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Oxidative stress as an indicator of the costs of reproduction among free-ranging rhesus macaques

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ABSTRACT

Sex differences in longevity may reflect sex-specific costs of intra-sexual competition and reproductive effort. As male rhesus macaques experience greater intrasexual competition and die younger, we predicted males would experience greater oxidative stress than females, and that oxidative stress would reflect sex-specific measures of reproductive effort. Males, relative to females, had higher 8-OHdG and malondialdehyde concentrations, markers of DNA oxidative damage and lipid peroxidation, respectively. Older macaques had lower 8-OHdG levels than younger ones, suggesting oxidative stress decreases in parallel with known age-related declines in reproductive investment. Among males, a recent period of social instability affected oxidative status: males who attacked others at higher rates had higher 8-OHdG levels. Multiparous lactating females with daughters had higher 8-OHdG levels than those with sons. No differences in antioxidant capacity were found. These results lend initial support for using oxidative stress markers to assess trade-offs between reproductive effort and somatic maintenance in primates.

SUMMARY STATEMENT (for email alerts etc.)

Measures of oxidative stress reveal higher DNA oxidative damage among males than females; among mothers raising daughters than sons; and show enduring costs of intense physical aggression for males.

INTRODUCTION

Reactive oxygen species (ROS) are a normal by-product of aerobic cellular metabolism, but oxidative damage to important biomolecules (including DNA) has been linked to pathological processes, reproduction, and aging (Costantini, 2014). Oxidative stress is a potential mediator of the relationship between increased reproduction and decreased lifespan (Selman et al., 2012) and inter-individual variation in oxidative stress can provide important insights into the processes that lead to individual and sex differences in reproduction and mortality (Costantini, 2014; Metcalfe and Alonso-Alvarez, 2010).

In this study we examined whether measures of oxidative stress can reveal sex-specific costs of reproduction in free-ranging male and female rhesus macaques (*Macaca mulatta*). Male rhesus macaques have a shorter life span than females (Clutton-Brock and Isvaran, 2007), thus males are expected to exhibit higher levels of oxidative damage than females. However, since males experience higher mortality during the mating season while female mortality is higher during the birth season (Hoffman et al., 2008), sex differences in oxidative damage may depend on the time period in which oxidative stress is measured.

In rhesus macaques, reproductive investment peaks in young adults (6-12 years of age: Dubuc et al., 2014; Hoffman et al., 2010) therefore we predicted that younger adults would show greater oxidative damage than older adults. Among lactating females, we hypothesized that levels of oxidative damage would be negatively associated with offspring age, as the energetic costs of lactation in primates tend to be highest at the beginning of infant life (Emery Thompson et al., 2012). We also predicted higher oxidative damage in mothers with daughters relative to mothers with sons, as interbirth intervals are longer (suggestive of greater maternal investment) following the birth of daughters than following the birth of sons (Maestripieri, 2001). For males, we hypothesized that reproductive effort during the preceding mating season (March – June 2013), measured by mean rate of copulations and mean rate of agonistic interactions won would correlate positively with oxidative damage, as higher mating effort leads to loss of energetic condition (Higham et al. 2011). We also examined whether oxidative status related to male agonistic effort during a period of unusually high social instability (22 July – 18 August 2013; Supplementary Figure 1) to test the prediction that investment in high-intensity agonistic competition compromises male oxidative status. As blood collections could

only be made several months after the breeding season (December 2013-February 2014), the intervening period of male social instability might be predicted to exert a greater effect on variation in oxidative stress, as aggressive competition during this period was unusually intense, with rates of agonistic behaviours exceeding those observed during the mating season (Supplementary materials).

In our study, we characterised individual oxidative status in plasma samples via three assays: two assays to measure oxidative damage (DNA oxidative damage and lipid peroxidation) and one assay to assess antioxidant defences (total antioxidant capacity). We also examined the potential confounding effect of social status in both sexes, as more dominant males were previously found to have lower plasma lipid peroxidation before the start of the mating season (Georgiev et al., 2015).

RESULTS & DISCUSSION

We collected blood samples (N = 31) from our study subjects during last 2 – 3 months before the start of the mating season – a period when females were still lactating and males had not yet started to experience increases in androgens, associated with the start of mating (Supplementary figure 1). Lactating females had significantly (17.7%) lower plasma concentrations of 8-OHdG, a marker of DNA oxidative damage, than males ($F_{2,28} = 6.33$, Adj. $R^2 = 0.26$, $P = 0.005$; effect of male sex: $\beta = 0.13 \pm 0.06$, $t = 2.2$, $P = 0.04$; Figure 1A). Across both sexes (N = 31 individuals; 14 males, 17 females), younger adults had higher levels 8-OHdG than older ones ($\beta = -0.02 \pm 0.01$, $t = -2.3$, $P = 0.03$; Figure 1B). The interaction between age and sex was not significant, suggesting that age had a similar effect on oxidative status in both sexes ($F_{3,27} = 4.22$, Adj. $R^2 = 0.24$, $P = 0.014$; interaction age*sex: $\beta = -0.01 \pm 0.02$, $t = -0.55$, $P = 0.59$). Sex had a small effect on malondialdehyde (MDA) concentration, a marker of lipid peroxidation ($F_{1,30} = 5.44$, Adj. $R^2 = 0.12$, $P = 0.03$), with males having higher levels than females ($\beta = 0.04 \pm 0.02$, $t= 2.4$, $P = 0.03$; Supplementary Figure 2A). Adding age to the model, however, worsened its fit ($F_{2,30} = 2.76$, Adj. $R^2 = 0.10$, $P = 0.08$) as age was not significantly associated with MDA ($\beta = 0.001 \pm 0.002$, $t= 0.35$, $P = 0.73$; Supplementary Figure 2B). Neither age nor sex were associated with total antioxidant capacity, measured in Trolox equivalents ($F_{2,31} = 1.16$, Adj. $R^2 = 0.01$, $P = 0.33$; Supplementary Figure 3).

Oxidative status was not related to dominance rank among the females, or among the males (Supplementary Table 1). We thus did not consider the effect of

rank further in our analyses. Among lactating females, 8-OHdG levels were not related to offspring age (Kendall rank correlation: $\tau = 0.258$, N = 17, P = 0.16). Mothers with daughters tended to have higher DNA oxidative damage than mothers with sons (W = 54, N = 17, P = 0.08). After excluding the only primiparous mother (who had a son, as well as one of the highest levels of 8-OHdG concentration) from the analysis, the effect of offspring sex became significant: multiparous mothers with daughters (N= 9) had significantly higher levels of DNA oxidative damage than multiparous mothers with sons (N= 7) (W = 53, P = 0.02, Fig. 2A). Lipid peroxidation and total antioxidant capacity did not relate to offspring age or to offspring sex (Supplementary Table 1).

For males, total copulation rates during the 2013 mating season (March-June) did not relate to subsequent levels of 8-OHdG, MDA, or total antioxidant capacity (Supplementary materials). Male agonistic investment (rate of dominating others and rate of attacking others) during the mating season was not related to any measure of subsequent oxidative status (Supplementary materials). Male rates of dominating others during the period of social instability following the overthrow of the alpha male did not correlate with subsequent oxidative status (Supplementary Table 1). Males who physically attacked others at higher rates, however, exhibited higher subsequent 8-OHdG levels ($\tau = 0.5$, N =11, P = 0.041, Fig. 2B) but not lipid peroxidation or total antioxidant capacity (Supplementary Table 1).

The finding that males had higher levels of 8-OHdG and MDA than lactating mothers supports the hypothesis that sex differences in life span reflect weaker selection on longevity among males in polygynous species (Clutton-Brock and Isvaran, 2007). In rhesus macaques, direct male competition is less pronounced than in many polygynous primates, yet male mortality peaks during the breeding season (Hoffman et al., 2008) and energy availability appears to constrain mating effort (Higham et al., 2011). Genetic data reveal significant, though moderate, reproductive skew, with lifetime reproductive success better predicted by variation in reproductive rate than longevity (Dubuc et al., 2014). Due to constraints imposed by the timing of trapping, both males and females were sampled during the latter part of the birth season, when females were lactating experiencing high reproductive costs (Hoffman et al., 2008). The fact, even during the birth season, oxidative damage was still higher in males than in females illustrates that physiological costs of intra-sexual competition

for males are significant year-round even in the absence of pronounced direct competition.

In our study, the greater levels of 8-OHdG in younger adults are consistent with the higher reproductive rates experienced in this age class, particularly among males (Dubuc et al., 2014). A similar relationship between age and oxidative damage measured during the non-mating season has been shown among male mandrills, *Mandrillus sphinx* (Beaulieu et al., 2014). Young adult males may face additional costs due to migration out of their natal group. Finally, the youngest animals in our dataset are probably still undergoing somatic growth, and this may also contribute to the negative correlation between age and oxidative damage (Nussey et al., 2009). We cannot, however, exclude the possibility of phenotypic correlations, such that the few individuals surviving past the age of 12 may have been those who were best able to avoid or counter oxidative damage.

Multiparous females with daughters had higher levels of 8-OHdG than mothers of sons. This is consistent with maternal investment being higher in daughters than in sons in rhesus macaques (Maestripieri, 2001). The only primiparous mother in our sample had one of the highest levels of 8-OHdG, which is consistent with the observation that first-time mothers experience higher costs of reproduction than multiparous females (Bercovitch et al., 1998; Hinde, 2007).

Among males, rates of copulation and dominance interactions during the preceding mating season did not correlate with oxidative status measured several months later. As oxidative status could only be assessed several months following the mating season, males may have recovered from the somatic damage of the mating season in preparation for the next. Nevertheless, we found that the rate at which males physically attacked others during the month following the overthrow of the alpha male, a period temporarily closer to blood sample collection, was related positively to 8-OHdG levels. Unusually, rates of contact aggression given during this socially unstable month were more than twice as high than during mating season (Supplementary Figure 4). Such intense agonistic engagement was likely more salient in affecting the overall condition of males than the competition they experienced during the mating season several months earlier. Indirect measures of social instability have also been linked to increased oxidative damage among high-ranking male mandrills (Beaulieu et al., 2014) . Taken together, these findings suggest that intense

male-male competition during periods of social instability may result in significant physiological costs, the recovery from which can take more than a few months.

Oxidative stress may result from an increase in production of potentially damaging reactive oxygen species (ROS) and/or a decreased ability to resist damage via antioxidants. In our study, differences in oxidative damage between and within the sexes were most likely related to the former, as levels of antioxidant capacity did not vary with any of the variables we investigated. This is consistent with the high metabolic costs of activities such as lactation and aggression. Additionally, where null effects on oxidative damage were found, as in the case of dominance rank, we can also tentatively conclude that this was not because individuals producing more oxygen radicals were simply more effective at neutralizing them.

Finally, it is worth noting that only one of the results – that males have higher oxidative damage than females – was born out by both markers of oxidative damage used in this study: 8-OHdG and MDA. Similar to others (Sharick et al., 2014), we suggest that assessing oxidative damage via multiple bio-markers may provide a more complete, albeit more difficult to interpret, picture of variation in individual oxidative status.

In conclusion, in rhesus macaques, a moderately polygynous species, sex differences in the costs of reproduction are reflected in pattern of 8-OHdG concentration, a marker of DNA oxidative damage. Within-sex variation in oxidative stress was also associated, to some extent, with variation in maternal investment and in male agonistic effort. This provides promising evidence to compel future work in primates to track changes in oxidative status longitudinally to allow a more detailed, proximate examination of how inter- and intra-individual variation in reproductive effort may relate to oxidative costs.

MATERIALS AND METHODS

We studied the free-ranging, provisioned rhesus macaques on Cayo Santiago, a small island off the coast of Puerto Rico. All experimental procedures were approved by IACUC of the University of Puerto Rico. Our study group, Group S, numbered 133 individuals including 15 adult males (≥ 5 years old) and 42 adult females (≥3 years old) during the mating season (28 of which gave birth during the latter part of the study), the remainder being non-reproducing infants, juveniles, and adolescents. We collected blood samples from 14 adult males and 17 lactating females (Supplementary materials). Behavioural data on all adult males were

collected from March to November 2013 and were used to quantify mating effort, agonistic engagement and social status (Supplementary materials). Matriline ranks, which are stable over many years, were established with behavioural data available from previous studies (Balasubramaniam et al., 2014; Mandalaywala et al., 2014). Females were classified as belonging to high-ranking (the top two) or low-ranking matrilines (Supplementary materials). Precise ages for all adults, and birth records for infants born during the 2013 birth season were available from long-term records of the Caribbean Primate Research Center (CPRC) of The University of Puerto Rico. Animals were trapped for plasma sample collection between December 2013 and February 2014. We measured oxidative status via three commercially available assay kits (two for oxidative damage and one for antioxidant capacity) at the University of New Mexico. We measured DNA oxidative damage (8-OHdG concentration) with the 'New 8-OHdG Check' kit (catalogue No. KOG-200S/E, Japan Institute for the Control of Aging/Genox Corp.). We measured lipid peroxidation via the OXItek TBARS assay kit (ZaptoMetrix Corporation # 0801192) as malondialdehyde (MDA) concentration equivalents (nmol/ml). Total antioxidant capacity was measured via the Cayman Chemical Antioxidant Assay Kit (Item No. 709001) in millimolar Trolox equivalents. All samples were assayed in duplicate and only those with coefficient of variation (CV) below 15% were considered (Supplementary materials).

We log10-transformed values of oxidative status assays before parametric analysis to ensure normality of residual distribution. We fitted three separate multiple regression models with each of the three oxidative stress measures as dependent variables, and individual age and sex as predictors (N = 31 – 34 samples). Initially we also added the interaction between sex and age but as it was not significant, we reported the main effects removing the interaction from the model. Because of the small sample size and associated low statistical power, we conducted bivariate nonparametric analysis for the male (N=11-14) and female (N=16-17) datasets separately. Nonparametric tests were conducted using untransformed assay values. All tests were two-tailed and significance set at 0.05.

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COMPETING INTERESTS

None.

AUTHOR CONTRIBUTIONS

AVG and DM conceived the study. AVG and TMM collected data and samples. AVG, MET, and TMM analysed data/samples. AVG wrote a first draft of the manuscript and all authors contributed to revisions.

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Figures

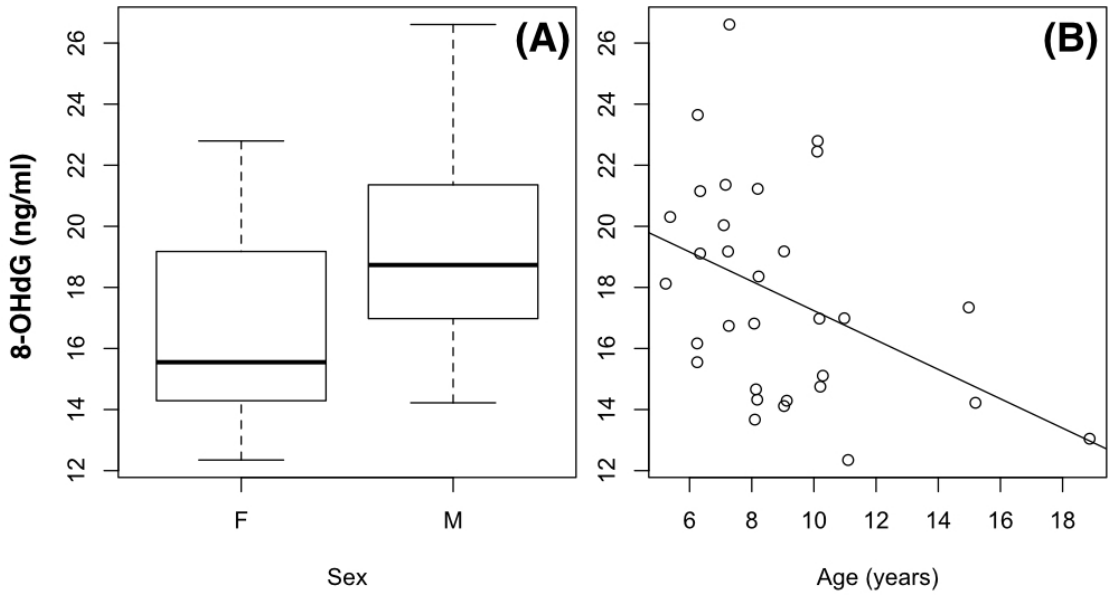

Figure 1. Inter-individual variation in DNA oxidative damage (8-OHdG concentration) among free-ranging rhesus macaques on Cayo Santiago (N = 31 individuals). Both sex (A) and age (B) independently affected oxidative damage.

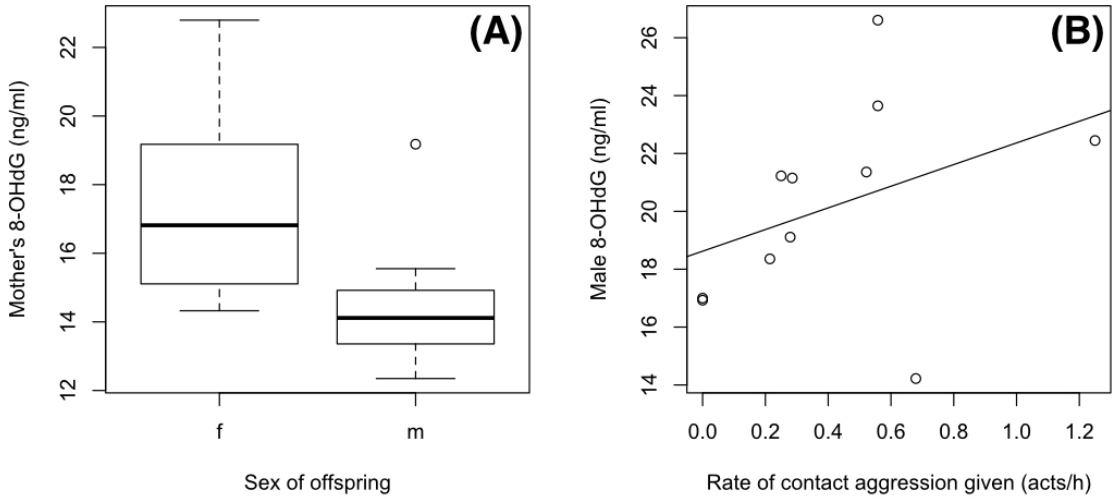

Figure 2. Factors affecting oxidative damage among lactating multi-parous females and adult males. Effect of (A) sex of offspring on oxidative damage among mothers (N = 16), and (B) mean rates of contact aggression given during a period of social instability (July – August 2013) on male oxidative damage (N = 11 males) measured several months later (December 2013 – February 2014). Regression line is for illustrative purposes. Two of the data points in panel B were offset minimally along the y-axis to avoid over-plotting.