Comparative Biochemistry and Physiology, Part A 155 (2010) 205–210

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Increased oxidative stress in barn swallows from the Chernobyl region

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ARTICLE INFO

Article history:
Received 20 June 2009
Received in revised form 27 October 2009
Accepted 29 October 2009
Available online 5 November 2009

Keywords:
Antioxidant capacity
Barn swallow
Chernobyl
Oxidative stress
Radioactive contamination
Reactive oxygen species

ABSTRACT

The Chernobyl nuclear accident produced the largest unintended release of radionuclides in history, with dramatic consequences for humans and other organisms. Exposure to ionizing radiation is known to reduce circulating and stored levels of specific antioxidants in birds and humans, thus potentially increasing oxidative stress. However, overall effects of radioactive exposure on oxidative status have never been investigated in any free ranging vertebrate. We measured plasma antioxidant capacity and concentration of reactive oxygen metabolites in adult barn swallows (*Hirundo rustica*) from colonies with variable background radiation levels in the Chernobyl region in Ukraine and Belarus. We predicted that antioxidants would decrease while reactive oxygen metabolites would increase with exposure to increasing levels of radiation at the breeding sites. Consistent with this expectation, radiation level positively predicted plasma concentration of reactive oxygen metabolites, whereas no significant covariation was found with non-enzymatic plasma antioxidant capacity. An index of oxidative stress was also larger in barn swallows exposed to high contamination levels. Thus, radioactive contamination appeared to be responsible for the increased generation of reactive oxygen metabolites and the imbalance between reactive oxygen metabolites and non-enzymatic plasma antioxidant capacity.

1. Introduction

Oxidative stress arises any time endogenous reactive oxygen species (ROS) are left unquenched by enzymatic and non-enzymatic antioxidant defences (Halliwell and Gutteridge, 2007). Organisms need to balance their energy requirements for fuelling biological processes against the potential toxicity of ROS generated by any activity as by-products of metabolic processes (Beckman and Ames, 1998; Finkel and Holbrook, 2000; Halliwell and Gutteridge, 2007). However, no prediction can readily be drawn from metabolic rate to infer oxidative damage, as organisms have evolved a complex set of antioxidant mechanisms to face oxidative challenges (Halliwell and Gutteridge, 2007; Costantini, 2008; Monaghan et al., 2009).

A promising field of research for testing mechanistic and functional hypotheses concerning natural variation in antioxidant defences is investigation of the consequences of exogenous sources of oxidative stress (e.g. pollutants, radioactive contamination; Monaghan et al., 2009). Radioactive contamination can deplete antioxidants because of their utilization for quenching ROS arising as a consequence of radiation (Iliakis, 1991), or because of their utilization for repairing DNA damage due to radiation exposure (Moody and Hassan, 1982; Imlay and Linn, 1988). However, no attempt has been made to assess the consequences of radioactive contamination for overall antioxidant status of wild populations of animals or humans. On the other hand, studies using the accident at Chernobyl power plant in 1986 as a source of potentially deleterious effects on antioxidant levels have shown that barn swallows (*Hirundo rustica*) living in radioactively contaminated areas have reduced circulating and stored levels of specific antioxidants (i.e. carotenoids and vitamins A and E; Møller et al., 2005a). These results substantially confirmed evidence from medical studies showing that individual exposure to radioactive contamination negatively impacted antioxidant defences of humans exposed to radioactive contamination in Chernobyl (Ben-Amotz et al., 1998; Ivaniota et al., 1998; Neyfakh et al., 1998a,b; Kumerova et al., 2000). In the barn swallow, concentrations of carotenoids and vitamins in eggs were also depleted in Chernobyl compared to an uncontaminated site (Møller et al., 2005a), thus suggesting that a reduction in maternal antioxidant levels may have trans-generational effects on offspring development and fitness. These findings were also consistent with results from a nest-box experiment with great tits (*Parus major*), where concentrations of total yolk carotenoids and vitamins A and E were reduced in a dose-dependent manner with increasing level of radiation at the nest boxes (Møller et al., 2008). Finally, an inter-specific survey of species richness and abundance in the Chernobyl region further showed that species using a large amount of antioxidants for sexual signalling,

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doi:10.1016/j.cbpa.2009.10.041

self-maintenance or maternal allocation to eggs might be more susceptible to the damaging effects of low-level environmental radiation in terms of population decline (Møller and Mousseau, 2007a). Thus, reductions in antioxidant level might have serious consequences for reproductive decisions in terms of clutch size and even whether to reproduce or not, and in determining the fate of natural populations in perturbed ecological conditions (Møller et al., 2005b, 2008; Møller and Mousseau, 2007b).

However, previous results obtained while studying the consequences of radioactive contamination in Chernobyl were based on only a few components of the antioxidant defence system, such as carotenoids or antioxidant vitamins. The antioxidant system is a multi-faceted complex, with different components responding differently to the same oxidative challenges (Monaghan et al., 2009). A recent analysis of the covariation among three groups of antioxidants (uric acid, vitamin E and four carotenoids) and an overall measure of antioxidant capacity (Trolox-equivalent antioxidant capacity or TEAC) found that the degree to which each antioxidant predicted total antioxidant capacity in adult individuals varied significantly both within and among species (Cohen and McGraw, 2009). The authors concluded that ecological studies attempting to examine antioxidant function should therefore use multiple measures and avoid the assumption that dietary antioxidants are essentially the most crucial defence against oxidative damage (Cohen and McGraw, 2009; Monaghan et al., 2009). To avoid one-sided evaluations and to correctly assess the balance between damage prevention by the antioxidant system and ROS generation, a more integrated approach is therefore needed (Finkel and Holbrook, 2000; Halliwell and Gutteridge, 2007; Monaghan et al., 2009).

In this study, we measured antioxidant capacity, oxidative damage and oxidative stress in barn swallows from different sites in the Chernobyl region. We tested the hypothesis that radioactive contamination can impair overall antioxidant status in wild populations of barn swallows living in this region. The barn swallow is a long-distance migratory species and is therefore an ideal model species for testing the relationship between radiation exposure and antioxidant status. In fact, migratory behaviour is known to impair antioxidant defences owing to intense metabolic activity required by flight effort (Costantini et al., 2007) and it has been shown that migratory species are experiencing a stronger decline in abundance in radioactively contaminated areas around Chernobyl compared to resident species, likely because of the depletion of antioxidants defences required to neutralize free radicals (Møller and Mousseau, 2007a).

2. Materials and methods

2.1. Field procedures

Using mist nets, we captured barn swallows at the peak of reproduction just before and during the laying period for most individuals (Møller, 1994; Møller et al., 2005b). Captures were conducted in 2007–2008, between June 1st and 15th, during daytime (ca. 10:00 h. to 15:00 h.).

We measured several phenotypic characters in adult males and females (including beak length, wing length, tail length, tarsus length, keel length, and body mass) with a ruler, a digital caliper, or a Pesola AG (Baar, Switzerland) spring balance, as described in Møller (1994). We also carefully checked all females for a fully developed brood patch. A blood sample was collected by puncturing the ulnar vein immediately after capture and collecting the blood in heparinized capillary tubes. Blood samples were then centrifuged for 10 min at 16,000 g and stored at −80 °C to serve for oxidative stress measurement. Background radiation level was measured using a hand-held dosimeter (Inspector, SE International, Summertown, Tennessee, USA) and later cross-validated with official measurements (Shestopalov, 1996). Based on our field measurements, we classified sampling sites as low-, intermediate- or high-level. Mean background radiation level was 0.074 (range 0.02–0.15) μSv/h in the low-level contamination sites (Farm 1 (1), Farm 2 (2), Farm 3 (3), Rudnia (6), Dytiaku (8), Pysky (9) and Voronkov (10)), 0.455 (range 0.45–0.46) μSv/h in the two intermediate-level sites (Farm 4 (4) and Farm 5 (5)), and 2.9 μSv/h in the high-level site (Vesniane (7)) (numbers in parentheses refer to the numbers used in Fig. 1). Background radiation in uncontaminated sites in Ukraine is around 0.03 μSv/h.

2.2. Plasma antioxidant capacity (TAC)

The plasma antioxidant barrier includes both exogenous (e.g. ascorbate, tocopherols, carotenoids) and endogenous (e.g. uric acid, enzymes) compounds. The total antioxidant capacity (TAC) of plasma was measured using the OXY-Adsorbent test (Diacron, Grosseto, Italy) that uses a colorimetric determination to quantify the ability of the plasma antioxidant barrier to cope with the oxidant action of hypochlorous acid (HClO). Similarly to the Trolox-equivalent antioxidant capacity or TEAC (Cohen and McGraw, 2009), TAC reflects levels of circulating antioxidants including vitamin C and E, carotenoids and uric acid, but does not account for levels of enzymatic antioxidants.

The plasma (10 μL) was diluted 1:100 with distilled water. A 10 μL aliquot of this solution was added to 1 mL of a known-titre HClO solution in plastic photometer cuvettes. The solution was gently mixed and incubated at 37 °C for 10 min. At the end of the incubation time, 10 μL of an alkyl-substituted aromatic amine (N,N-diethyl-*p*-phenylenediamine) solubilised in a chromogenic mixture were added. Such amine is oxidized by the residual HClO and converted into a pink-coloured derivative. Colour intensity is directly proportional to the HClO excess and inversely related to the antioxidant capacity of tested plasma. The absorbance of the coloured solution was measured at 546 nm using a dedicated photometer (Free, Diacron). One standard sample of known TAC was used as reference. The absorbance of a blank sample (10 μL of distilled water) was subtracted from that of the tested samples. Antioxidant capacity per unit volume of plasma was expressed in μM of HClO neutralised by the antioxidants contained in the sample. Measurement repeatability was estimated on 15 samples (12 samples run in duplicate and three samples run in triplicate) by means of a one-way ANOVA with individual as the main effect. This analysis showed that TAC measures were highly consistent within individual ($F_{14,32} = 28.79$, $P < 0.001$, adjusted $R^2 = 0.926$). Concentration for samples assayed more than once were calculated as the mean of the measures.

2.3. Reactive oxygen metabolites (ROMs) and oxidative stress

Reactive oxygen metabolites (ROMs) are peroxidation products (primarily hydroperoxides, ROOH) of reactive oxygen species (ROS). They can be considered markers of early oxidative damage, as their cleavage by metals leads to the generation of two highly reactive prooxidants, the alkoxyl (R–O.) and alkylperoxyl (R–OO.) radicals, which in turn can promote an oxidative cascade (Costantini, 2008). ROMs are relatively more stable than ROS (that can last as short as a few seconds) and therefore they can be detected and quantified by analytical procedures (Iorio, 2004). The plasma concentration of ROMs was measured by the d-ROMs test (Diacron). The plasma (10 μL) was diluted with 1 mL of a solution containing an acetate buffer (pH 4.8). Then 20 μL of an alkyl-substituted aromatic amine (N, N-diethyl-*p*-phenylenediamine) solubilised in a chromogenic mixture were added. The solution was incubated for 75 min at 37 °C. During incubation, the acidic pH of the acetate buffer favours the release of iron from plasma proteins. Iron ions (Fe^{2+} and Fe^{3+}) catalyse the cleavage of ROOH generating alkoxyl (R–O.) and peroxyl (R–OO.) radicals. Such radicals are able to oxidize the alkyl-substituted aromatic amine (N,N-diethyl-*p*-phenylenediamine) solubilised in the chromogen, thereby producing a pink-coloured derivative

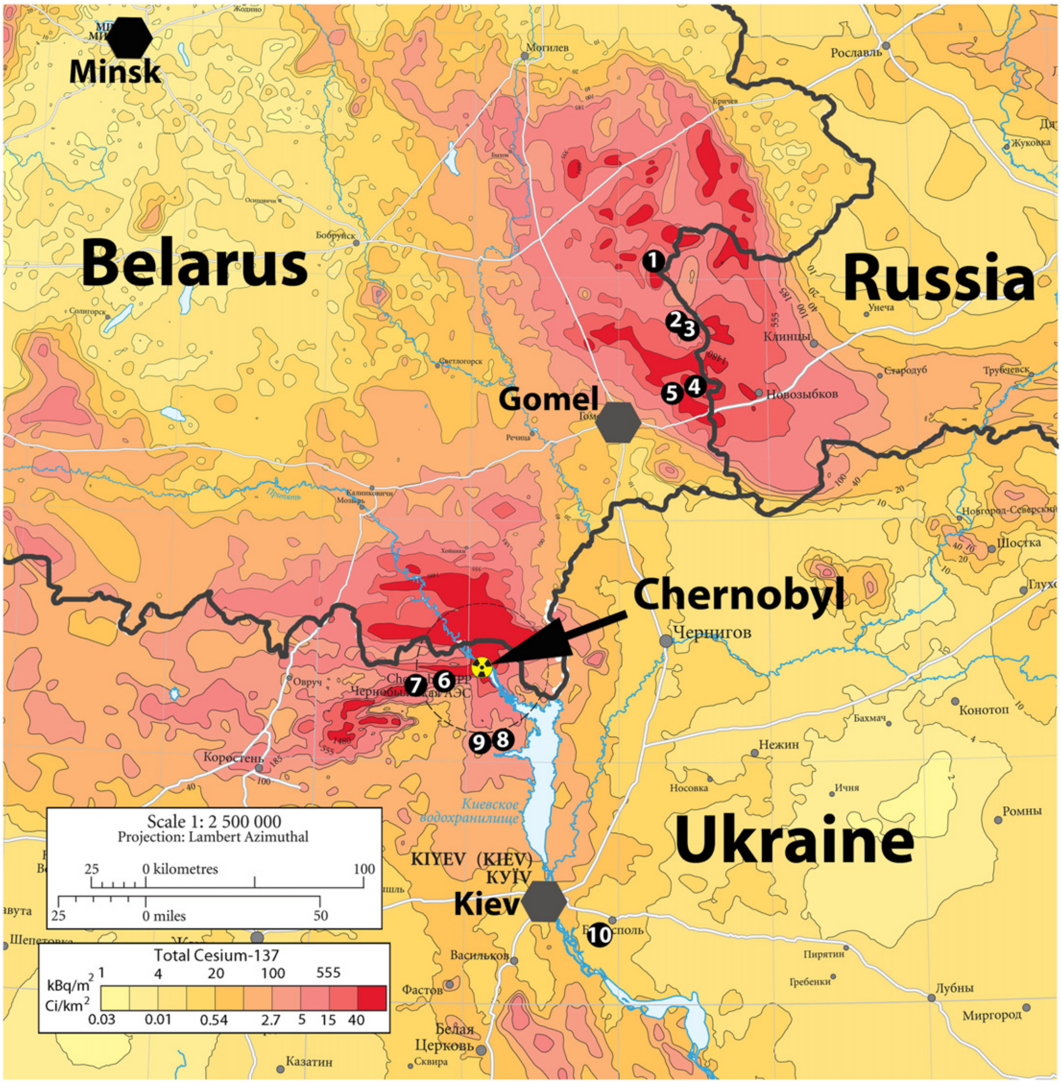

Fig. 1. Location of the study sites and levels of radioactive contamination in Ukraine and Belarus. 1: Farm 1 (53° 04′ N, 31° 18′ E), 2: Farm 2 (52° 47′ N, 31° 27′ E), 3: Farm 3 (52° 45′ N, 31° 31′ E), 4: Farm 4 (52 34′ N, 31° 32′ E), 5: Farm 5 (52° 32′ N, 31° 22′ E), 6: Rudnia (51° 17′ N, 29° 47′ E), 7: Vesniane (51° 18′ N, 29° 37′ E), 8: Dytiaku (51° 07′ N, 30° 09′ E), 9: Pisky (51° 05′ N, 30° 03′ E), and 10: Voronkov (50° 12′ N, 30° 49′ E). Modified from: European Union, 1998. Atlas of Caesium Deposition on Europe after the Chernobyl Accident. EU Publication EUR 16733, Bruxelles.

whose colour intensity is directly proportional to the concentration of ROMs. Absorbance was measured at 505 nm using a dedicated photometer (Free, Diacron). One standard sample and one blank sample (i.e. 10 µL of distilled water) were processed and used as reference for the tested samples.

The results of d-ROMs test are expressed as mM of H_2O_2 equivalents. Measurement repeatability as estimated by means of a one-way ANOVA with individual as the main effect on showed that ROMs values were repeatable within individual ($F_{8,9}=2.87$, $P=0.069$, adjusted $R^2=0.468$). Concentration values for samples assayed twice were calculated as the mean of the two measures.

The ratio of ROMs concentration to the TAC was used as an index of oxidative stress (Costantini and Dell'Omo, 2006; Costantini et al., 2006, 2007). Oxidative stress was calculated as ROMs/TAC × 1000, according to Costantini et al. (2006). Higher values indicate higher oxidative stress.

2.4. Statistical analyses

We mainly relied on linear mixed model analyses of variance where sex and radiation level (as a three-level factor) were included as fixed effects. Collection site was included in all analyses as random factor. Year and individual identity were also entered as random factors in the analyses of TAC to account for differences between years and for non-independence of nine individuals that were sampled in both years. Parameters were estimated using restricted maximum likelihood (REML) and degrees of freedom were computed using Satterthwaite's approximation. Data on ROMs concentration and oxidative stress were log-transformed before the analyses to attain normality and homoscedasticity. Statistical tests were performed using SPSS 13.

3. Results

Data on TAC were obtained for a total of 247 individuals from sites with low contamination levels, 45 individuals from sites with intermediate contamination levels, and 30 individuals exposed to high level of contamination (see Fig. 2). These data were normally distributed and homoscedastic, and were analyzed in linear mixed models in relation to contamination level (3-levels factor) and sex as fixed effects, with year and site as random effects. Individual identity was also included as a random factor to account for non-independence of TAC data obtained from 9 individuals that were sampled in both years.

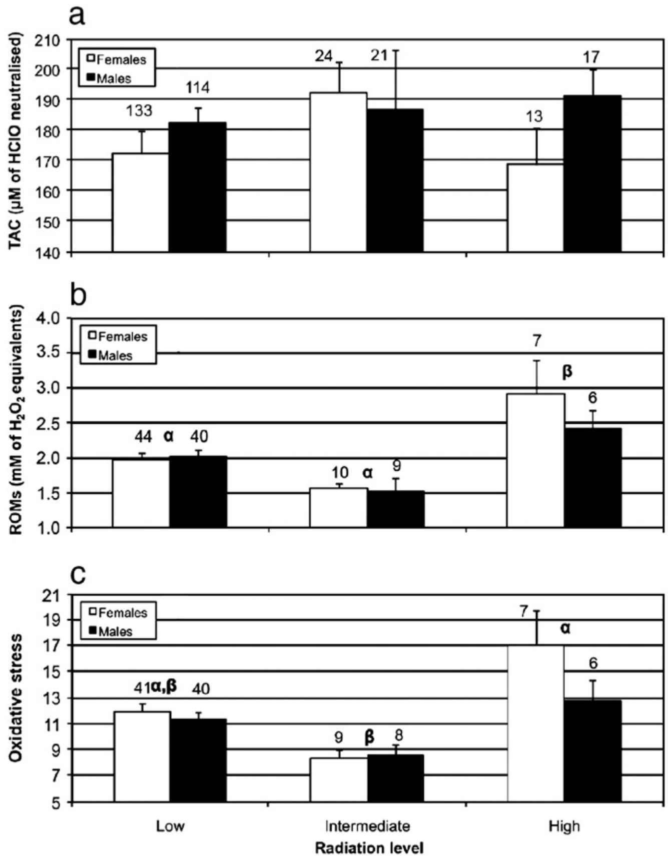

Fig. 2. Mean (+ SE) (a) antioxidant capacity (TAC), (b) reactive oxygen metabolites (ROMs) concentration and (c) ratio of the two variables (ROMs/TAC × 1000) for each level of radioactive contamination (low, intermediate and high-level) in male and female barn swallows. See Materials and methods section for further details. Sample size is indicated above bars for each group. For variables where the effect of radiation level was significant, different Greek letters indicate significant differences between levels of radioactive contamination, after Bonferroni correction for multiple testing.

We found no significant differential effect of radiation level on TAC of males or females (interaction between sex and contamination level: $F_{2,314} = 2.10$, $P = 0.124$), nor a significant main effect of radiation level ($F_{2,7.77} = 0.03$, $P = 0.971$). However, females had lower TAC levels than males, independently of radiation level ($F_{1,314} = 5.13$, $P = 0.024$; Fig. 2).

Measures of ROMs (log-transformed to ensure normality and homoscedasticity) were obtained for 84 individuals from sites with low contamination levels, 19 individuals from sites with intermediate contamination levels, and 13 individuals from highly contaminated sites (see Fig. 2). Data were analysed using linear mixed model analysis with contamination level and sex as fixed effects and site as a random factor. As all individuals were sampled only once in 2008, neither individual identity nor year were included in this model. The analysis disclosed no significant sex by contamination level effect ($F_{2,110} = 0.27$, $P = 0.765$). However, contamination level had a highly significant effect on ROMs values ($F_{2,110} = 7.43$, $P = 0.0009$; Fig. 2). Post-hoc comparisons with Bonferroni correction showed that ROMs values were significantly higher in individuals from sites with high compared to low or intermediate radiation levels ($P<0.03$ in both comparisons). No significant difference was found between sites with low compared to intermediate radiation levels ($P = 0.061$). The difference between mean (untransformed) ROMs values exposed to high radiation levels compared to those exposed to intermediate or low radiation levels pooled corresponded to 1.2 standard deviations of the values recorded among the latter. In this analysis, sex had no significant effect on ROMs concentrations ($F_{1,110} = 0.39$, $P = 0.532$).

In addition, we analysed the covariation between ROMs and TAC values. The analysis was performed by means of a linear mixed model where TAC was the response variable, sex and contamination level were entered as fixed effects, site as a random factor, and ROMs values as a covariate, together with the two-way interaction terms among ROMs, sex or radiation level. None of the interaction terms significantly predicted TAC (interaction between: sex and ROMs: $F_{1,101} = 0.62$, $P = 0.431$; radiation level and ROMs: $F_{2,101} = 0.73$, $P = 0.484$; sex and radiation level: $F_{2,101} = 0.81$, $P = 0.448$). In a simplified model excluding these non-significant interactions, ROMs values positively predicted TAC ($F_{1,106} = 5.76$, $P = 0.018$, slope = 13.65 (5.689)), while no significant effect of sex or contamination level was detected ($P>0.17$ in both cases).

Finally, we analysed estimates of oxidative stress (see Materials and methods) obtained in 2008 on a sample of 81 individuals from sites with low contamination levels, 17 individuals from sites with intermediate contamination levels, and 13 individuals from highly contaminated sites. The individuals included in this analysis were those included in TAC and ROMs analyses for which both measures could be obtained. Measures of oxidative stress (log-transformed) were not significantly predicted by the sex by radiation level interaction ($F_{2,102} = 0.63$, $P = 0.533$). Oxidative stress was maximal in heavily contaminated areas and showed a significant variation in relation to radiation level ($F_{2,5.22} = 8.92$, $P = 0.021$; Fig. 2). Post-hoc tests with Bonferroni correction disclosed a significant difference only between birds from sites with heavy compared to intermediate contamination levels ($P = 0.025$), whereas the difference between sites with low contamination and those with either intermediate or high contamination level was non-significant ($P>0.06$ in both comparisons). In this analysis, sex did not significantly predict oxidative stress ($F_{1,102} = 1.38$, $P = 0.243$).

Morphological traits (including length of bill, keel, tarsus, wing and outermost rectrices, and body mass) of the individuals included in the analyses of TAC did not vary among radiation levels (F values always associated to $P>0.10$; details not shown) at analyses of variance with year, site and individual as random factors, and sex as a fixed effect. In addition, body mass did not vary according to radiation level while controlling for the effect of body size as indexed by tarsus or keel length. Thus, mean phenotype of the individuals did not vary among radiation levels. Moreover, presence of a brood patch in females (indicating breeding status) did not differ among radiation levels (details not shown). Similar results were obtained when the analyses of these features were restricted to individuals for which we had information on ROMs or oxidative stress. Thus, there was no indication that the observed significant variation in ROMs or oxidative stress in relation to radiation level could be caused by other sources of phenotypic variation, at least as assessed by the traits we could measure under the constraints imposed by working in sites with high levels of radioactive contamination.

4. Discussion

In this study, we analysed total non-enzymatic antioxidant capacity (TAC), concentration of reactive oxygen metabolites (ROMs) and oxidative stress, as indexed by the ratio of these two variables, in barn swallows from several sites in the Chernobyl region, with variable levels of radioactive contamination. We found that radiation level positively predicted ROMs values, while no significant covariation was found with TAC. Oxidative stress was also increased in barn swallows exposed to high radioactive contamination.

Contrary to our hypothesis, plasma TAC did not vary according to radiation level. This finding is in contrast with previous studies of antioxidant defences of barn swallows and other bird species inhabiting the radioactively contaminated area around Chernobyl (Møller et al., 2005b, 2008; Møller and Mousseau, 2006, 2007a). However, previous conclusions about the antioxidant status of

Chernobyl barn swallows were largely based on carotenoids and other dietary antioxidants, rather than on integrated measures of overall antioxidant status. The role of carotenoids in antioxidant protection has increasingly been questioned recently by experimental (e.g. Cohen et al., 2007), correlational (e.g. Isaksson et al., 2007) and meta-analytical studies (Costantini and Møller, 2008; see also Møller et al., 2000; Hartley and Kennedy, 2004). Although it has been shown that supplementation with beta-carotene reduced the deleterious consequences of radiation exposure in Chernobyl children (Ben-Amotz et al., 1998), findings based on carotenoid levels and other dietary antioxidants should thus be interpreted cautiously. Moreover, a high antioxidant capacity may indicate not only a better antioxidant status, but also the up-regulation of antioxidant defences as a response to an increase in oxidant production (Monaghan et al., 2009). Several mechanisms can account for this up-regulation. Antioxidant defences can be enhanced through mobilization of stored antioxidants or increased production of endogenous antioxidants (Surai, 2002). Moreover, a rather permanent, beneficial up-regulation of antioxidant defences may also arise as a consequence of a chronic exposure to low-dose radiation (a phenomenon termed 'hormetic effect'; Rattan, 2008). Consequently, relatively high levels of antioxidants are not necessarily associated with a better antioxidant state, as actual oxidative stress depends on abundance of ROS with which these defences have to cope (Finkel and Holbrook, 2000; Halliwell and Gutteridge, 2007). In our study, ROMs values positively predicted plasma TAC, thus suggesting that barn swallows from highly contaminated areas might be actively up-regulating their antioxidant defences, rather than having them compromised by the oxidative challenge. The mechanism through which this up-regulation might take place, and particularly whether it involves mobilization of stored antioxidants or an increased intake of nutrients, remains to be elucidated.

Performance impairment resulting from oxidative damage can only be determined by relating TAC to the oxidative challenge in the same tissue (Monaghan et al., 2009). In our study, both ROMs and oxidative stress values were significantly affected by contamination level, with higher oxidative damage and oxidative stress in barn swallows associated with high radiation level.

This effect can be due to two, non-mutually exclusive phenomena. First, radiation can affect oxidative status by increasing the production of ROS (Iliakis, 1991), thus disrupting the prevention of their toxic effects by the antioxidant defences. Second, radiation can increase oxidative stress because antioxidants are allocated to repair the DNA damage arising as a consequence of exposure to radiation (Collins and Horváthová, 2001; Møller et al., 2005a). This latter explanation is supported in our model species by direct and indirect evidence concerning the effects of radioactive exposure on genetic integrity. In barn swallows from the Chernobyl region microsatellite mutation rates were shown to be higher by a factor two to ten compared to samples from a Ukrainian and an Italian control population (Ellegren et al., 1997), as well as in a range of other species (review in Møller and Mousseau, 2006). Mutation level, as indexed by sperm abnormality, negatively correlated with circulating and stored levels of antioxidants in barn swallows from Chernobyl, suggesting that variation in mutation rate might be dependent on individual differences in antioxidants availability (Møller et al., 2005a). Furthermore, we recently documented that barn swallows from the Chernobyl region have an increased level of DNA damage, as estimated by the single cell gel electrophoresis test (or comet assay), compared to birds from relatively uncontaminated control sites (Bonisoli-Alquati, submitted for publication). However, our test procedure in that study did not allow us to discriminate between different damage categories. Modifications of the protocol should thus be implemented to recognize oxidized nitrogenous bases, thus clarifying the mechanistic process linking genetic damage to depleted antioxidant resources (Collins and Horváthová, 2001; Bonisoli-Alquati, submitted for publication).

Although both ROMs and oxidative stress values were maximal in birds from highly-contaminated areas, the difference between low- and intermediate-level sites, although marginally non-significant after the Bonferroni correction, was in the opposite direction to a priori predictions. At present, we have no straightforward explanation for this pattern. It is possible however, that variation in reproductive behaviour or in age structure among populations exposed to different radiation levels accounts for this counter-intuitive patterns.

We found that female barn swallows had lower TAC than males. Barn swallows are known to provision large amounts of antioxidants to their eggs (e.g. Saino et al., 2003). This active allocation, as indicated by the larger concentrations of these compounds in eggs than in maternal plasma (Saino et al., 2003), has to be traded against the use of these same compounds for protecting the tissues of adult females. Such a trade-off has been suggested by a decline in carotenoids levels of female barn swallows during laying (Ninni et al., 2004) and in yolk concentration of some antioxidant compounds during the laying sequence (Saino et al., 2003). Maternal antioxidant transfer to the eggs to buffer ROS generation during embryonic growth may thus entail a cost for mothers in terms of a reduced capacity to deal with oxidative threats. Support for the interpretation that egg-laying might account for a depletion of antioxidant defences in females compared to males comes from an inter-specific survey that we conducted in the Chernobyl region to investigate the relationship between specific abundance, life-history traits and radiation exposure (Møller and Mousseau, 2007a). Relative egg mass was found to predict species-specific sensitivity to the deleterious effects of radiation, with species laying relatively large eggs for a given body size declining more strongly than others (Møller and Mousseau, 2007a).

Alternatively, this finding might be due to the sex-specific pattern of dispersion in our study species. The same inter-specific survey demonstrated that species with longer dispersal distance were more susceptible to the deleterious consequences of radiation (Møller and Mousseau, 2007a). In the barn swallow, females are the dispersing sex, and might therefore experience larger consequences of radioactive exposure because of higher physical activity or more frequent contact with novel antigens that would require immunostimulants such as carotenoids and antioxidant vitamins to be allocated to immune response rather than to antioxidant defences (Møller and Mousseau, 2007a).

The effects of radiation we observed are likely to be relevant to individual fitness and to the fate of wild populations of barn swallows in the Chernobyl region, owing to the plausible role of oxidative stress as a mediator of life-history trade-offs (Catoni et al., 2008; Monaghan et al., 2009). Counter-strategies to defy oxidative stress are expected to entail physiological costs for individuals (Monaghan et al., 2009). Due to their generation by virtually any biological process, ROS have been suggested as likely mediators of life history trade-offs (Monaghan et al., 2009), underlying the so-called 'cost of reproduction' (Alonso-Alvarez et al., 2004) and the deleterious consequences of rapid growth (Metcalfe and Monaghan, 2001), as well as ensuring honesty in sexual signalling (von Schantz et al., 1999). Therefore, an increase in oxidative stress levels can entail negative consequences for a wide range of behavioural and life-history traits. Oxidative stress is also known to participate in the senescence-related decline in physiological performance (Harman, 1956; Beckman and Ames, 1998; Finkel and Holbrook, 2000; Sohal et al., 2002; Monaghan et al., 2008), and to have a role in the onset of many pathologies (including atherosclerosis, autoimmune diseases and cancer; Halliwell and Gutteridge, 2007). Finally, correlative evidence from our previous studies in Chernobyl suggests that availability of antioxidants might be a determinant of reproductive success for barn swallows exposed to radioactive contamination (Møller et al., 2005b), and species-specific requirements for antioxidants may be a contributing factor in driving population declines of some bird species in the region (Møller and Mousseau, 2007a).

Overall, our findings suggest that radioactive contamination in the Chernobyl region might be damaging barn swallows through an effect on oxidative stress. Factors other than radioactive contamination are unlikely to have caused the observed patterns, as ecological conditions in the sampling sites were otherwise consistent and farming practice has deteriorated in Ukraine and Belarus irrespective of radiation level. However, we recently demonstrated that the abundance of invertebrates decreased with increasing radiation at forest sites around Chernobyl, in a study where we controlled for several ecological factors, including soil type, habitat and height of vegetation (Møller and Mousseau, 2009). Although the taxa surveyed in the study are not part of the barn swallow's diet, a reduction in available preys could contribute to explain variation in oxidative status in the barn swallow if its main preys (namely Diptera and Hemiptera; Møller, 1994) are similarly affected by radioactive contamination. Future studies utilizing the Chernobyl area as a 'natural' field laboratory will relate oxidative stress levels to behavioural and life-history traits, thus providing insight into the mechanistic and functional processes linking oxidative stress to life-history plasticity and evolution, and clarifying the exact magnitude of the worst nuclear accident in history.

Acknowledgments

The authors gratefully acknowledge the help of D. Costantini, G. Milinevski, I. Chizhevsky, O. Bondarenko, A. Cavalleri, S. Ostermiller, J. Kenney-Hunt, and the Chernobyl EcoCenter. The authors acknowledge financial support from the CNRS (France), The National Geographic Society, the Samuel Freeman Charitable Trust, the NATO CLG program, the Fulbright Program, and the School of the Environment and the College of Arts and Sciences of the University of South Carolina. The manuscript greatly benefited from the comments of three reviewers, which we thankfully acknowledge here.

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