\chi^2_1 = 1{\cdot}09$, $P = 0{\cdot}30$). There was a non-significant trend for higher SOD activities in the pre-season phase compared with the post-season ($\chi^2_1 = 3{\cdot}64$, $P = 0{\cdot}06$). SOD activities were not significantly predicted by group size, age, capture-to-bleed lag or the time since sunset (all $\chi^2_1 < 2{\cdot}76$, $P > 0{\cdot}12$).

Female residual TEAC was significantly predicted by the interaction between dominance status and season

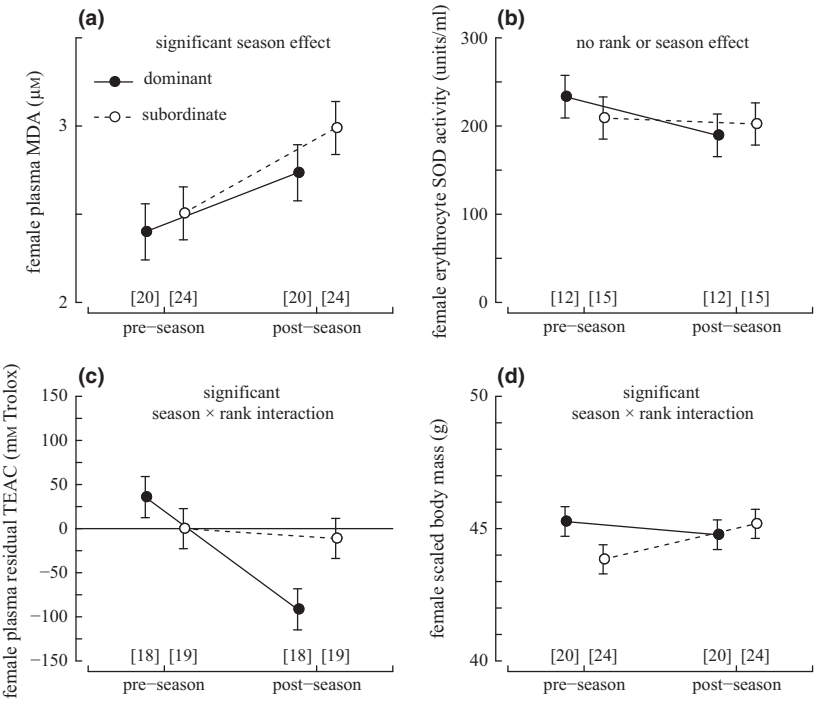

Fig. 2. Female rank-related differences in oxidative status and scaled body mass, before (pre) and after (post) the breeding season, in dominants (filled circles and solid lines) and subordinates (open circles and dotted lines): (a) oxidative damage (malondialdehyde, MDA), (b) intracellular enzymatic antioxidant protection (superoxide dismutase, SOD), (c) non-enzymatic antioxidant protection (non-uric acid residual TEAC) and (d) scaled body mass. Points show model predicted means ± SE from the interaction of season phase and dominance status, while controlling for other significant predictors. Numbers in parentheses are sample sizes (numbers of females sampled). The annotations above the lines highlight associations between rank or season phase and the response variable, in each case.

phase (Fig. 2c, $\chi^2_1 = 6{\cdot}39$, $P = 0{\cdot}011$, $n = 74$ captures of 37 females). Dominant females showed significant reductions in residual TEAC over the course of the breeding season, while subordinate females did not, leaving dominants with significantly lower residual TEAC than subordinates after the breeding season. The emergence of this rank-related difference during the breeding season was confirmed using paired *t*-tests to compare dominant females' residual TEAC to the mean residual TEAC of their own female subordinates, using the social groups for which data were available. In the pre-season phase, dominant females' residual TEAC did not significantly differ from those of their female subordinates ($t_5 = 0{\cdot}15$, $P = 0{\cdot}89$), while in the post-season phase, they did differ ($t_5 = 3{\cdot}09$, $P = 0{\cdot}015$). Residual TEAC in females was not significantly predicted by group size, age, capture-to-bleed lag or time since sunset (all $\chi^2_1 < 1{\cdot}94$, $P > 0{\cdot}16$).

Scaled body mass

There was a significant interaction between dominance status and season phase in the model of female scaled body masses (Fig. 2d, dominance × season phase interaction: $\chi^2_1 = 9{\cdot}54$, $P = 0{\cdot}002$, $n = 88$ captures of 44 females). Dominant females had larger scaled masses than subordinates before the breeding season, but subordinates increased in scaled body mass over the course of the season, such that no rank-related difference was evident in the post-season phase. This relationship was further investigated using paired *t*-tests to compare a dominant female's scaled body mass to the mean scaled body mass of her female subordinates in the pre- and post-season phase, using the groups for which data were available. Such a comparison revealed no evidence of a difference between dominant and subordinate females in the post-season ($t_{10} = 0{\cdot}58$, $P = 0{\cdot}57$), while there was a trend towards dominant females being heavier than their subordinates in the pre-season ($t_{10} = 2{\cdot}07$, $P = 0{\cdot}065$). Female scaled body mass was not significantly affected by group size, age or time since sunset (all $\chi^2_1 < 0{\cdot}77$, $P > 0{\cdot}38$).

SOCIAL DOMINANCE AND OXIDATIVE STATUS AMONG MALES

Oxidative damage

Dominance status did not significantly predict male plasma concentrations of MDA ($\chi^2_1 = 0{\cdot}01$, $P = 0{\cdot}94$, $n = 96$ captures of 48 males), and this was not dependent on season phase (Fig. 3a, dominance × season phase interaction: $\chi^2_1 = 0{\cdot}46$, $P = 0{\cdot}50$; season phase: $\chi^2_1 = 0{\cdot}51$, $P = 0{\cdot}47$). Furthermore, plasma MDA concentration was not significantly predicted by group size, age, capture-to-bleed lag or time after sunset (all $\chi^2_1 < 0{\cdot}58$, $P > 0{\cdot}48$) in males.

Enzymatic and non-enzymatic antioxidant protection

Similarly, erythrocyte SOD activity was not predicted by dominance status ($\chi^2_1 = 0{\cdot}01$, $P = 0{\cdot}93$, $n = 62$ captures of 31 males), or season phase (either as a single term: $\chi^2_1 = 0{\cdot}08$, $P = 0{\cdot}78$; or in the interaction with dominance status: Fig. 3b, $\chi^2_1 = 0{\cdot}34$, $P = 0{\cdot}56$). Group size, age, capture-to-bleed lag and time after sunset were also non-significant predictors of male SOD activity (all $\chi^2_1 < 0{\cdot}37$, $P > 0{\cdot}54$).

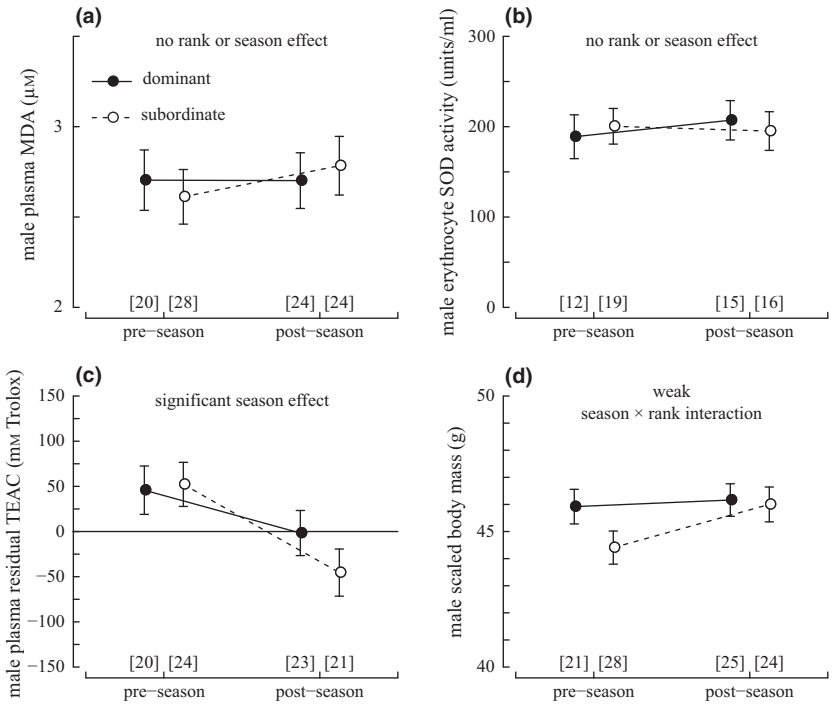

Fig. 3. Male rank-related differences in oxidative status and scaled body mass, before (pre) and after (post) the breeding season, in dominants (filled circles and solid lines) and subordinates (open circles and dotted lines): (a) oxidative damage (malondialdehyde, MDA), (b) intracellular enzymatic antioxidant protection (superoxide dismutase, SOD), (c) non-enzymatic antioxidant protection [residual Trolox-equivalent antioxidant capacity TEAC)] and (d) scaled body mass. Points show model predicted mean ± SE from the interaction of season phase and dominance status, while controlling for any significant predictors. Numbers in parentheses are sample sizes (number of males sampled); values for pre and post-season do not precisely match because four subordinate males became dominant during the breeding season. The annotations above the lines highlight associations between rank or season phase and the response variable, in each case.

Male plasma residual TEAC was not significantly predicted by dominance status, either as a single term ($\chi^2_1 = 0{\cdot}11$, $P = 0{\cdot}74$, $n = 88$ captures of 44 males), or in interaction with breeding season phase (Fig. 3c $\chi^2_1 = 2{\cdot}89$, $P = 0{\cdot}089$). However, after controlling for other important predictors (see below), residual TEAC did differ significantly between pre- and post-breeding season phases ($\chi^2_1 = 4{\cdot}96$, $P = 0{\cdot}026$): males had stronger non-enzymatic antioxidant protection before the breeding season than after it. Male residual TEAC was negatively associated with group size ($\chi^2_1 = 6{\cdot}92$, $P = 0{\cdot}009$) and time since sunset ($\chi^2_1 = 15{\cdot}93$, $P < 0{\cdot}001$) and positively associated with capture-to-bleed lag ($\chi^2_1 = 5{\cdot}11$, $P = 0{\cdot}024$). Male residual TEAC was not significantly predicted by age ($\chi^2_1 = 0{\cdot}10$, $P = 0{\cdot}76$).

Scaled body mass

Dominant and subordinate males did not differ significantly in scaled body mass ($\chi^2_1 = 2{\cdot}59$, $P = 0{\cdot}11$, $n = 98$ captures of 49 males), while controlling for the significant negative correlation with time since sunset ($\chi^2_1 = 13{\cdot}55$, $P < 0{\cdot}001$). While there was no significant effect of the season phase × dominance interaction (Fig. 3d, $\chi^2_1 = 3{\cdot}44$, $P = 0{\cdot}064$), there was a trend reflecting the result found in females: dominant males did not gain body mass during the season, while subordinate males did. Season phase as a single term was also of borderline significance in predicting scaled body mass ($\chi^2_1 = 3{\cdot}70$, $P = 0{\cdot}054$), with a tendency towards higher body mass in the post-season. There was no significant correlation between either group size or age and male scaled body mass (both $\chi^2_1 < 2{\cdot}13$, $P > 0{\cdot}14$).

Discussion

This study is a rare investigation of the association between social dominance and oxidative status in a wild social vertebrate. Our results suggest that rank-related differences in oxidative status only emerged after the breeding season, which strongly suggests they reflect, rather than cause, disparities in reproductive effort between dominants and subordinates. Neither dominant males nor females entered the breeding season with stronger oxidative status than their same-sex subordinates. However, both sexes showed declines in non-enzymatic antioxidant protection over the course of the breeding season. In males, this decline was mild, independent of rank and not accompanied by a rise in oxidative damage. By contrast, in females, the decrease in antioxidant defences was marked but restricted to dominant females, while all females showed increases in oxidative damage. While dominants of both sexes tended to start the season with greater scaled body masses than their subordinates, these differences were no longer apparent by the end of the season. We discuss our findings and their implications for key hypotheses regarding patterns of oxidative status in animal societies.

At the start of the breeding season, neither dominant males nor females differed from their same-sex subordinates in oxidative status. The equivalent oxidative status among dominants and subordinates counters the hypothesis that rank-related differences in intrinsic quality or competitive ability leave dominants with stronger antioxidant defences, and, furthermore, that such differences could underpin their markedly higher reproductive rates. Dominant individuals might also have been expected to differentially upregulate their antioxidant defences in preparation for the breeding

season (Beaulieu *et al*. 2011; van de Crommenacker, Komdeur & Richardson 2011), when their heavier investment in territory defence, mate attraction and guarding, egg production, incubation and nestling care may differentially challenge their oxidative status relative to those of subordinates. Indeed, dominant females (and to a certain extent dominant males) *did* show larger scaled body masses than their subordinates before the breeding season, suggesting that they may benefit from differential access to some resources over winter and/or pre-emptively lay down energetic reserves prior to breeding, but showed no comparable patterns in circulating antioxidant levels. Our findings contrast, therefore, with evidence that strong antioxidant protection and low levels of oxidative damage do predict greater reproductive effort in some vertebrates (Beaulieu *et al*. 2011; van de Crommenacker, Komdeur & Richardson 2011; Heiss & Schoech 2012; Stier *et al*. 2012) and, moreover, that dominant individuals in barn swallows and some eusocial insects can experience stronger antioxidant defences and lower oxidative damage than lower-ranked individuals (Haddad, Kelbert & Hulbert 2007; Aamodt 2009; Vitousek, Stewart & Safran 2013). Dominants in our population may simply be unable to pre-emptively elevate their antioxidant levels because antioxidant-rich dietary resources are scarce during the lengthy dry non-breeding season. Alternatively, dominant sparrow weavers might not benefit more than subordinates from pre-emptively upregulating antioxidant protection if, for example, (i) they can avoid elevated ROS production despite higher reproductive effort through mechanisms such as mitochondrial uncoupling (Barja 2007), or (ii) subordinates benefit similarly from upregulated protection, if they anticipate acquiring dominance themselves [particularly as new dominants frequently breed without help (Harrison *et al*. 2013b)]. Finally, it is conceivable that dominants in our study species pre-emptively store additional antioxidants in their core tissues, whose levels may not be reflected in the circulating markers measured here (Veskoukis *et al*. 2009; Garratt *et al*. 2012).

While males did exhibit declines in their levels of antioxidant protection over the course of the breeding season, there was no evidence to suggest that dominant males suffered differential declines in any aspect of their oxidative status. This is perhaps surprising, as dominant males produce dawn song more frequently, and for longer, than subordinate males (York 2012), closely guard the dominant female (Harrison *et al*. 2013b), and contribute more to territorial defence than subordinate males and comparably to nestling care (Lewis 1981; Wingfield & Lewis 1991; A. J. Young, unpublished data). As a result, dominant males were expected to be differentially exposed to oxidative stress following the breeding season. However, investment in these behaviours may decline towards the end of the breeding season [e.g. song production (York 2012)], allowing dominant males to recover any deficits in oxidative status before our post-season sampling took place. Alternatively, subordinate males may be suffering an equal oxidative burden over the course of the breeding season, arising from physiological costs associated with prospecting and competing for dispersal and reproductive opportunities (e.g. Young, Spong & Clutton-Brock 2007; Young & Monfort 2009).

After the breeding season, females showed evidence of increased oxidative damage to lipids and a tendency towards weaker enzymatic antioxidant protection. While this apparent increase in oxidative stress among females was independent of dominance status, we also found evidence of marked rank-related differences in the relationship between breeding season phase and antioxidant defences in females. Over the course of the breeding season, subordinate females showed no reduction in their plasma antioxidant protection, while dominant females exhibited marked reductions, leaving the levels of dominants significantly lower than those of subordinates in the post-breeding period. This finding cannot be attributed to differences in either group size or territory quality that might be correlated with dominance (e.g. because some dominants live with no subordinates on poor quality territories), because it was also evident in within-group comparisons of dominant females and their own subordinates. The emergence of this rank-related difference in oxidative status during the breeding season is therefore likely to reflect costs of reproduction paid primarily by dominant females. In this species, dominant females are the only birds that lay and incubate eggs, are the primary provisioners of nestlings and contribute more to territorial defence than subordinate females (Lewis 1981; Wingfield & Lewis 1991; Harrison *et al*. 2013b). As such, their differential decline in antioxidant defences during the breeding season is consistent with evidence that reproduction can promote oxidative stress (Bergeron *et al*. 2011; Stier *et al*. 2012; Fletcher *et al*. 2013). Whether the loss of antioxidant protection exhibited by dominant females is the result of antioxidant deposition in eggs (Blount, Houston & Møller 2000; Blount *et al*. 2004; van de Crommenacker, Komdeur & Richardson 2011), oxidative damage sustained whilst offspring provisioning (Stier *et al*. 2012; Fletcher *et al*. 2013), or ROS generation during aggression or territorial defence (Rammal, Bouayed & Soulimani 2010) should be a focus for future studies.

While reductions in antioxidant protection among dominant females need not necessarily be costly in themselves (Costantini & Verhulst 2009), they do raise the possibility that dominant females are suffering oxidative damage either to core tissues (Veskoukis *et al*. 2009; Garratt *et al*. 2012) or to biomolecules whose damage products were not measured here (e.g. proteins and DNA). The reduced antioxidant defences of dominant females at the end of the breeding season may also be expected to entail downstream costs as they are likely to suffer poor food availability during the non-breeding winter period. While compensatory behavioural and physiological mechanisms may allow dominant females to recover, if they are unable to do so, their antioxidant deficits could impair future reproductive effort and survival, while their same-sex subordinates avoid such ill effects.

Selection for group-living may conceivably arise in part from potential benefits of sociality for oxidative status. For example, individuals in large social groups frequently forage more efficiently (Beauchamp 1998) or (in cooperative species) contribute less to reproduction (Crick 1992; Heinsohn 2004), and these benefits could be reflected by lower oxidative damage or stronger antioxidant protection. On the contrary, our study provides no evidence of an oxidative benefit of group-living, finding instead that male antioxidant protection was *weaker* in larger groups. This finding may instead reflect additional oxidative challenges faced by individuals in large groups. For example, conflict over reproductive opportunities can promote endocrine stress responses (Creel 2001; Sapolsky 2005; Young *et al*. 2006), which frequently result in oxidative stress (Costantini, Marasco & Møller 2011). Individuals in large groups can also suffer from increased disease transmission (Côté & Poulin 1995; Møller *et al*. 2001), which can incur an oxidative cost (Costantini & Møller 2009). Such a cost may be particularly heavy if elevated competition reduces access to key dietary antioxidant resources (Catoni, Peters & Martin Schaefer 2008). Group-living may therefore comprise a complex suite of oxidative benefits and challenges, in which future studies should examine in greater detail.

Together, our findings suggest that, prior to breeding, dominants do not exhibit lower levels of oxidative damage or stronger antioxidant protection than their subordinates, suggesting that rank-related disparities in oxidative physiology are unlikely to underpin the differential reproductive effort shown by dominants in animal societies with high reproductive skew. Nor do dominants of either sex appear to pre-empt the oxidative costs that may arise from reproductive effort by differentially upregulating their antioxidant protection at the start of the breeding season. On the contrary, rank-related differences in oxidative status emerged *following* differences in reproductive effort, with the physiological burdens falling most heavily on dominant group members, in a sex-specific manner. Our findings are among the first of their kind for social vertebrates (van de Crommenacker, Komdeur & Richardson 2011; van de Crommenacker *et al*. 2012) and echo evidence from rank-related comparisons of the endocrine stress physiology of high skew cooperative breeders (Creel 2001) in suggesting that social dominance in such species may entail hidden physiological costs, with downstream implications for the patterns of health and ageing in societies. Indeed, our results raise the possibility that the increased longevity of dominants reported in some cooperatively breeding vertebrates (Arnold & Owens 1999; Dammann & Burda 2006) might actually be in spite of, and not due to, rank-related differences in antioxidant protection.

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Data accessibility

Data deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.ss1d1 (Cram, Blount & Young 2014).

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