Article Type: Research Papers



# Large-brained birds suffer less oxidative damage

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jeb.12920

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## Abstract

Large brains (relative to body size) might confer fitness benefits to animals. Although the putative costs of well-developed brains can constrain the majority of species to modest brain sizes, these costs are still poorly understood. Given that the neural tissue is energetically expensive and demands antioxidants, one potential cost of developing and maintaining large brains is increased oxidative stress ('oxidation exposure' hypothesis). Alternatively, because large-brained species exhibit slow-paced life histories, they are expected to invest more into self-maintenance such as an efficacious antioxidative defence machinery ('oxidation avoidance' hypothesis). We predict decreased antioxidant levels and/or increased oxidative damage in large-brained species in case of oxidation exposure, and the contrary in case of oxidation avoidance. We address these contrasting hypotheses for the first time by means of a phylogenetic comparative approach based on an unprecedented dataset of 4 redox state markers from 85 European bird species. Large-brained birds suffered less oxidative damage to lipids (measured as malondialdehyde levels) and exhibited higher total non-enzymatic antioxidant capacity than small-brained birds, while uric acid and glutathione levels were independent of brain size. These results were not altered by potentially confounding variables and did not depend on how relative brain size was quantified. Our findings partially support the 'oxidation avoidance' hypothesis and provide a physiological explanation for the linkage

of large brains with slow-paced life histories: reduced oxidative stress of large-brained birds can secure brain functionality and healthy lifespan, which are integral to their lifetime fitness and slow-paced life history.

*Keywords:* antioxidants; brain size; life history; lipid peroxidation; oxidative stress.

## Introduction

A range of fitness benefits have been associated with large brain sizes (relative to body mass) and these served as primary explanation for their evolution. For instance, the 'cognitive-buffer' hypothesis proposes that large brains confer enhanced cognitive skills and flexible behavioural repertoires, which ultimately buffer them against the extrinsic hazards of mortality (e.g. starvation, parasites, predation and social stress; Allman *et al.*, 1993; reviewed by Sol, 2009). Ample comparative evidence demonstrates that big-brained species excel in terms of learning, cognition, innovation and behavioural coping with environmental stressors, and enjoy enhanced survival and longer lifespan in changing or novel environments (e.g. Shultz *et al.*, 2005; Sol *et al.*, 2005, 2007, 2012; González-Lagos *et al.*, 2010; Maklakov *et al.*, 2011; Benson-Amram *et al.*, 2016; reviewed by Sol, 2009). Considering these salutary effects, it is odd that only a small fraction of homeotherm vertebrates possess considerably larger brains than expected from their body mass (Parker, 1990; Isler & van Schaik, 2009a; b). Costs of a highly-developed central nervous system should be responsible for constraining the evolution of large brains relative to body mass. Yet, except the high energetic needs of brain development and maintenance, there is scant evidence about putative costs, even though these are imperative to understand brain size evolution (Martin, 1981; Parker, 1990; Isler & van Schaik, 2006b, 2009a; Navarrete *et al.*, 2011).

The costs of enlarged brains postulated so far are often grounded on the high metabolic expenses of developing and maintaining the neural tissue, which require high rates of oxygen consumption, ion pumping and neurotransmitter synthesis (Parker, 1990; Aiello & Wheeler, 1995; Isler & van Schaik, 2006b). This cost can be paid directly by increased metabolic rate (Parker, 1990; Ricklefs, 2004; Isler & van Schaik, 2006b; but see Isler & van Schaik, 2006a) and/or increased maternal metabolic turnover (Martin, 1981; Isler & van Schaik, 2009a; Barton & Capellini, 2011). It can also be manifested as energetic trade-offs implying that allocation into the brain tissue is saved to the detriment of investment into intestines, other visceral organs, locomotion, adipose tissue or production of soma and progeny (Aiello & Wheeler, 1995; Isler & van Schaik, 2006a, 2009a; b; Barton & Capellini, 2011; Navarrete *et al.*, 2011; Kotrschal *et al.*, 2013).

Oxidative homeostasis, another component of the physiological network, was also found to play a role in brain development and functioning within species (Gilgun-Sherki *et al.*, 2001; Halliwell, 2001; Lin & Beal, 2006; Dröge & Schipper, 2007). However, this topic was seldom addressed in evolutionary biology (see Buchanan *et al.*, 2013 and references therein) and thus the understanding of the brain–redox state nexus remained elusive especially in comparisons across several species. Oxidative stress arises when the generation of reactive oxygen species (ROS) by cellular respiration overwhelms the defence capacity of the antioxidative system (reviewed in detail in Monaghan *et al.*, 2009; Pamplona & Costantini, 2011). Oxidative stress state implies that vital cellular components (proteins, lipids and DNA) can be damaged, and the accumulation of unrepaired oxidative damages was suggested to play at least some role in ageing and a plethora of age-related disorders (around 150) at the organism level in general and nervous system in particular (Finkel & Holbrook, 2000; Barja, 2004; Halliwell & Gutteridge, 2007; Buttemer *et al.*, 2010; Clausen *et al.*, 2010; Speakman *et al.*, 2015).

Here we ask for the first time whether oxidative state measured in the blood tissue is associated with brain size in birds. We propose two alternative hypotheses linking brain size and redox physiology. The 'oxidation exposure' hypothesis argues that an elevated oxidation exposure might arise in large-brained organisms for at least three reasons. First, the high metabolic expenses of large brains might exacerbate the oxidative insults, all else in the redox system being equal (Beckman & Ames, 1998; see also Burger *et al.*, 2008); though the metabolic theory of oxidative stress and ageing is progressively devalued (Hulbert *et al.*, 2007; Speakman & Selman, 2011; Speakman *et al.*, 2015). Second, brain development and maintenance demands large amounts of antioxidants (e.g. glutathione; Galván & Møller, 2011), which might be traded off with the antioxidant protection against oxidation challenges. Third, the neural tissue demands high blood oxygen and glucose levels, which are sources of ROS production (Aiello & Wheeler, 1995; Gilgun-Sherki *et al.*, 2001; Halliwell, 2001; Galván & Møller, 2011).

Alternatively, the 'oxidation avoidance' hypothesis postulates that species with large brains should exhibit adaptations that better retard an oxidative stress state to be settled. Better oxidation avoidance can help to shield out potentially long-lasting and adverse carry-over effects of oxidative stress. Given that large-brained species feature a slow pace-of-life, and hence rely on a long reproductive life to improve their fitness (Sol *et al.*, 2007, 2012; Sol, 2009; van Schaik *et al.*, 2012), they should gain fitness benefits by investing into self-maintenance such as oxidative homeostasis (Halliwell & Gutteridge, 2007; Monaghan *et al.*, 2009). The rationale behind this hypothesis is that oxidative homeostasis is, on one hand, conducive to brain functionality and cognitive resilience, which are integral to the fitness and slow-paced life history of large-brained species (Buchanan *et al.*, 2013), and, on the other hand, might permit longer reproductive lifespan (Finkel & Holbrook, 2000; Halliwell & Gutteridge, 2007; Buttemer *et al.*, 2010; Salmon *et al.*, 2010; Speakman *et al.*, 2015).

We contrasted the 'oxidation exposure' and 'oxidation avoidance' hypotheses by predicting that the former holds if species with large relative brain size feature reduced antioxidant defence and/or enhanced oxidative damage as measured in the blood tissue, while the latter is supported if these relationships are reversed. For this, we conducted a comprehensive phylogenetic comparative analysis based on an unprecedented dataset of four blood redox state markers of 85 European bird species that belong to 37 families and 13 orders (based on taxonomy by Gill & Donsker, 2016). Three markers describe the non-enzymatic antioxidant defence (total antioxidant status, uric acid and total glutathione), and one marker shows the oxidative damage of lipids (malondialdehyde). Lipid peroxidation was measured for the first time spanning a wide range of wild-living avian taxa, while the antioxidant parameters were the first time assayed for such a large number of European bird species. These markers were chosen because (1) non-enzymatic antioxidants, besides antioxidant enzymes, are deployed to combat free radical insults and might play a role in ageing (Cohen *et al.*, 2007), (2) glutathione is the most significant intracellular, endogenous, non-enzymatic antioxidant with multifaceted physiological effects (Meister & Anderson, 1983; Galván & Alonso-Alvarez, 2008) including brain development and maintenance (Gilgun-Sherki *et al.*, 2001; Halliwell, 2001; Galván & Møller, 2011) and is also integral to the ageing process (Maher, 2005; Rebrin & Sohal, 2008), and (3) malondialdehyde, which results from the peroxidative degeneration of polyunsaturated fatty acids by ROS, is a widely used marker of oxidative stress (Del Rio *et al.*, 2005; Halliwell & Gutteridge, 2007; Monaghan *et al.*, 2009), can act as a ROS itself and consequently can trigger oxidative cascades and perpetuate lipid peroxidation (Barja, 2004; Del Rio *et al.*, 2005; Halliwell & Gutteridge, 2007), and was also linked to ageing (Spiteller, 2007; Pamplona, 2008).

## Materials and methods

### Fieldwork

We sampled 544 birds belonging to 85 species in Romania between 2011 and 2013 (Table S1). These species represent a diverse set of European birds as they belong to 37 families and 13 orders (based on taxonomy by Gill & Donsker, 2016). Only a small fraction of species is represented by one sampled individual (11 out of 85, 13%; Table S1). The majority of the species were captured at multiple locations (totally 42 in eight counties) and/or during multiple sampling occasions (Table S1). Species were sampled in their breeding phase from late April until early July. The breeding phase was confirmed by females exhibiting clear brood patches. After capture, we banded the birds with metal rings (to exclude repeated sampling of the same individuals) and determined their age (only adults were considered) and sex (except for 28 species that are sexually monomorphic). Then, we took a blood sample (range 30–300 µL, depending on body size) by brachial venepuncture into heparinized capillaries. The blood sample drawn from small-sized species usually allowed only one aliquot, therefore different redox state markers were measured from different samples, which makes the sample size to vary per marker per species (Table S1). For the welfare of birds and potential stress-sensitivity of redox markers, bleeding took place as fast as possible after the bird hit the net (bleeding time within 15 min; mean = 9.34 min, SD = 4.72). All birds were released in good condition after sampling. The samples were kept in dark cooling boxes at around 4°C for less than 10 hs until spun (for 5 mins at 5,000 rpm) to separate the plasma and erythrocyte fractions. The plasma fraction was visually scored for the degree of haemolysis on a 4-point scale (none, weak, intermediate, strong), then plasma was partitioned into aliquots for each marker and all aliquots and the erythrocytes were stored at –50°C until assaying. Laboratory assays (see below) were done following the same protocol and by the same person (LP). The abovementioned standardisation of sample collection and assaying

minimized the heterogeneity in data due to geographic region, life-history stage, bird's age, stress exposure (samples only from Romania, breeding season and adults, and collected within a short time, respectively) and laboratory protocols. Trapping by mist nets and blood sampling was done as licensed by the Romanian Academy of Sciences (permission no. 2257) and in accordance with current Romanian laws of animal welfare.

**Biochemical assays**

We measured three antioxidant markers (total antioxidant status, TAS, uric acid, UA, and total glutathione, tGSH) and a marker of peroxidative damage to lipids (malondialdehyde, MDA, by HPLC). Detailed protocols can be found in the Supporting Information and Bókony *et al.* (2014). UA significantly positively correlates with TAS (species-level correlation: $r$ = 0.32, 95% CI: 0.12–0.50, $t_{82}$ = 3.07, $P$ = 0.003) in agreement with previous studies (Cohen *et al.*, 2008). To remove the contribution of UA to TAS, we extracted the TAS residuals from an ordinary least squares regression (Cohen *et al.*, 2008) of individual-level measures of TAS on UA and then averaged this per species.

**Brain size**

Information on brain mass was obtained from a previously assembled dataset (Sol *et al.*, 2010). Despite concerns raised about combining brain measurements from different sources, this has proved negligible in comparisons across species (Sol *et al.*, 2010). For seven species we had no species-specific brain mass data and thus we instead used genera mean brain mass; however, this did not alter the results (see the Supporting Information). To account for the allometric effects of body mass on brain mass, body mass data were collected from (Dunning, 2008) and the allometric exponent was estimated as the slope of the log brain size on log

body mass by means of phylogenetic generalized least squares (PGLS) regression. Brain size scales to body mass$^{0.65}$ (log brain mass = –2.48 + 0.65 × log body mass); the scaling exponent 0.65 being very close to the hypothetic negative allometric exponent 0.67. Consequently, we derived a mass-corrected brain size dividing original brain mass with body mass$^{0.65}$ (see also Sol *et al.*, 2008). The mass-corrected brain size thus reflects the size of the brain relative to what is expected from body size. The results found by other two alternative measures of relative brain size are coincident and can be found in the Supporting Information.

**Confounding variables**

We ruled out the potentially confounding effects of sampling stress (expressed as time elapsed between hitting the net and bleeding), degree of haemolysis, sampling date, body mass, life history and breeding latitude. Details on their relevance and methods are provided in the Supporting Information.

**Statistical analyses**

All statistical analyses were carried out in R 3.2 (R Core Team, 2015). To assess whether redox state markers are suitable for multispecies comparison, we tested the species-specificity of these traits by partitioning variance into among- and within-species components using the function 'ICCest' from the R package 'ICC' (Wolak *et al.*, 2011). Since the precision of within-species variance estimation is dependent on sample size, the estimation of intraclass correlation coefficient (ICC; a measure of species-specificity here) in comparative studies with unbalanced sample sizes across species is not equivocal. To cope with this problem, as a first step, species with only one individual sampled per focal redox marker were excluded. Subsequently, for the remaining species, we randomly picked two individuals out of the total

number of available measurements per species and computed ICC. This routine was iterated 1,000 times, so final ICCs are the average of the 1,000 ICC estimates. Then we restricted ICC calculations to species with at least three, four and five individuals sampled within species. ICC was computed using raw, non-transformed values. We also used an alternative approach building a model for the entire individual-level dataset (i.e. without restriction to any particular sample size within species) with species ID being set as random factor. The variance was partitioned to among- and within-species components ($V_a$ and $V_w$, respectively) and ICC was computed as $V_a / (V_a + V_w)$.

To test the 'oxidation exposure' and 'oxidation avoidance' hypotheses, we modelled each of the 4 redox markers as a function of relative brain size and the three potential confounding variables (body mass, developmental time and LCB) as covariates. Additionally, because the redox state markers are expected to covary, those three markers that were not the response in the actual model were also entered as covariates to assess whether they are related to relative brain size independently of each other. Sex was omitted from models since one third of the species could not be sexed in the field. However, the significant species-specificity of redox markers (see Results) indicates that conspecifics are more similar to each other than to other species, independent of their sex. All variables were log-transformed to meet the normality assumption, and were scaled to zero mean and unit SD to gain comparable parameter estimates. The full models were reduced to minimal adequate models (MAMs) by backward stepwise elimination with the more permissive criterion of $P < 0.1$ in order to retain marginal explanatory terms as well (i.e. $0.05 < P < 0.1$). Although body mass and developmental period were correlated, the fact that their relationship with the response variables were consistent whether the other life-history trait was present or absent in the model suggests that collinearity was not an issue in our models.

To account for the dependence of species due to shared evolutionary ancestry, we built PGLS models in which phylogenetic signal (Pagel's $\lambda$; Pagel, 1997, 1999) was estimated by maximum likelihood. To control for phylogeny and uncertainties in phylogenetic construction, we retrieved 1,000 phylogenetic trees from birdtree.org (Jetz *et al.*, 2012), with the backbone tree of (Hackett *et al.*, 2008), which were merged into an ultrametric consensus phylogenetic tree using the SumTrees program (Sukumaran & Holder, 2010). Each PGLS model was based on the entire species pool and we weighted models by within species sample size (i.e. sampling effort) of the focal response variable as implemented in the 'gls' function of the R package 'nlme' (Pinheiro *et al.*, 2015). This was meant to avoid the possibility that well- or poorly-sampled species might alter the results. Furthermore, all analyses were repeated by only using species with at least three individuals sampled within species for the focal response variable, the level at which each redox state marker proved to be species-specific (see Table S2).

Phylogenetic signal of redox parameters was computed using the 'phylosig' function of the R package 'phytools' (Revell, 2012). Strong and weak signal (i.e. $\lambda$ approaches 1 and 0, respectively) indicates that evolution conforms to or deviates from the Brownian motion model, respectively.

Additional statistical methods can be found in the Supporting Information.

## Results

The redox state markers were found to be species-specific (i.e. have significant ICC values) with both approaches (i.e. based on the entire dataset or with sample size restrictions); though, the ICC of non-enzymatic antioxidant markers were generally low (Table S2). The phylogenetic signal of MDA was marginally (Pagel's $\lambda = 0.57$, $P = 0.087$) and that of tGSH

significantly ($\lambda$ = 0.46, $P$ = 0.01) different from zero, while it did not differ from zero for the other two antioxidant markers (TAS: $\lambda$ = 0.00, $P$ = 1.0; UA: $\lambda$ = 0.24, $P$ = 0.37).

The levels of UA and MDA were weakly negatively, while the concentration of tGSH was significantly positively related to body mass. TAS and UA levels increased with higher degree of lipid peroxidative damage (i.e. MDA concentration; Table 1). In addition, residual TAS was also elevated in species with higher MDA level (PGLS, $\beta \pm$ SE = 0.08 ± 0.03, $t_{84}$ = 2.47, $P$ = 0.016, Pagel's $\lambda$ = 0.04).

Species with large relative brain size featured significantly higher TAS and significantly lower peroxidative damage to lipids (expressed by the concentration of MDA) in concordance with the 'oxidation avoidance' hypothesis (Table 1, Fig. 1; see Table S3 for full models). UA and tGSH, however, were unrelated to relative brain size. These results were robust to the confounding effects of body mass, developmental time, LCB and redox state covariates (Table 1), and held regardless of multiple changes in the modelling procedure (see Sensitivity analyses and Tables S4, S5 and S6 in the Supporting Information).

## Discussion

We found that the four redox state markers are species-specific, i.e. conspecifics resemble each other more (even if they differ in e.g. sampling stress, sampling day or breeding status) than other species, which is a primary requisite of phylogenetic comparisons. We showed that bird species with disproportionally larger brains relative to body mass suffer lower oxidative damage to cell lipids (MDA) and have higher total non-enzymatic antioxidant capacity (TAS) in the peripheral blood during the energetically demanding period of reproduction. However, two individual antioxidant compounds, plasma uric acid (UA) and erythrocyte glutathione (GSH), were not related to brain size. These findings lend partial support for the 'oxidation

avoidance' hypothesis and refute the hypothesis that large brains bring 'oxidation exposure' costs.

MDA is a reactive carbonyl species, a di-aldehyde intermediate of lipids' peroxidative decomposition. Cardiolipin, the most frequent phospholipid in mitochondrial membranes, has central role in cellular bioenergetics and is highly susceptible to oxidation (Paradies *et al.*, 2011). Therefore, higher lipid peroxidation levels might imply cardiolipin depletion, cellular energetic decay and loss of functionality (Paradies *et al.*, 2011). Furthermore, MDA is a prime promoter of oxidative vicious cycles by perpetuating lipid peroxidation and causing oxidative damages to proteins and DNA as well (Barja, 2004; Halliwell & Gutteridge, 2007). These oxidative damages are unwanted *per se* because of their adverse effects on neural and organismal capacity, but also derange other crucial physiological setting points like immune effectors and the insulin/IGF-1 signalling that can also cause further damages for instance in the brain (Dröge & Schipper, 2007). These multifaceted negative effects of MDA can ultimately jeopardize mitochondrial, cellular and organismal functioning and accelerate ageing (Pamplona, 2008). Species with large relative brain size thus seem to protect better their odds by mitigating such oxidation costs during the oxidatively challenging breeding period.

Studies involving transgenic model organism increasingly suggest that antioxidants might play a minor role in combating oxidative stress and silencing (neural) ageing (e.g. Barja, 2004; Speakman *et al.*, 2015). However, comparative evidence in wild-living birds shows that the level of antioxidants is elevated in species exposed to increased oxidative insults (Cohen *et al.*, 2008). Consistent with this latter study, we found that species with higher levels of MDA also exhibit higher concentrations of TAS in their bloodstream. Therefore, boosted levels of non-enzymatic antioxidants might play a role in mitigating oxidative damage of large-brained species. However, this does not deny that adaptations

other than non-enzymatic antioxidant defence might also alleviate oxidative stress in large-brained species, including lower ROS generation rate (Pamplona *et al.*, 2005), higher activity of antioxidant enzymes (Clausen *et al.*, 2010), lowered cardiolipin content (Paradies *et al.*, 2011) and lower receptor density for glucocorticoids or IGF-1 (Gilgun-Sherki *et al.*, 2001; Dröge & Schipper, 2007).

Our results show that the concentration of tGSH is unrelated to brain size, even though a positive association was previously postulated (Galván & Møller, 2011). GSH is a tri-peptide ubiquitous in virtually all cells, a key intracellular antioxidant *per se* due to the thiol group and it is also required as substrate by the antioxidant enzyme glutathione peroxidase (Gilgun-Sherki *et al.*, 2001). GSH preserves brain functionality during ageing and protects the blood–brain barrier (Halliwell, 2001) and the level of reduced GSH decreases with ageing in brain and other tissues as well (Dröge & Schipper, 2007). The downside of GSH-mediated antioxidant potential is its difficulty to bypass the blood–brain barrier (Gilgun-Sherki *et al.*, 2001). Interestingly, vitamin C and E, which are significant components of TAS, can cross the blood–brain barrier, have large and average concentrations in the brain, respectively, and both were found to be important in neural protection against oxidative insults and neurodegenerative diseases, alone or in cocktails (Gilgun-Sherki *et al.*, 2001). These differences among antioxidants might explain the positive association between TAS and brain size and the lack of association between tGSH and brain size.

At least three mutually non-exclusive explanations can be put forward for the negative covariation between brain size and oxidative stress. First, certain components of the total non-enzymatic antioxidants are derived from food. If a large relative brain size enhances the ability to discriminate and choose food resources richer in antioxidants (e.g. via visual acuity; Schaefer *et al.*, 2008 and/or diet generalism; Overington *et al.*, 2011; Ducatez *et al.*, 2015; Sol *et al.*, 2016), this should lead to an association between oxidative damage and relative

brain size. Second, given that glucocorticoids induce oxidative stress (Costantini *et al.*, 2011), large-brained species might suffer less glucocorticoid-induced oxidative stress if they cope with stress by means of cognitive mechanisms (e.g. anticipation or innovation) rather than by a glucocorticoid response (Lendvai *et al.*, 2013). Finally, the overall energy consumption rates in large-brained animals might be lower than generally assumed: (Pontzer *et al.*, 2014) reported that primates expend on average only 50% of the energy expected for a mammal of similar mass. This does not contradict that a large brain consumes more energy, as, despite their lower total energy expenditure, primates have basal metabolic rates similar to those of other mammals; however, it does suggest that overall they might suffer less oxidative damage. Whether this is also true in birds requires further analyses (but see Isler & van Schaik, 2006b).

An important contribution of our findings is that they provide a physiological explanation for the evolutionary linkage between large relative brain size and slow-paced life histories (Sol, 2009; van Schaik *et al.*, 2012) (Fig. 2). In large-brained and long-lived species selection should favour, on one hand, adaptations that ensure a homeostatic development and maintenance of the nervous system and, on the other hand, adaptations that promote longevity (Barja, 2004; van Schaik *et al.*, 2012; Buchanan *et al.*, 2013). Improved oxidation avoidance is a good candidate mechanism to meet both requirements. On one hand, it promotes brain functionality and cognitive resilience, which has high relevance for the fitness of slow-living large-brained species (Buchanan *et al.*, 2013). On the other hand, the ageing process might be at least partly contingent upon the redox state (Finkel & Holbrook, 2000; Halliwell & Gutteridge, 2007; Salmon *et al.*, 2010; Galván *et al.*, 2015; Speakman *et al.*, 2015), and the accumulation of ROS-induced oxidative injuries (e.g. mtDNA mutations) in post-mitotic tissues such as the brain are considered relevant causes of ageing in both mammals and birds (Gilgun-Sherki *et al.*, 2001; Barja, 2004; Hyun *et al.*, 2006). This is

corroborated by our data as we found higher TAS and lower MDA levels in bird species that live longer and feature slow-paced life history (Vágási *et al*., in prep.). Thus, big-brained birds might benefit not only from reduced extrinsic mortality risk via cognitive buffering, but also from reduced intrinsic hazards via oxidation avoidance. We speculate that this might have implications for the more frequent occurrence of large brains in birds as opposed to mammals (Isler & van Schaik, 2009b). The peculiarities of the avian redox physiological system confer them two-fold longer lifespan relative to size-matched mammals (Hulbert *et al.*, 2007; Costantini, 2008). Because species with large relative brains have reduced reproductive rates and lifespan partially levels off this loss (Isler & van Schaik, 2009b), the longer (reproductive) lifespan of birds might contribute to the higher frequency of large brains in birds.

In sum, our study provides support for the hypothesis that large brain size relative to body mass coevolves with an improved resistance to oxidative stress in birds. To assess the generality of our findings, the covariation between brain size and redox state needs to be further validated in other organisms. Taking the outstanding differences in redox physiology and ageing between birds and mammals (Costantini, 2008), but also in brain size (Isler & van Schaik, 2009b), mammals are an obvious choice for future tests of the hypotheses. It would also be insightful to delve into the proposed mechanisms and to measure redox state markers in post-mitotic tissue as well.

## Acknowledgements

We owe a debt of gratitude to J. Veres-Szászka, K. Sándor, B. Bakó, M. Csiszár and A. Fülöp for fieldwork, M. Banciu and A. Sesarman for biochemical advice and the directorate of the Botanical Garden Cluj for permitting bird capturing. We thank the editor Judith Mank, two

anonymous reviewers, Neil Metcalfe and Alan A. Cohen for their help to improve the quality of the manuscript. This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program'. The research infrastructure was supported by a CNCSIS research grant (PN II. RU TE 291/2010) of the Romanian Ministry of Education and Research. During writing, CIV was financed by the Postdoctoral Fellowship Programme and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences, PLP by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and DS by a Proyecto de Investigación CGL2013-47448-P. We declare we have no conflicting interests.

## Data accessibility

Supporting Information Table S1 shows the species, sample sizes per each oxidative stress variable, the number of sampling locations and dates, mean and SD of sampling dates, and body and brain mass data. The species means of the four oxidative state markers are archived at Dryad repository: DOI xyz.

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## Tables and figure captions

**Table 1** Minimal adequate PGLS models about the relationship between redox state markers (i.e. antioxidants and oxidative damage) and brain size. Pagel's $\lambda$ of each model is provided in brackets beside the response variables. $N = 84$ species for each model. Significant effects are marked in bold.

| Response | Predictor | $\beta \pm$ SE | $t$ | $P$ |
|---|---|---|---|---|
| TAS ($\lambda = 0.00$) | Intercept | 1.04 ± 0.17 | 5.98 | < 0.001 |
| | LCB | 0.09 ± 0.05 | 1.81 | 0.074 |
| | Relative brain size | 0.12 ± 0.05 | 2.42 | **0.018** |
| | MDA | 0.26 ± 0.03 | 7.59 | **< 0.001** |
| UA ($\lambda = 0.32$) | Intercept | 5.54 ± 2.01 | 2.75 | 0.007 |
| | Body mass | –1.16 ± 0.59 | 1.97 | 0.053 |
| | MDA | 3.04 ± 0.36 | 8.40 | **< 0.001** |
| tGSH ($\lambda = 0.28$) | Intercept | 10.61 ± 1.05 | 10.08 | < 0.001 |
| | Body mass | 3.11 ± 0.72 | 4.34 | **< 0.001** |
| MDA ($\lambda = 0.66$) | Intercept | 1.00 ± 0.44 | 2.24 | 0.028 |
| | Body mass | –0.25 ± 0.13 | 1.88 | 0.064 |
| | Relative brain size | –0.32 ± 0.12 | 2.75 | **0.007** |
| | TAS | 0.83 ± 0.18 | 4.66 | **< 0.001** |
| | UA | 0.10 ± 0.02 | 5.74 | **< 0.001** |

**Fig. 1** The relationship between relative brain size and (a) total antioxidant status and (b) oxidative damage (MDA). The slopes are extracted from MAMs presented in Table 1. Dot sizes are proportional to within species sample size of oxidative markers plotted without bearing on the fit.

**Fig. 2** The brain size–oxidative state–life history triangle. The association of large relative brain size with slow life-history pace might be underpinned by the oxidative stress being lower in large-brained species and higher in species with fast life-history pace.

**Fig. 1**

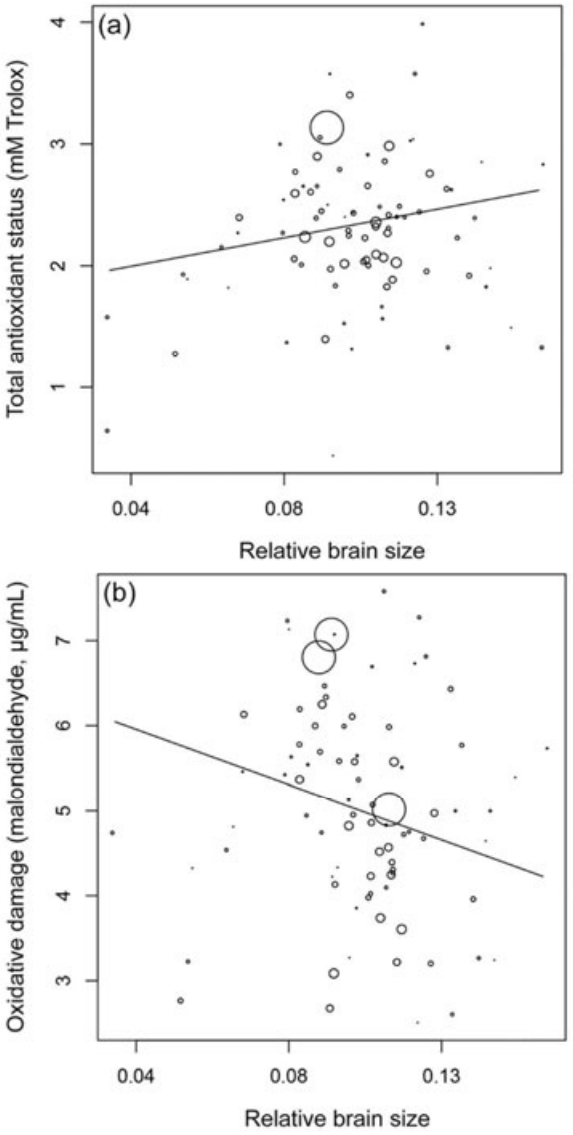


**Fig. 2**