Oxidative costs of cooperation in cooperatively breeding Damaraland mole-rats

Running title: Oxidative costs of cooperation

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ABSTRACT

Within cooperatively breeding societies, individuals adjust cooperative contributions to maximise indirect fitness and minimize direct fitness costs. Yet, little is known about the physiological costs of cooperation, which may be detrimental to direct fitness. Oxidative stress, the imbalance between reactive oxygen species (by-products of energy production) and antioxidant protection, may represent such a cost when cooperative behaviours are energetically demanding. Oxidative stress can lead to the accumulation of cellular damage, compromising survival and reproduction, thus mediating the trade-off between these competing life history traits. Here we experimentally increased energetically demanding cooperative contributions in captive Damaraland mole-rats (*Fukomys damarensis*). We quantified oxidative stress related effects of increased cooperation on somatic and germline tissues, and the trade-off between them. Increased cooperative contributions induced oxidative stress in females and males, without increasing somatic damage. Males accumulated oxidative damage in their germline despite an increase in antioxidant defences. Finally, oxidative damage accumulation became biased towards the germline, while antioxidant protection remained biased towards the soma, suggesting that males favour the maintenance of somatic tissues, i.e. survival over reproduction. Our results show that, heightened cooperative contributions can ultimately affect direct fitness through oxidative stress costs, which may represent a key selective pressure for the evolution of cooperation.

Keywords: Cooperation; fitness; costs; oxidative stress; life history trade-offs; Damaraland mole-rats.

Subject Category: Behaviour

Subject Areas: behaviour, physiology, evolution

1. INTRODUCTION

Individual variation in cooperative behaviour is inherently associated with variation in benefits and costs accrued. In many cooperatively breeding species, where helpers postpone reproduction and assist the breeders in raising their offspring and/or maintaining their territory [1,2], the benefits of cooperative behaviours have been intensely studied [3–10]. Additionally, several types of cooperative behaviours have been shown to be energetically demanding [11–17], and are thus expected to generate costs. Indeed, higher cooperative contributions are linked to higher metabolic rate [16,17] and lower growth rate [3,15], weight gain [12,13,18] or foraging time [19–22]. However, it remains largely unexplored whether cooperative behaviours carry important physiological costs capable of affecting Darwinian fitness.

Oxidative stress may represent a physiological cost of cooperative behaviours, which can ultimately decrease an individual's fitness. Oxidative stress is an imbalance between reactive oxygen species (ROS; inevitable by-products of energy production) and antioxidant protection, in favour of the former [23]. Under oxidative stress, ROS are more likely to oxidise important biomolecules and accumulation of oxidative damage may disrupt cell and tissue functioning, compromising survival and/or reproduction [24–27]. Cooperative contributions are supported by appropriate tuning of metabolism and energy production [17], and may induce oxidative costs due to such energetic demands [24,28–30, but see 31]. In cooperatively breeding birds, recent correlative work suggests that social status is a good predictor of oxidative stress at the end of the breeding season, when cooperation is intense [32–34]. However, whether this is the consequence of differences in social status or of their associated differences in energetically demanding cooperative contributions remains unclear.

Upon increased energetic demands, individuals may face a trade-off between protecting somatic or germline tissues against damaging effects of oxidative stress, reflecting the trade-off between survival and reproduction [34–36]. Noticeably, the germline, and particularly sperm cells, are highly sensitive to ROS due to a high proportion of polyunsaturated fatty acids in their membranes, and vulnerable DNA [37,38]. Hence, competing demands between somatic and germline functions in terms of protection against ROS may represent a physiological mechanism underlying the survival/reproduction trade-off [39]. Optimal investment in either function may be plastic and depend on reproductive prospects, such that individuals with low/uncertain opportunities may benefit from increased investment in somatic functions, preserving survival and thus future reproduction [40,41]. Strikingly, whether changes in cooperative contributions affect the trade-off between somatic and germline functions remains untested.

Here, we experimentally tested whether increases in energetically demanding cooperative contributions (i) affect oxidative balance; (ii) affect the trade-off between somatic and germline tissues and (iii) are dependent on initial oxidative status, in captive Damaraland mole-rats (*Fukomys damarensis*). Damaraland mole-rats are cooperative breeders with only one female and up to three males monopolizing reproduction [35–37], whereas the breeders' offspring (so-called helpers) are unlikely to breed while staying in their natal colony [35,37–39], but do produce functional sperm [40]. This subterranean rodent dwells in niches where burrowing behaviours - to maintain and expand the tunnel system in search for food, which is then shared by all colony members [41] - are the most pronounced and energetically demanding form of cooperation [17]. All members of the colony contribute to burrowing activities, and variation in cooperative contributions, within and between individuals, has been previously reported [37,42,43].

Oxidative balance was assessed in a somatic tissue in both males and females, and in the germline of the males. To account for the complexity of the oxidative balance system [44], we described oxidative status through: (i) the activity of the antioxidant superoxide dismutase (SOD), a key enzymatic antioxidant in the first line of defence against ROS [45]; (ii) the ratio between oxidized and reduced glutathione (GSSG/GSH) which provides a measure of cellular oxidation and ROS formation [46], highlighting oxidative threats that disturb the oxidative balance [47]. In harmony with SOD, GSH reduces ROS producing GSSG [45]. And (iii) the concentration of malondialdehyde (MDA), a marker of lipid peroxidation (i.e. oxidative damage) [45].

Based on the hypothesis that expression of energetically demanding cooperative behaviours stimulates ROS formation, we predicted that experimentally increasing cooperative contributions would lead to increased antioxidant protection (SOD) and cellular oxidation (GSSG/GSH). We expected stable or increased levels of oxidative damage (MDA) to be related to a good or poor antioxidant protection, respectively. Finally, we predicted that upon an oxidative threat, helpers would bias their investment towards somatic tissues and thus favour survival, while breeders would largely invest in their germline, favouring reproduction.

2. METHODS

(a) Study animals and husbandry

We used six wild-caught colonies of Damaraland mole-rats (79 individuals: 36 females, 43 males; mean group size = 13.2 individuals), maintained in captivity for at least two years at the Kuruman River Reserve, Northern Cape, South Africa. Breeding status (breeder/helper) of males was determined through genetic paternity analysis of the offspring born in captivity as late as 3 months after the end of the experiment, following [48]. Paternity analysis revealed the presence of one breeding male in one colony, two breeding males in four colonies and three breeding males in one colony used in this study. Female breeders were identified by the presence of elongated nipples and the obvious pregnancies and parturitions observed in each colony. Only one female breeder was present in each of the experimental colonies. To allow individual identification, animals carried subcutaneous RFID microchips and unique dye marks.

Each colony was individually housed in standardized artificial tunnel systems made of modified PVC pipes covered with a transparent PET sheet allowing behavioural observations. Each tunnel system contained a nest box, food storage areas, and a large plastic waste box, for animals to discard unwanted material. Three vertical pipes, hereafter referred to as sand dispensers, were placed close to the extremities of the tunnel systems to provide sand to the colonies [Fig. S2 in 43].

Every morning, tunnel systems were cleaned and nest material (small pieces of paper towel) was provided away from the nest. Twice daily, animals were fed *ad libitum* with large non-transportable and small transportable items of sweet potatoes and cucumbers. Food items were provided behind the sand dispensers, which were filled on the same occasion, to encourage burrowing activities.

(b) Experimental design

Sand provisioning was manipulated to experimentally increase burrowing contributions. Each colony was subjected to a control and a sand treatment, each lasting 8 days and separated by an 8-day resting period (Figure S1). Treatment order was balanced across colonies. Daily, sand dispensers were filled twice (at 7:00 and 19:00) during the control treatment and 12 times (i.e. every hour from 7:00 to 19:00) during the sand treatment, resulting in the daily provisioning of 16 kg and 92 kg (range: 70.4 – 112 kg) of sand, respectively. Although these amounts of sand do not necessarily reflect the amounts burrowed by wild colonies, they illustrate Damaraland mole-rats' ability to adjust cooperative contributions according to the existing needs. To control

for possible hourly disturbances during the sand treatment, the sand dispensers were touched every hour during the control treatment.

(c) Behavioural observations

Behavioural observations were carried out on days two and seven of each treatment (Figure S1). A scan protocol [49] was used to record the behaviour of each colony member every 4 minutes, from a list of pre-defined behaviours (Table S1). Scan observations lasted 12 hours, resulting in the collection of 180 behavioural data points for each individual. Observations were shared equally between two observers with shifts of 2 to 4 hours. Five behavioural measures were considered for our analyses: active, non-cooperation, burrow, food carry and nest build. Active referred to any behaviour displayed while outside of the nest; non-cooperation grouped all behaviours that are not of cooperative nature (see below); burrow included all behaviours related tunnel excavation; food carry referred to transportation of food pieces; nest building included preparation and transportation of nest material (Table S1). The latter three are cooperative activities [43].

(d) Sampling procedures

Blood and ejaculates were collected from subjects anaesthetised with isoflurane, two days before the start and on the last day of each treatment (day 8) (Figure S1). Blood was collected from all individuals older than 6 months, while ejaculates were collected from males older than 8 months. A vein from a pre-warmed foot was pierced using a sterile 16G needle and blood was collected into lithium-heparinized tubes and centrifuged immediately for 5 minutes at 2000 *g*. Plasma was separated from the cell pellet and both fractions were kept at -80℃ until laboratory analysis. After blood sampling, ejaculates were collected by electro-ejaculation (supplemental methods), following minor adjustments to the procedure described by [50]. Ejaculate cell density was assessed when ejaculate volume was greater than that needed for oxidative markers quantification. Ejaculates were loaded into a counting-chamber under a microscope and cell density was calculated using cell count adjusted for the area examined, the volume of the chamber used and the final dilution. Subjects were then removed from anaesthesia, weighed and placed back in their original tunnel system, once full mobility was regained.

(e) Quantification of oxidative stress markers

SOD was quantified in erythrocyte and ejaculate homogenates; MDA in erythrocytes, ejaculate and plasma; GSH and GSSG in erythrocytes. SOD activity was determined, using a commercial kit (Cayman Chemical), following the supplier's instructions and adjusting the samples' final dilution (supplemental methods). Concentrations of GSH and GSSG were determined following minor adjustments to the description of Mora et al. [51] (supplemental methods).

Total MDA was quantified following Mendonça et al. [52], applying minor modifications (supplemental methods).

(f) Statistical analysis

All analyses and graphical representations were done using the software R (version 3.4.0) [53]. Whenever possible, (generalized) linear mixed-effects models ((G)LMM) were used, with the package *lme4* [54]. When measurements of colony and/or individual were repeated, these were specified as random effects. An observation level random effect (OLRE) was added in the presence of overdispersion of GLMMs residuals [55]. Covariates specified in interaction terms were scaled to allow interpretation of their main effects [56]. Full models were used for interpretation. P-values for fixed effects were extracted from ANOVA tables using Wald F tests with Kenward-Roger approximation of degrees of freedom, from the package *car* [57]. Statistical significance was set at $p < 0.05$.

(i) Effect of treatment on behaviour and body condition

To confirm that the sand treatment specifically increased energetically demanding cooperative contributions, the proportions of active, non-cooperation and burrow were used as response variables in distinct binomial GLMMs (n=77 after exclusion of two pups younger than 3 months). Rare behaviours (nest build and food carry) were analysed using Wilcoxon signed-rank tests with continuity corrections. All GLMMs included treatment, sex, breeding status and all possible interactions as predictors to assess possible differences between breeders and helpers of both sexes. Body mass, day of treatment and their interaction with treatment were also included.

To determine the effect of treatment on body condition, the change in body mass throughout each treatment was used as response variable in a LMM including treatment, sex, breeding status and all possible interactions as predictors. To control for regression to the mean effects [58], body mass at the beginning of the week was included as a covariate.

(ii) Effect of treatment on oxidative stress in blood

The final measure (day 8) of each somatic oxidative marker (SOD, GSSG/GSH, GSSG, GSH, MDA) was ln-transformed and set as response variable in distinct LMMs including treatment, sex, breeding status and all possible interactions, body mass and its interaction with treatment as well as the interaction between treatment and the initial (day -2) measure of the respective marker were specified as predictors.

We determined whether individuals going through the treatment sequence sand-control (Figure S1b) experienced carry-over effects (i.e. the 8-day resting period was not sufficient for oxidative markers to return to a baseline level similar to that encountered before the sand

treatment). The initial (day -2) measure of each somatic oxidative marker for these individuals was ln-transformed and set as response variable in distinct LMMs with treatment as a predictor.

(iii) Effect of treatment on oxidative stress in ejaculates

The final (day 8) measure of each germline oxidative marker (SOD, MDA) was ln-transformed and set as response variable in distinct LMMs. Due to smaller sample sizes, only body mass and the interaction between treatment and breeding status were specified as predictors. Due to a reduced number of ejaculates collected before the start of each treatment, initial measures were not included. An additional LMM investigated whether final ejaculate cell density was affected by treatment, which could be the underlying mechanism for the observed effects on oxidative markers.

(iv) Effect of treatment on the trade-off between somatic and germline tissues

The ratio between ejaculate and erythrocyte final (day 8) levels for each marker was used as response variable in two distinct LMMs. Positive ratios indicate biased antioxidant protection (SOD) or damage accumulation (MDA) towards the germline, while negative ratios indicate a bias towards the soma. Model structure followed the one described in *(c)*.

(v) Relationship between initial oxidative stress and changes in cooperative contributions

We tested whether individuals better equipped to cope with an oxidative challenge, either due to higher initial antioxidant levels or lower initial oxidative damage, could raise cooperative contributions to a greater extent. The difference in the percentage of burrow between the two treatments (sand – control) was set as response variable in six LMMs (one for each marker), including the initial measure of the respective marker (day -2 of the sand treatment), and its interaction with sex and breeding status as well as initial body mass as predictors. To control for regression to the mean effects, percentage of burrow during the control treatment was included as a covariate [58].

3. RESULTS

(a) Effect of treatment on behaviour and body condition

Animals were significantly more active when more sand was provided to the colony, compared to the control treatment (Table S2a). Such increase in individual activity was specifically driven by the significant increase in burrowing behaviours: the proportion of burrowing doubled during the sand treatment (Table S2b; Figure 1), while other cooperative and non-cooperative activities remained unchanged (Nest Build females: V = 145, p = 0.6; males: V = 142.5, p = 0.6; Food Carry females: V = 117, p = 1; Figure S2a, b), or even decreased (Food Carry males: V = 382.5, p = 0.008, Figure S2a; Non-Cooperation: Table S2c; Figure S2c). The increase in individual burrowing activities, caused by increased sand provisioning, did not drive individuals to exhaustion as the expression of these behaviours throughout the week did not differ between treatments (Treatment * Day: all models p > 0.1).

Weekly changes in body mass did not differ between treatments (Table S3).

(b) Effect of treatment on oxidative stress in blood

Cellular oxidation (GSSG/GSH) in erythrocytes was elevated upon increased burrowing activities (Table S4a, Figure 2a). This effect was driven by a greater rise in oxidized (GSSG) than in reduced (GSH) glutathione (45.5% and 14.8%, respectively; t = -2.7, p = 0.008; Table S4b, c; Figure 2b, c). Females, but not males, showed a decrease in erythrocyte lipid damage (MDA) during the sand treatment (Table S5a; Figure S3; least-squares means comparisons with Tukey adjustment; females: t = 2.8, p = 0.037; males: t = -0.7, p = 0.9). Increased burrowing activities did not affect erythrocyte antioxidant SOD activity (Table S5b) nor plasma MDA levels (Table S5c).

The initial levels of all oxidative stress markers were positively correlated with their final levels. Other variables of interest such as sex, breeding status and body mass appeared to have little effect on oxidative stress markers (Table S4 to S5).

The initial (day -2) oxidative marker levels did not differ between treatments in individuals that started the experiment with the sand treatment (all p > 0.3), showing that the 8-day resting period was sufficient to avoid potential carry-over effects.

(c) Effect of treatment on oxidative stress in ejaculates

Independently of breeding status, SOD and MDA in ejaculates were elevated upon increased burrowing activities (Table S6; Figure 3a, b). These effects were not driven by changes in sperm density as this did not differ between treatments ($F_{1,14}$ = 2.3, p = 0.2).

(d) Effect of treatment on the trade-off between somatic and germline tissues

Upon increased burrowing activities and independently of breeding status, SOD and MDA were both elevated in the germline to a greater degree than in the soma, resulting in a higher ratio germline/soma during the sand treatment (Table S7, Figure 3c, d). SOD activity was biased towards somatic tissues in both treatments (negative ratio), while MDA levels were relatively balanced during the control treatment and became biased towards the germline during the sand treatment (positive ratio).

(e) Relationship between initial oxidative stress and changes in cooperative contributions

The initial levels of oxidative stress markers did not significantly predict changes in the proportion of time spent burrowing between the two treatments (Tables S8, S9). An initial model revealed a trend for the interaction between initial plasmatic MDA and sex ($p = 0.053$), where males showed a negative (and females a positive) relationship between initial plasmatic MDA and changes in burrowing activities. Visual inspection of this interaction suggested that one single male may be responsible for this result, and its removal from the model rendered the interaction non-significant (Table S9c).

4. DISCUSSION

Our results offer the first experimental demonstration of an oxidative cost of energetically demanding cooperative behaviours. Increased energetically demanding cooperative contributions generated oxidative stress in somatic and germline tissues, as witnessed by elevated cellular oxidation and oxidative damage, respectively. Upon increased cooperative contributions, Damaraland mole-rat males favoured somatic over germline functions, revealing the greater cost of cooperation and illustrating a trade-off prioritizing survival over reproduction. The specific effect of our sand manipulation on burrowing behaviours makes us confident that the oxidative costs observed were driven by the increased energetically demanding cooperative contributions. The lack of an effect of increased cooperative contributions on body condition, in a captive setting, highlights the possibility that the oxidative costs of cooperation in the wild may be exacerbated due to limited resources and harsher environment conditions [59–61]. Together, our results provide evidence on a physiological mechanism underlying the greater cost of cooperative behaviours – that induced to reproduction through oxidative stress.

Increased cooperative contributions, and their associated energetic demands, induce an oxidative threat to somatic tissues, as witnessed by the increased levels of cellular oxidation (GSSG/GSH) in erythrocytes [47,62]. This effect was driven by increased oxidized glutathione (GSSG), supporting the view of reduced glutathione's (GSH) important role in neutralizing ROS [24]. Despite the extensive oxidation of GSH (into GSSG), its concentration was also increased during the sand treatment, likely through *de novo* synthesis [45], suggesting an investment in somatic antioxidant protection upon heightened cooperative contributions.

Individuals were able to deal with the oxidative threat imposed to the soma by increased cooperative contributions, avoiding accumulation of somatic oxidative damage. Firstly, increased cooperative contributions did not affect antioxidant SOD activity in erythrocytes, suggesting that this was within sufficient levels to neutralize the exceeding superoxide anions formed upon higher energetic demands [63,64]. Secondly, regardless of sex and breeding status, increased cooperative contributions did not increase blood oxidative damage to lipids (MDA). Such results could be suggestive of individual adjustments of cooperative contributions according to their oxidative status, yet, initial oxidative markers did not predict the extent to which individuals increased cooperative contributions during the sand treatment. Studies in the wild often suggest that individual condition and oxidative status affect energetically demanding cooperative contributions [13,32,33,65], an effect that may have been shadowed in our study by captive conditions and *ad libitum* food provision. Direct manipulations of individuals'

oxidative balance [66] are needed to experimentally assess the role of oxidative status on cooperative contributions.

Female Damaraland mole-rats are better equipped than males to prevent and even reduce somatic oxidative damage upon increased cooperative contributions. While males were able to prevent increased somatic lipid damage, females reduced erythrocyte damage levels at the end of the sand treatment. Sex differences in antioxidant defences not measured in our study [67] and/or efficiency of mechanisms repairing oxidative damage to lipids [27] could explain this reduction in MDA levels in females, and should be addressed in future studies. Decreased oxidative damage may represent an adaptive oxidative shielding response of females to the possibility of encountering foreign mates of neighbouring colonies in the wild [68].

The greater oxidative costs of energetically demanding cooperative behaviours may be those imposed to the germline, with potential detrimental effects on direct fitness. Although the increased SOD activity in ejaculates suggests a protection response against ROS, increased cooperative contributions also resulted in elevated oxidative damage in ejaculates, possibly compromising individuals' reproductive success. Indeed, high levels of lipid peroxidation in the sperm are associated with decreased sperm quality [69] due to reduced motility and ability to fuse with oocytes [70,71]. Although Damaraland mole-rat helpers do not reproduce within their natal colony, reproductive opportunities in the wild may arise when the underground tunnel systems of two colonies become connected, for example due to animals extending their tunnels in search of new food sources. In such situations, compromised sperm quality can negatively affect direct fitness of male helpers. The prediction that male breeders may invest in germline protection to a greater extent than helpers was not supported by our study. Instead, our results may highlight a lack of reproductive competition between the males, allowing them to withstand some level of damage to the germline.

Our study supports the hypothesis that oxidative stress mediates a trade-off between survival and reproduction through a trade-off between somatic and germline tissues [72]. Males accumulated oxidative damage in the germline to a greater extent than in the somatic tissue, suggesting that overall antioxidant protection has been traded-off. Oxidative damage integrates the negative effects of ROS and the protective effects of antioxidants and repair mechanisms [45] and thus provides reliable information about the outcome of a potential trade-off between somatic and germline tissues. Upon an oxidative challenge, Damaraland mole-rat males prioritize survival over reproduction by avoiding increased oxidative damage to the soma, at the expense of damage to the germline. For male helpers, such strategy may be adaptive because reproduction is unlikely while staying in their natal colony [35,37–39], and investment in

survival may result in increased indirect fitness and increased prospects of future reproduction [73,74]. For male breeders, the lack of reproductive competition may have favoured investment in survival as long as reproduction is not fully compromised (i.e. spermatozoa remain capable of fertilization). Aligned with these results, spermatozoa characteristics are similar in Damaraland mole-rat helpers and breeders [40], suggesting that breeding status may not play a significant role in germline investment in this species.

Although heightened cooperative contributions induce oxidative costs capable of leading to fitness reductions in the short-term, whether they do so in the long-term [4,75] remains to be confirmed. Individuals may avoid the long-term build-up of oxidative damage through two non-mutually exclusive mechanisms. Firstly, individuals can adjust cooperative contributions according to their past contributions and current condition [15,76,77]. Secondly, individuals can adjust their oxidative stress physiology [31], though upregulation of antioxidant production and oxidative damage repair mechanisms, and changes of lipid composition of cellular membranes [30,78–80]. Longer-term experimental manipulations of cooperative contributions would be extremely valuable to address this question.

Our study provides the first experimental demonstration that oxidative stress represents a key physiological cost of energetically demanding cooperative behaviours, susceptible to ultimately compromise individuals' fitness. Furthermore, assessing such costs in somatic and germline tissues is of great importance to gain insight into the physiological mechanisms underpinning one of the best studied life history trade-offs, that between survival and reproduction. Future studies will gain from addressing this trade-off in females. Finally, these findings have implications on our appreciation of the selective pressures posed by energetically demanding behaviours on the evolution of cooperation in animal societies.

Ethics. All protocols were approved by the Animal Ethics Committee at the University of Pretoria (EC093-14).

Data accessibility. Data and R code used in this study can be assessed on https://datadryad.org/stash/share/GMK1FJwpCU4lhEMydsL0-DIEoJhJ_2dIPOnrPa4×7Lww.

Authors' contributions: R.M., P.V., N.C.B. and F.H. designed experiments; R.M. and N.K. collected data; R.M., G.G. and A.V. conducted laboratory analysis; R.M., P.V. and F.H. conducted statistical analysis; R.M. authored the manuscript with contributions from all co-authors.

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References

1. Clutton-Brock TH. 2016 *Mammal Societies*. West Sussex, UK: Wiley.
2. Koenig WD, Dickinson JL. 2016 *Cooperative breeding in vertebrates: studies of ecology, evolution, and behavior*. Cambridge University Press.
3. Taborsky M. 1984 Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. *Anim. Behav.* **32**, 1236–1252. (doi:10.1016/S0003-3472(84)80241-9)
4. Reyer H-U. 1984 Investment and relatedness: a cost/benefit analysis of breeding and helping in the pied kingfisher (*Ceryle rudis*). *Anim. Behav.* **32**, 1163–1178.
5. Brown JL, Brown ER, Brown SD, Dow DD. 1982 Helpers: effects of experimental removal on reproductive success. *Science (80-.).* **215**, 421–422.
6. Hodge SJ, Manica A, Flower TP, Clutton-Brock TH. 2008 Determinants of reproductive success in dominant female meerkats. *J. Anim. Ecol.* **77**, 92–102. (doi:10.1111/j.1365-2656.2007.01318.x)
7. Russell AF, Brotherton PNM, McIlrath GM, Sharpe LL, Clutton-Brock TH. 2003 Breeding success in cooperative meerkats: effects of helper number and maternal state. *Behav. Ecol.* **14**, 486–492. (doi:10.1093/beheco/arg022)
8. Clutton-Brock TH, Russell AF, Sharpe LL, Brotherton PNM, McIlrath GM, White S, Cameron EZ. 2001 Effects of helpers on juvenile development and survival in meerkats. *Science (80-.).* **293**, 2446–2449. (doi:10.1126/science.1061274)
9. Moehlman PD. 1979 Jackal helpers and pup survival. *Nature* **277**, 382–383. (doi:10.1038/277382a0)
10. Russell AF, Young AJ, Spong G, Jordan NR, Clutton-Brock TH. 2007 Helpers increase the reproductive potential of offspring in cooperative meerkats. *Proc. R. Soc. B* **274**, 513–520. (doi:10.1098/rspb.2006.3698)
11. Rowley I. 1978 Communal activities among white-winged choughs *Corcorax melanorhamphus*. *Ibis (Lond. 1859).* **120**, 178–197. (doi:10.1111/j.1474-919X.1978.tb06774.x)
12. Heinsohn R, Cockburn A. 1994 Helping is costly to young birds in cooperatively breeding white-winged choughs. *Proc. R. Soc. London B* **256**, 293–298.
13. Clutton-Brock TH *et al.* 1998 Costs of cooperative behaviour in suricates (*Suricata suricatta*). *Proc. R. Soc. B* **265**, 185–190. (doi:10.1098/rspb.1998.0281)
14. Canestrari D, Marcos JM, Baglione V. 2007 Costs of chick provisioning in cooperatively breeding crows: an experimental study. *Anim. Behav.* **73**, 349–357.

(doi:10.1016/j.anbehav.2006.04.013)

15. Russell AF, Sharpe LL, Brotherton PNM, Clutton-Brock TH. 2003 Cost minimization by helpers in cooperative vertebrates. *Proc. Natl. Acad. Sci.* **100**, 3333–3338. (doi:10.1073/pnas.0636503100)
16. Grantner A, Taborsky M. 1998 The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *J. Comp. Physiol. B* **168**, 427–433. (doi:10.1007/s003600050162)
17. Lovegrove BG. 1989 The cost of burrowing by the social mole rats (Bathyergidae) *Cryptomys damarensis* and *Heterocephalus glaber*: the role of soil moisture. *Physiol. Zool.* **62**, 449–469. (doi:10.2307/30156179)
18. Hodge SJ. 2007 Counting the costs: the evolution of male-biased care in the cooperatively breeding banded mongoose. *Anim. Behav.* **74**, 911–919. (doi:10.1016/j.anbehav.2006.09.024)
19. Price EC. 1992 The costs of infant carrying in captive cotton-top tamarins. *Am. J. Primatol.* **26**, 23–33. (doi:10.1002/ajp.1350260106)
20. Schradin C, Anzenberger G. 2001 Costs of infant carrying in common marmosets, *Callithrix jacchus*: an experimental analysis. *Anim. Behav.* **62**, 289–295. (doi:10.1006/anbe.2001.1767)
21. Sánchez S, Peláez F, Gil-Bürmann C, Kaumanns W. 1999 Costs of infant-carrying in the cotton-tap tamarin (*Saguinus oedipus*). *Am. J. Primatol.* **48**, 99–111. (doi:10.1002/(SICI)1098-2345(1999)48:2<99::AID-AJP2>3.0.CO;2-6)
22. Heinsohn RG, Cockburn A, Cunningham RB. 1988 Foraging, delayed maturation, and advantages of cooperative breeding in white-winged choughs, Corcorax melanorhamphos. *Ethology* **77**, 177–186. (doi:10.1111/j.1439-0310.1988.tb00202.x)
23. Sies H. 1985 Oxidative stress: introductory remarks. In *Oxidative stress* (ed H Sies), pp. 1–8. London, UK: Academic Press Inc.
24. Davies KJA, Quintanilha AT, Brooks GA, Packer L. 1982 Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* **107**, 1198–1205. (doi:10.1016/S0006-291X(82)80124-1)
25. Finkel T, Holbrook NJ. 2000 Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239–247. (doi:10.1038/35041687)
26. Harshman LG, Zera AJ. 2007 The cost of reproduction: the devil in the details. *Trends Ecol. Evol.* **22**, 80–86. (doi:10.1016/j.tree.2006.10.008)

27. Monaghan P, Metcalfe NB, Torres R. 2009 Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol. Lett.* **12**, 75–92. (doi:10.1111/j.1461-0248.2008.01258.x)
28. Bejma J, Ji LL. 1999 Aging and acute exercise enhance free radical generation in rat skeletal muscle. *J. Appl. Physiol.* **87**, 660–664.
29. Leeuwenburgh C, Heinecke JW. 2001 Oxidative stress and antioxidants in exercise. *Curr. Med. Chem.* **8**, 829–838. (doi:10.2174/0929867013372896)
30. Radak Z, Chung HY, Koltai E, Taylor AW, Goto S. 2008 Exercise, oxidative stress and hormesis. *Ageing Res. Rev.* **7**, 34–42. (doi:10.1016/j.arr.2007.04.004)
31. Speakman JR, Garratt M. 2013 Oxidative stress as a cost of reproduction: beyond the simplistic trade-off model. *BioEssays* **36**, 93–106. (doi:10.1002/bies.201300108)
32. van de Crommenacker J, Komdeur J, Richardson DS. 2011 Assessing the cost of helping: the roles of body condition and oxidative balance in the Seychelles warbler (*Acrocephalus sechellensis*). *PLoS One* **6**, e26423. (doi:10.1371/journal.pone.0026423)
33. Cram DL, Blount JD, Young AJ. 2014 Oxidative status and social dominance in a wild cooperative breeder. *Funct. Ecol.* **29**, 229–238. (doi:10.1111/1365-2435.12317)
34. Cram DL, Blount JD, Young AJ. 2015 The oxidative costs of reproduction are group-size dependent in a wild cooperative breeder. *Proc. R. Soc. B* **282**, 20152031. (doi:10.1098/rspb.2015.2031)
35. Burland TM, Bennett NC, Jarvis JUM, Faulkes CG. 2004 Colony structure and parentage in wild colonies of co-operatively breeding Damaraland mole-rats suggest incest avoidance alone may not maintain reproductive skew. *Mol. Ecol.* **13**, 2371–2379. (doi:10.1111/j.1365-294X.2004.02233.x)
36. Burland TM, Bennett NC, Jarvis JUM, Faulkes CG. 2002 Eusociality in African mole-rats: new insights from patterns of genetic relatedness in the Damaraland mole-rat (*Cryptomys damarensis*). *Proc. R. Soc. B* **269**, 1025–1030. (doi:10.1098/rspb.2002.1978)
37. Bennett NC, Jarvis JUM. 1988 The social structure and reproductive biology of colonies of the mole-rat, *Cryptomys damarensis* (Rodential, Bathyergidae). *J. Mamalogy* **69**, 293–302.
38. Bennett NC, Faulkes CG. 2000 *African mole-rats: ecology and eusociality*. Cambridge, UK: Cambridge University Press.
39. Jarvis JUM, Bennett NC. 1993 Eusociality has evolved independently in two genera of bathyergid mole-rats - but occurs in no other subterranean mammal. *Behav. Ecol.*

Sociobiol. **33**, 253–260.

40. Faulkes CG, Trowell SN, Jarvis JU, Bennett NC. 1994 Investigation of numbers and motility of spermatozoa in reproductively active and socially suppressed males of two eusocial African mole-rats, the naked mole-rat (*Heterocephalus glaber*) and the Damaraland mole-rat (*Cryptomys damarensis*). *J. Reprod. Fertil.* **100**, 411–416. (doi:10.1530/jrf.0.1000411)
41. Jarvis JUM, Bennett NC, Spinks AC. 1998 Food availability and foraging by wild colonies of Damaraland mole-rats (*Cryptomys damarensis*): implications for sociality. *Oecologia* **113**, 290–298. (doi:10.1007/s004420050380)
42. Bennett NC. 1990 Behaviour and social organization in a colony of the Damaraland mole-rat *Cryptomys damarensis*. *J. Zool.* **220**, 225–247. (doi:10.1111/j.1469-7998.1990.tb04305.x)
43. Zöttl M, Vullioud P, Mendonça R, Torrents Ticó M, Gaynor D, Mitchell A, Clutton-Brock T. 2016 Differences in cooperative behavior among Damaraland mole rats are consequences of an age-related polyethism. *Proc. Natl. Acad. Sci.* **113**, 10382–10387. (doi:10.1073/pnas.1607885113)
44. Hõrak P, Cohen AA. 2010 How to measure oxidative stress in an ecological context: methodological and statistical issues. *Funct. Ecol.* **24**, 960–970.
45. Halliwell B, Gutteridge JMC. 2007 *Free radicals in biology and medicine*. Oxford, UK: Oxford University Press, USA. (doi:10.1016/0891-5849(91)90055-8)
46. Jones DP. 2006 Redefining oxidative stress. *Antioxid. Redox Signal.* **8**, 1865–1879. (doi:10.1089/ars.2006.8.1865)
47. Flohé L. 2013 The fairytale of the GSSG/GSH redox potential. *Biochim. Biophys. Acta* **1830**, 3139–3142. (doi:10.1016/j.bbagen.2012.10.020)
48. Burland TM, Bishop JM, O'Ryan C, Faulkes CG. 2001 Microsatellite primers for the African mole-rat genus Cryptomys and cross-species amplification within the family Bathyergidae. *Mol. Ecol. Notes* **1**, 311–314. (doi:10.1046/j.1471-8278.2001.00086.x)
49. Altmann J. 1974 Observational study of behavior: sampling methods. *Behaviour* **49**, 227–267. (doi:10.1080/14794802.2011.585831)
50. Fasel NJ, Helfenstein F, Buff S, Richner H. 2015 Electroejaculation and semen buffer evaluation in the microbat *Carollia perspicillata*. *Theriogenology* **83**, 904–910. (doi:10.1016/j.theriogenology.2014.11.030)
51. Mora AR, Meniri M, Glauser G, Vallat A, Helfenstein F. 2016 Badge size reflects sperm oxidative status within social groups in the house sparrow *Passer domesticus*.

Front. Ecol. Evol. **4**, 67. (doi:10.3389/fevo.2016.00067)

52. Mendonça R, Gning O, Cesaré C Di, Lachat L, Bennett NC, Helfenstein F, Glauser G. 2017 Sensitive and selective quantification of free and total malondialdehyde in plasma using UHPLC-HRMS. *J. Lipid Res.* **58**, 1924–1931. (doi:10.1194/jlr.D076661)
53. Team RC. 2017 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2016.
54. Bates D, Maechler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48.
55. Zuur AF, Hilbe J, Ieno E. 2013 *A beginner's guide to GLM and GLMM with R: a frequentist and bayesian perspective for ecologists*. Highland Statistics Limited.
56. Schielzeth H. 2010 Simple means to improve the interpretability ofregression coefficients. *Methods Ecol. Evol.* **1**, 103–113. (doi:10.1111/j.2041-210X.2010.00012.x)
57. Fox J, Weisberg S. 2011 *An {R} companion to applied regression*. Second. Thousand Oaks CA: Sage.
58. Kelly C, Price TD. 2005 Correcting for regression to the mean in behavior and ecology. *Am. Nat.* **166**, 700–707. (doi:10.1086/497402)
59. Costantini D. 2010 Effects of diet quality on growth pattern, serum oxidative status, and corticosterone in pigeons (Columba livia). *Can. J. Zool.* **88**, 795–802. (doi:10.1016/j.cbpa.2010.02.021)
60. Costantini D, Marasco V, Møller AP. 2011 A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J. Comp. Physiol. B* **181**, 447–456. (doi:10.1007/s00360-011-0566-2)
61. Feoli AM *et al.* 2006 Effects of protein malnutrition on oxidative status in rat brain. *Nutrition* **22**, 160–165. (doi:10.1016/j.nut.2005.06.007)
62. Urso ML, Clarkson PM. 2003 Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* **189**, 41–54. (doi:10.1016/S0300-483X(03)00151-3)
63. Vaanholt LM, Speakman JR, Garland Jr T, Lobley GE, Visser GH. 2008 Protein synthesis and antioxidant capacity in aging mice: effects of long-term voluntary exercise. *Physiol. Biochem. Zool.* **81**, 148–157. (doi:10.1086/525289)
64. Selman C, McLaren JS, Collins AR, Duthie GG, Speakman JR. 2002 Antioxidant enzyme activities, lipid peroxidation, and DNA oxidative damage: the effects of short-term voluntary wheel running. *Arch. Biochem. Biophys.* **401**, 255–261. (doi:10.1016/S0003-9861(02)00050-4)
65. Eden SF. 1987 When do helpers help? Food availability and helping in the moorhen,

Gallinula chloropus. *Behav. Ecol. Sociobiol.* **21**, 191–195. (doi:10.1007/BF00303210)

66. Koch RE, Hill GE. 2017 An assessment of techniques to manipulate oxidative stress in animals. *Funct. Ecol.* **31**, 9–21. (doi:10.1111/1365-2435.12664)
67. Costantini D, Verhulst S. 2009 Does high antioxidant capacity indicate low oxidative stress? *Funct. Ecol.* **23**, 506–509. (doi:10.1111/j.1365-2435.2009.01546.x)
68. Blount JD, Vitikainen EIK, Stott I, Cant MA. 2016 Oxidative shielding and the cost of reproduction. *Biol. Rev.* **91**, 483–497. (doi:10.1111/brv.12179)
69. Helfenstein F, Losdat S, Møller AP, Blount JD, Richner H. 2010 Sperm of colourful males are better protected against oxidative stress. *Ecol. Lett.* **13**, 213–222. (doi:10.1111/j.1461-0248.2009.01419.x)
70. John Aitken R, Clarkson JS, Fishel S. 1989 Generation of Reactive Oxygen Species, Lipid Peroxidation, and Human Sperm Function. *Biol. Reprod.* **41**, 183–197. (doi:10.1095/biolreprod41.1.183)
71. Suleiman SA, Ali ME, Zaki ZMS, El-Malik EMA, Nasr MA. 1996 Lipid peroxidation in human sperm motility: protective role of vitamin E. *J. Androl.* **17**, 530–537.
72. Mora AR, Meniri M, Gning O, Glauser G, Vallat A, Helfenstein F. 2017 Antioxidant allocation modulates sperm quality across changing social environments. *PLoS One* **12**, e0176385. (doi:10.1371/journal.pone.0176385)
73. Perrin N, Sibly R. 1993 Dynamic models of energy allocation and investment. *Annu. Rev. Ecol. Syst.* **24**, 379–410. (doi:10.1146/annurev.es.24.110193.002115)
74. Drenos F, Kirkwood TBL. 2005 Modelling the disposable soma theory of ageing. *Mech. Ageing Dev.* **126**, 99–103. (doi:10.1016/j.mad.2004.09.026)
75. Stacey PB, Koenig WD, editors. 1990 *Cooperative breeding in birds: long term studies of ecology and behaviour*. Cambridge, UK: Cambridge University Press. (doi:10.1017/CBO9780511752452.014)
76. Meade J, Hatchwell BJ. 2010 No direct fitness benefits of helping in a cooperative breeder despite higher survival of helpers. *Behav. Ecol.* **21**, 1186–1194. (doi:10.1093/beheco/arq137)
77. Sanderson JL, Young AJ, Hodge SJ, Kyabulima S, Walker SL, Cant MA. 2014 Hormonal mediation of a carry-over effect in a wild cooperative mammal. *Funct. Ecol.* **28**, 1377–1386. (doi:10.1111/1365-2435.12307)
78. Costantini D. 2008 Oxidative stress in ecology and evolution: lessons from avian studies. *Ecol. Lett.* **11**, 1238–1251. (doi:10.1111/j.1461-0248.2008.01246.x)
79. Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA. 2007 Life and death: metabolic

rate, membrane composition, and life span of animals. *Physiol. Rev.* **87**, 1175–1213. (doi:10.1152/physrev.00047.2006.)

80. Radak Z, Taylor AW, Ohno H, Goto S. 2001 Adaptations to exercise-induced oxidative stress: from muscle to brain. *Exerc. Immunol. Rev.* **7**, 90–107.