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The effects of radiation on sperm swimming behavior depend on plasma oxidative status in the barn swallow (*Hirundo rustica*)

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ABSTRACT

Sperm are highly susceptible to reactive oxygen species (ROS) that can damage sperm DNA and structure, resulting in reduced fertilizing capacity. Exposure to radioactive contamination can also impair sperm swimming behavior and fertilizing ability, both through a reduction of sperm DNA integrity and via an increased generation of reactive oxygen species (ROS). However, the relationship between individual oxidative status and sperm swimming behavior has never been investigated in any wild population of animals exposed to radioactive contamination. We studied the motility of sperm collected from barn swallows, *Hirundo rustica*, breeding under different levels of radioactive contamination following the Chernobyl accident in 1986, in relation to individual oxidative status. We tested the hypothesis that the degree of impairment of sperm swimming behavior by radioactive contamination depended on plasma antioxidant capacity, the level of reactive oxygen metabolites (ROMs) and oxidative stress (*sensu* Costantini et al. 2006), a better oxidative status being associated with higher sperm motility. Sperm behavior parameters were subjected to principal component (PC) analysis, which extracted four PCs explaining 86% of the variance in sperm motility. PC2, representing sperm with high track velocity and ample lateral head displacement, was significantly predicted by the interaction between radiation level and either oxidative damage or oxidative stress. Contrary to our predictions, the highest values of PC2 were associated with relatively high radiation levels, particularly for high levels of either ROMs or oxidative stress. In addition, there was a tendency for values of PC3 (representing the percent of motile sperm) and PC4 (representing slow sperm with high beat cross frequency) to depend on the interaction between radiation level and total plasma antioxidant protection. Our results confirm the importance of oxidative status in determining the genetic and physiological outcome of exposure to radioactive contamination, complementing previous studies relating sperm abnormality to circulating levels of specific antioxidants. Our results also complement previous evidence that oxidative damage of sperm was negatively related to sperm motility, thus indicating a possible trade-off in quenching pro-oxidant compounds in the plasma and the seminal fluid.

1. Introduction

Reactive oxygen species (ROS), a class of pro-oxidants derived from O_2 that naturally occur as a result of oxidative metabolism, can damage macromolecules when left unquenched by the antioxidant system (Halliwell and Gutteridge, 2007). Sperm are particularly susceptible to the attack by ROS, owing to their limited antioxidant machinery, their high metabolic activity and the high polyunsaturated fatty acid (PUFA) content of their membranes (Sikka, 2001; Surai et al., 1998, 2001; Tremellen, 2008). Oxidative insult to sperm can damage the mitochondria, the cytoskeleton and the sperm axoneme, resulting in a reduction of energy availability and sperm motility (MacLeod, 1943; Jones et al., 1979; Wishart, 1984; de Lamirande and Gagnon, 1992; de Lamirande et al., 1997, 1998). As a result of this damage, less sperm reach the oocyte for fertilization (Kao et al., 2008). In addition, ROS can also prevent fertilization through a reduction in acrosin activity (Zalata et al., 2004) and/or an impairment of sperm-oocyte fusion (Aitken et al., 1989; Iwasaki and Gagnon, 1992).

Conversely, levels of antioxidants have been associated with a number of indices of sperm quality (review in Tremellen, 2008), and antioxidants supplementation is known to improve the quality of sperm in men (reviews in Agarwal et al., 2005; Tremellen, 2008), birds

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(Biswas et al., 2007) and fishes (Pike et al., 2010). An imbalance between the production of ROS and their neutralization by the antioxidant system, resulting in oxidative stress (*sensu* Costantini, 2008), is a leading cause of loss of fertility in humans (Aitken 1999; Tremellen, 2008) and domestic animals (Wishart, 1984; Surai et al., 1998, 2001).

In addition, oxidative stress can damage nuclear and mitochondrial DNA of sperm (Moody and Hassan, 1982; Imlay and Linn, 1988; Saleh et al., 2003; Li et al., 2006), increasing the frequency of aberrant sperm and adverse pregnancy outcomes (Shen and Ong, 2000; Aitken and Krausz, 2001; Morris et al., 2002). Sperm are likely to carry DNA damage owing to the down-regulation of their DNA repair systems and to the loss of the ability to undergo apoptosis during spermatogenesis (Sawyer et al., 2001, 2003; Lewis and Aitken 2005; Marchetti and Wyrobek, 2008). Consequently, ejaculated sperm may exhibit unusually high levels of damage to the nuclear and the mitochondrial genomes (Sawyer et al., 2001, 2003; Morris et al., 2002), with important trans-generational consequences (Aitken et al., 2009). Consistent with this scenario, antioxidant supplementation was also shown to improve sperm DNA integrity (Greco et al., 2005; Menezo et al., 2007), as well as the chances of fertilization (Suleiman et al., 1996; Tremellen et al., 2007).

Exposure to radiation can impair sperm swimming behavior, through a damaging effect on DNA integrity. Radiation has been shown to reduce the total number of sperm and the number of motile sperm in humans (Cheburkov and Cheburkova, 1993; Sakharov et al., 1996; Fischbein et al., 1997). Likewise, studies on rodents have demonstrated that high doses of radiation can severely damage spermatogonial stem cells (Meistrich, 1982) and increase the frequency of translocations in spermatocytes (Pomerantseva et al., 1997).

Radiation may also have an indirect, negative effect on sperm DNA via the production of an excess of ROS. Studies of humans and wild populations of animals in the Chernobyl region, where an accident at the local nuclear power plant in 1986 gave rise to the largest anthropogenic release of radionuclides in history, have shown a decreased abundance of antioxidant resources (Neyfakh et al., 1998; Ivaniota et al., 1998; Kumerova et al., 2000; Møller et al., 2005a, 2008a; Møller and Mousseau, 2007) and an increase in oxidative stress (Bonisoli-Alquati et al., 2010a), DNA damage (Frenzilli et al., 1998, 2001; Bonisoli-Alquati et al., 2010b) and mutation rate (Ellegren et al., 1997; Møller et al., 2005a; reviews in Møller and Mousseau, 2006; Yablokov et al., 2009; but see Furitsu et al., 2005).

Previous studies have shown that the frequency of sperm abnormalities in barn swallows (*Hirundo rustica*) from the Chernobyl region is negatively correlated with circulating and stored levels of specific antioxidants (i.e. vitamins C and E, carotenoids; Møller et al., 2005a), thus pointing to a role of antioxidants in protecting the sperm from the damaging effects of radiation. Importantly, we have shown also that radioactive contamination following the Chernobyl accident predicted features of sperm swimming behavior and that sperm motility was predictable in subsequent years based on radiation levels (Møller et al., 2008b).

However, the relationship of sperm swimming behavior with a more integrated index of individual oxidative status, rather than with the concentration of individual antioxidants, has never been addressed in any species exposed to different levels of radioactive contamination. This is despite the demonstration that oxidative stress is increased in barn swallows inhabiting areas where radioactive contamination is relatively high (Bonisoli-Alquati et al., 2010a). In this study we tested whether individual plasma oxidative status predicts the effects of radiation on sperm swimming behavior. To this aim, we measured overall plasma antioxidant capacity and levels of ROS metabolites, and used the ratio between these two measures to assess individual oxidative stress (Iorio, 2004; Costantini, 2008; see also Bonisoli-Alquati et al., 2010a). We correlated these data to previously collected information on sperm swimming behavior and radiation level, expecting the deleterious effects of radiation on sperm motility to be buffered in individuals with relatively high plasma levels of antioxidants (or, conversely, to be exacerbated by relatively high levels of oxidative stress). We analyzed sperm viability and motility based on computer assisted sperm analysis (CASA), as several studies in different animal taxa have shown that sperm motility and swimming behavior are major predictors of fertilization success (Froman et al., 1999; Garcia Gonzales and Simmons, 2005; Rudolfsen et al., 2008; Gasparini et al., 2010).

The barn swallow is a small, insectivorous passerine bird that commonly breeds in farms across Europe after migrating from the winter quarters in sub-Saharan Africa (Møller, 1994). In our study populations in Ukraine, males inhabiting radioactively contaminated areas have paler plumage coloration than males from control areas (Camplani et al., 1999). The frequency of albinistic feathers was also increased in individuals from contaminated areas (Møller and Mousseau, 2003), and the albinistic phenotype was found to be at least partly of germline origin and associated with reduced survival prospects (Ellegren et al., 1997). Overall, mutation rate (Ellegren et al., 1997), DNA damage (Bonisoli-Alquati et al., 2010b) and oxidative stress (Bonisoli-Alquati et al., 2010a) are all increased in barn swallows from the heavily contaminated sites around Chernobyl.

2. Material and methods

2.1. Study species and field procedures

We captured barn swallows (n = 88) in seven different farms in the Chernobyl region, Ukraine, during late May and early June 2005–2006 (Fig. 1), using mist nets placed across windows and doors. Birds were placed in cloth bags before being measured and weighed. We then collected a blood sample in heparinized capillary tubes. Subsequently, we collected one or two sperm samples from all males, using a simple massage technique. Sperm were collected in micro-capillaries and immediately transferred to the microscope chamber (see below). We collected our field measurements of background radiation at the ground level using a hand-held Inspector dosimeter (Model: Inspector, SE International, Inc., Summertown, TN, USA). Radiation levels were mainly due to gamma radiation and were on average 0.0379 mR/h (standard error (SE) = 0.0069; range 0.02–0.065 mR/h) at seven breeding sites (Fig. 1). Previous analyses have shown that our field measurements have strong positive relationships with officially published data (i.e. Shestopalov, 1996; see Møller et al., 2008b). Thus, our estimates of radioactive contamination at each breeding colony were comparable with other estimates. Admittedly, we do not have information on internal dose of the birds involved in this study. However, background radiation level accurately predicts average internal dose of individual birds from Chernobyl (Gaschak et al., 2008).

2.2. Sperm behavior analyses

The recording and analysis of sperm behavior have been described in details elsewhere (Møller et al., 2008b; see also Supplementary Material and Methods). Briefly, the sperm sample was transferred to a microscope chamber, without the experimenter having knowledge of the individual male. Here the sperm was diluted in a one-step procedure by adding 9 μL of Dulbecco's Modified Eagle Medium (D-MEM; Invitrogen, Carlsbad, CA, USA) to the sample. We then conducted video recording by means of a Sony CCD black-and-white video camera (XC-ST50CE PAL, Sony, Tokyo, Japan), mounted on an external negative phase-contrast microscope (Olympus CH30, Olympus, Tokyo, Japan) with a 10× objective. The video recordings were then downloaded for analysis to a PC. For each ejaculate, we recorded the mean values of (1) VAP (smoothed path velocity; μm/s), (2) VSL (straight-line velocity; μm/s), (3) VCL (curvilinear velocity; μm/s), (4) ALH (amplitude of lateral head displacement; μm), (5) BCF (beat cross frequency; Hz), (6) LIN (linearity, VSL/VCL) and (7) STR (straightness,

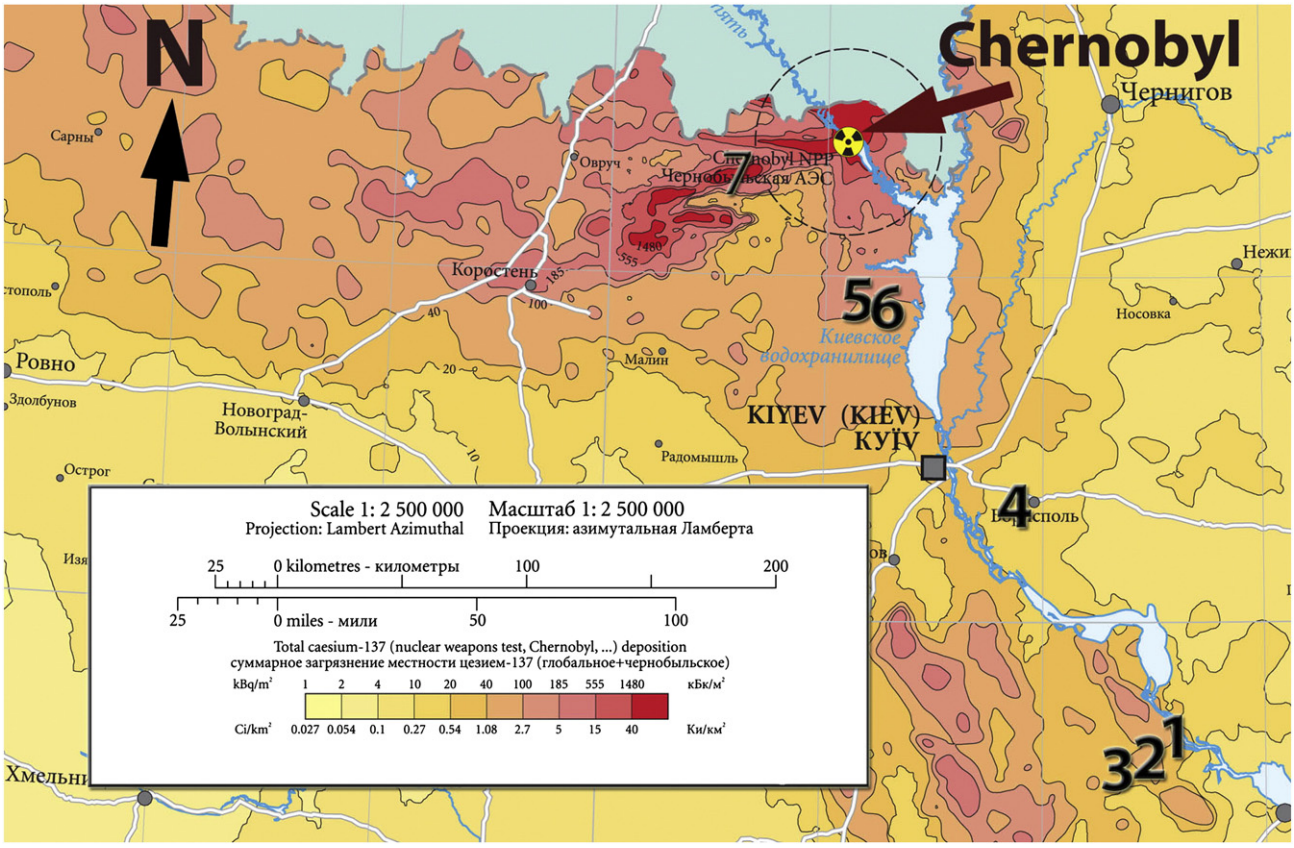

Fig. 1. Location of the study sites (1. Kanev, 2. Konongia, 3. Zalyska, 4. Ghovtnere, 5. Pisky, 6. Dytiatku, 7. Martinovichy). Adapted from De Cort et al. (1998).

VSL/VAP). Cells having a VAP<10 μm/s and a VSL<5 μm/s were excluded from the motility analysis, and counted as static. We used these measurements to estimate (8) the percentage of static sperm (the percentage of sperm that remained static), (9) the percentage of sperm with slow velocity (i.e. <10 μm/s), (10) the percentage of sperm with medium velocity (10–50 μm/s), (11) the percentage of sperm with high velocity (>50 μm/s) and (12) the percentage of progressive sperm (the percentage of all sperm that moved with STR>80 and VAP>50 μm/s).

2.3. Plasma antioxidant capacity (TAC), reactive oxygen metabolites (ROMs) and oxidative stress

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) according to a previously described protocol (Bonisoli-Alquati et al., 2010a). Briefly, this test uses a colorimetric determination to quantify the ability of the plasma antioxidant barrier to cope with the oxidant action of hypochlorous acid (HClO), an endogenously produced pro-oxidant. The test reflects levels of circulating antioxidants including vitamin C and E, carotenoids and uric acid, but does not account for levels of enzymatic antioxidants (see Costantini, in press).

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 pro-oxidants, 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), according to a previously described protocol (Bonisoli-Alquati et al., 2010a; see Supplementary Material and Methods). The results of d-ROMs test are expressed as mM H_2O_2.

The ratio of ROMs/TAC×1000 was used as an index of overall oxidative status (or 'oxidative stress'; *sensu* Costantini et al., 2006). Higher values indicate a higher unbalance between pro-oxidants and antioxidant resources. Oxidative stress values were log-transformed before analyses to reach normality.

2.4. Statistical analyses

We conducted principal component analysis (PCA) on the 12 sperm parameters by applying the varimax rotation. We then used the scores for the principal components (PCs) as response variables in linear mixed models. For each measure of individual oxidative status and each principal component, we used Akaike Information Criterion (AIC) scores to compare and rank the four different models including (i) only radiation level (as a covariate); (ii) only the measure of oxidative status (TAC, ROMs or oxidative stress; also as a covariate); (iii) both radiation level and the measure of oxidative status; or (iv) both variables together with their interaction term. Two models are assumed to be equally informative when the difference in their AIC scores was lower than 2.00 (Burnham and Anderson, 2002). Based on the hypothesis that background radiation affects sperm swimming behavior depending on individual oxidative status, we expect best-fit models to include both radiation levels and the measure of oxidative status. Statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

We included site and year as random factors in all analyses.

Table 1
Principal components of the 12 sperm variables. The eigenvalues associated with each principal component, together with the amount of variance explained are also indicated. Only the principal components with an eigenvalue >1.00 were extracted.

	PC1	PC2	PC3	PC4
Eigenvalue	5.45	2.05	1.75	1.13
Percent of variance	45.38	17.07	14.55	9.43
Cumulative percent of variance	45.38	62.45	77.00	86.43
Pct. static sperm	**−0.54**	−0.28	**−0.78**	0.02
Pct. slow sperm	0.14	−0.26	0.35	**0.69**
Pct. medium sperm	−0.14	0.01	**0.92**	0.04
Pct. rapid sperm	**0.74**	0.41	0.40	−0.21
Pct. progressive sperm	**0.84**	0.21	0.35	−0.14
VAP	**0.86**	0.42	−0.08	−0.14
VSL	**0.95**	0.20	−0.12	−0.12
VCL	0.21	**0.92**	−0.10	−0.02
ALH	−0.05	**0.79**	0.25	−0.02
BCF	−0.25	0.14	−0.22	**0.83**
STR	**0.85**	−0.15	0.03	0.15
LIN	**0.92**	−0.34	0.02	−0.01

Loadings with absolute value >0.50 are highlighted in bold. PC1 tended to be positively correlated with all variables indicating higher sperm velocity and was negatively correlated with the percent of static sperm; PC2 represented sperm with high track velocity (VCL), but also with ample lateral displacement of the head (ALH); PC3 reflected highly non-static sperm, moving with medium velocity; PC4 represents slow sperm with a high beat cross frequency (BCF). VAP (smoothed path velocity; μm/s), VSL (straight line velocity; μm/s), VCL (curvilinear velocity; μm/s), ALH (amplitude of lateral head displacement; μm), BCF (beat cross frequency; Hz), LIN (linearity, VSL/VCL) and STR (straightness, VSL/VAP) (see also Material and Methods).

3. Results

The PCA on the 12 sperm parameters extracted four PCs that accounted for >86% of the variance in our data (Table 1). PC1 was positively correlated with all variables indicating higher sperm velocity and negatively with the percent of static sperm; PC2 represented sperm with high track velocity (VCL), but also with ample lateral head displacement (ALH); PC3 reflected highly non-static sperm, moving with medium velocity, and PC4 represented slow sperm with high beat cross frequency (BCF) (Table 1).

For all PCs, the best-fit models always included both radiation level and the measure of oxidative status (Table 2).

Table 2
Akaike Information Criterion (AIC) values for the models of the four principal components of sperm swimming behavior. For each of the variables of oxidative status (total antioxidant capacity or TAC; reactive oxygen metabolites level or ROMs, and their ratio or Oxidative Stress), we compared the models including (i) only radiation (as a covariate), (ii) only the oxidative status variable (as a covariate), (iii) both variables or (iv) both variables and their interaction. The AIC values of the best-fit models are highlighted in bold. Two models are assumed to be equally informative when the difference in their AIC scores was lower than 2.00 (Burnham and Anderson, 2002; see Statistical Analyses for more details).

	PC1	PC2	PC3	PC4
Total antioxidant capacity (*TAC*) (*n* = 75)				
Rad	242.0	233.6	210.8	238.2
TAC	217.9	210.1	199.6	220.6
TAC + Rad	210.0	**200.1**	**187.2**	**213.7**
TAC + Rad + TAC × Rad	**212.1**	**201.5**	**187.0**	**212.6**
Reactive Oxygen Metabolites (*ROMs*) (*n* = 77)				
Rad	242.0	233.6	210.8	238.2
ROMs	213.0	201.9	195.5	196.5
ROMs + Rad	205.6	193.3	181.8	189.2
ROMs + Rad + ROMs × Rad	**196.8**	**176.3**	**176.2**	**182.7**
Oxidative stress (*Stress*) (*n* = 65)				
Rad	242.0	233.6	210.8	238.2
Stress	175.5	155.6	163.5	161.1
Stress + Rad	166.2	147.2	152.2	**143.1**
Stress + Rad + Stress × Rad	**156.9**	**132.0**	**143.1**	**143.6**

Table 3
Best-fit models for the principal components in relation to background radiation level (Rad), total antioxidant capacity (TAC) and their interaction (see also Table 2).

	d.f.	F	P	Estimate	Coefficient (SE)
PC1					
Rad	1,7.3	1.34	0.283		−12.23 (10.55)
TAC	1,70.4	1.10	0.299		-2.1×10^{-3} (2.0×10^{-3})
PC2					
Rad	1,4.2	7.01	0.055		22.37 (8.45)
TAC	1,70.8	0.33	0.568		-1.1×10^{-3} (1.1×10^{-3})
PC3					
Rad	1,63.2	0.31	0.582		13.10 (23.68)
TAC	1,68.4	3.52	0.065		8.6×10^{-3} (4.6×10^{-3})
TAC × Rad	1,68.7	2.80	0.099		−0.20 (0.11)
PC4					
Rad	1,62.0	2.96	0.090		51.74 (30.06)
TAC	1,68.6	1.79	0.185		7.6×10^{-3} (5.7×10^{-3})
TAC × Rad	1,68.8	3.21	0.077		−0.25 (0.14)

The best-fit models for PC1 scores did not include any significant effect of radiation level, TAC, ROMs or oxidative stress, neither alone nor in interaction with each other (Tables 3–5). In the best-fit models for PC2 scores, the scores were significantly predicted by the interaction between radiation level and ROMs (Table 4; Fig. 2). PC2 values were highest for highest levels of radiation and highest levels of ROMs. Thus, sperm had highest track velocity and highest lateral head displacement for increasing levels of radiation, in particular when plasma levels of ROMs were also high (Fig. 2). A qualitatively similar effect was disclosed by the analysis of PC2 scores as a function of radiation level and oxidative stress (Table 5), with highest PC2 scores for high levels of oxidative stress and relatively high radiation levels (Fig. 3). Thus, sperm had highest track velocity and amplest lateral head displacement for relatively high levels of radiation and high levels of plasma ROMs, even after accounting for TAC of plasma (Fig. 3).

PC3 scores were only marginally significantly predicted by the interaction between TAC levels and background radiation level (Table 3; Fig. 4). This effect implied that the percent of motile sperm was highest for the lowest level of background radiation and for highest plasma levels of antioxidants (Fig. 4). PC4 scores were also marginally non-significantly predicted by the interaction between background radiation levels and TAC (Table 3; Fig. 5). The coefficients

Table 4
Best-fit models for the four principal components in relation to background radiation level (Rad), reactive oxygen metabolites (ROMs) levels and their interaction (see Table 2).

	d.f.	F	P	Slope (SE)
PC1				
Rad	1,37.9	0.78	0.383	19.17 (21.71)
ROMs	1,64.0	2.57	0.114	0.73 (0.45)
ROMs × Rad	1,69.1	2.18	0.144	−16.88 (11.43)
PC2				
Rad	1,32.3	3.96	0.055	−41.03 (20.61)
ROMs	1,69.8	7.90	0.006	−1.13 (0.42)
ROMs × Rad	1,71.3	11.29	0.001	33.80 (10.06)
PC3				
Rad	1,41.5	0.02	0.899	−2.17 (16.93)
ROMs	1,64.0	1.01	0.3188	0.39 (0.38)
ROMs × Rad	1,67.5	1.21	0.275	−10.37 (9.43)
PC4				
Rad	1,31.4	0.16	0.693	5.47 (22.90)
ROMs	1,71.8	0.06	0.813	0.17 (0.42)
ROMs × Rad	1,72.0	0.00	0.984	0.21 (10.54)

Table 5
Best-fit models for the four principal components in relation to background radiation level (Rad), oxidative stress levels (or Stress, as indexed by the ratio of total antioxidant capacity to the reactive oxygen metabolites levels; see Material and Methods) and their interaction (see Table 2).

	d.f.	F	P	Slope (SE)
PC1				
Rad	1,6.88	3.45	0.106	−16.95 (9.13)
Stress	1,59.4	0.00	0.977	−0.05 (1.71)
Stress × Rad	1,59.7	0.05	0.829	8.83 (40.83)
PC2				
Rad	1,6.67	2.89	0.135	19.34 (11.37)
Stress	1,58.4	5.18	0.027	−3.11 (1.36)
Stress × Rad	1,58.8	6.62	0.013	84.05 (32.67)
PC3				
Rad	1,9.44	6.58	0.029	−21.87 (8.53)
Stress	1,58.9	0.23	0.633	−0.71 (1.48)
Stress × Rad	1,58.9	0.18	0.670	14.99 (35.03)
PC4				
Rad	1,7.96	0.18	0.670	6.99 (13.57)
Stress	1,57.9	6.58	0.029	0.81 (0.49)

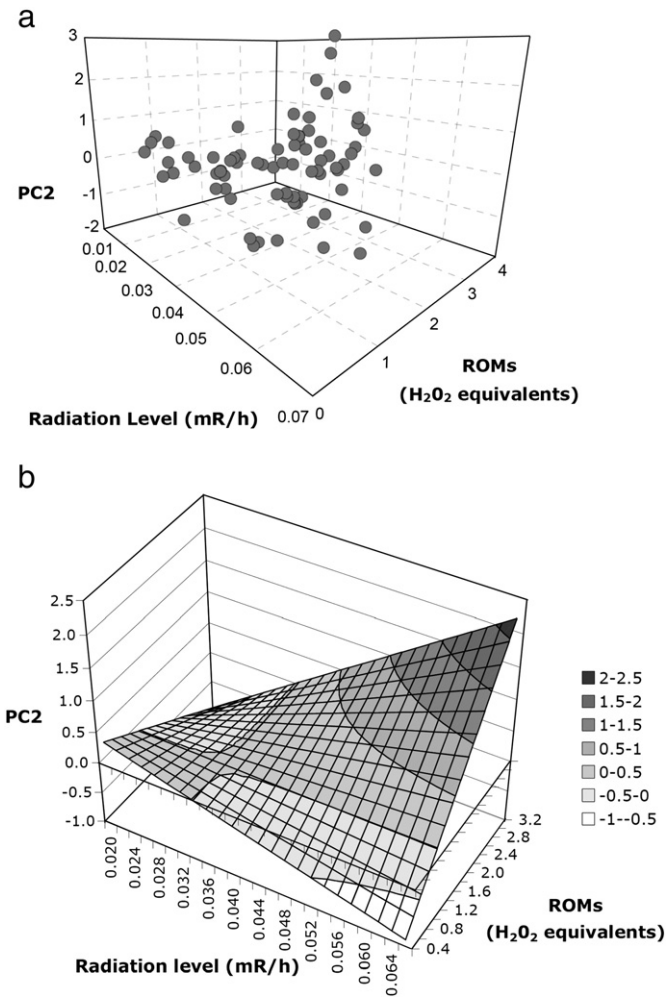

Fig. 2. Relationship between the PC2 scores, background radiation level and plasma levels of reactive oxygen metabolites (ROMs). In panel (a), individual values are shown (n = 77). In panel (b), a surface was interpolated based on the coefficients for the effects of background radiation level, ROMs levels and the interaction between the two in the best-fit model. The coefficients of the model are reported in Table 4. The loadings of PC2 scores on the original parameters of sperm motility and swimming behavior are reported in Table 1.

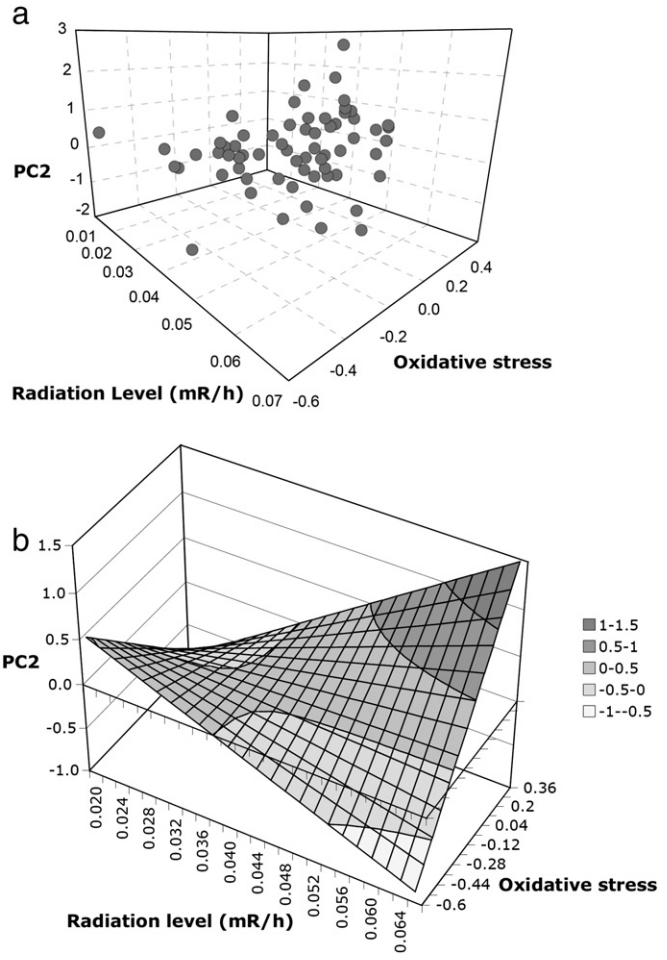

Fig. 3. Relationship between the PC2 scores, background radiation level and oxidative stress in the plasma. In panel (a), individual values of those individuals for which we could determine both TAC and ROMs levels are shown (n = 65). In panel (b), a surface was interpolated based on the coefficients for the effects of background radiation level, oxidative stress levels and the interaction between the two in the best-fit model. The coefficients of the model are reported in Table 5. The loadings of PC2 scores on the original parameters of sperm motility and swimming behavior are reported in Table 1.

for this effect implied that slow sperm with high beat cross frequency were most frequent for relatively high levels of radioactive contamination, particularly when plasma TAC was low (Fig. 5).

The effect of year was not significant in any analysis (P always >0.05). Similarly, there was no significant effect of site in any analysis (P always >0.05). The inclusion of body mass or body condition (as indexed by the residuals of the regression of body mass on tarsus length) did not qualitatively change the results of any of the best-fit models (details not shown).

4. Discussion

In this study we assessed the covariation between sperm motility and plasma total antioxidant capacity (TAC), oxidative damage (as indexed by ROMs level) and oxidative stress (as indexed by the ratio of the two measures; Costantini et al., 2006) in barn swallows under different levels of radioactive contamination. Consistently with our prediction that the deleterious effects of radiation on sperm motility would depend on individual oxidative status, the effect of chronic exposure to low-level radiation on different features of sperm swimming behavior depended on the indices of antioxidant status.

In particular, sperm swimming with highest track velocity and amplest lateral head displacement (i.e. highest values on the PC2) were associated with highest ROMs and relatively high background

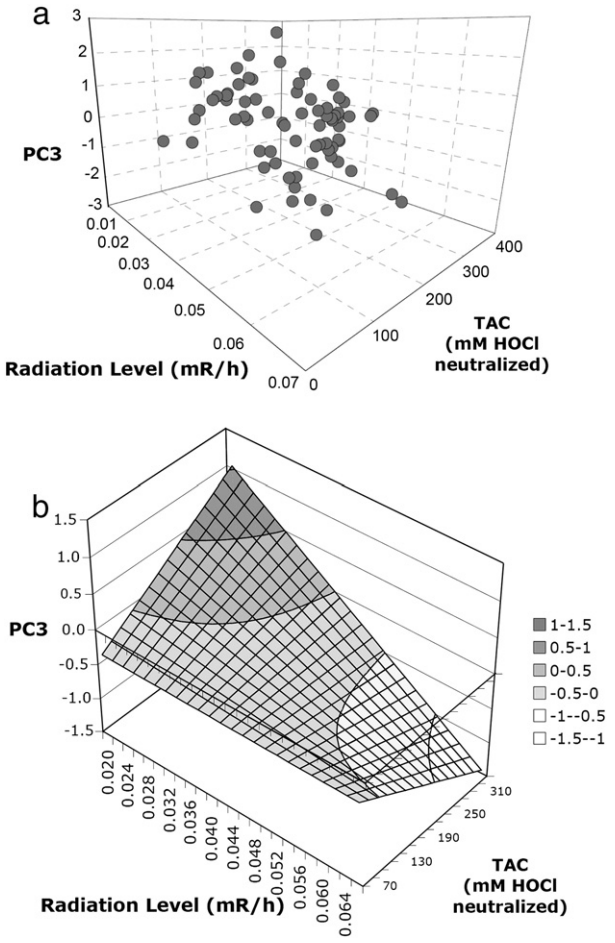

Fig. 4. Relationship between the PC3 scores, background radiation level and plasma total antioxidant capacity (TAC). In panel (a), individual values are shown (n = 75). In panel (b), a surface was interpolated based on the coefficients for the effects of background radiation level, TAC and the interaction between the two in the best-fit model. The coefficients of the model are reported in Table 3. The loadings of PC3 scores on the original parameters of sperm motility and swimming behavior are reported in Table 1.

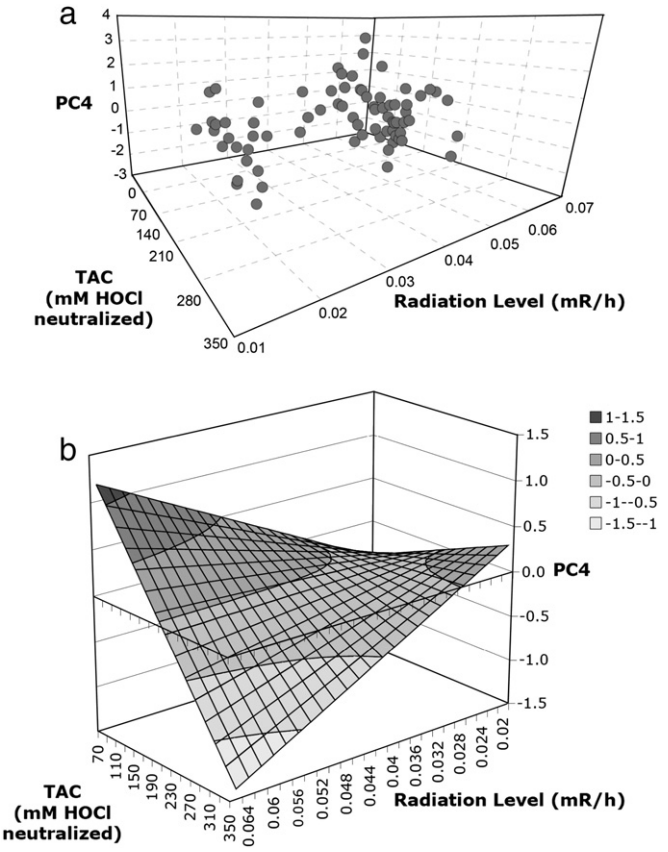

Fig. 5. Relationship between the PC4 scores, background radiation level and plasma total antioxidant capacity (TAC). In panel (a), individual values are shown (n = 75). In panel (b), a surface was interpolated based on the coefficients for the effects of background radiation level, TAC and the interaction between the two in the best-fit model. The coefficients of the model are reported in Table 3. The loadings of PC4 scores on the original parameters of sperm motility and swimming behavior are reported in Table 1.

radiation levels. Interestingly, this result held true even when analyzing oxidative stress (i.e. accounting for variation in antioxidant capacity of plasma; Fig. 3). Both track velocity and lateral head displacement are known to correlate with sperm penetration capacity of the cervical barrier (Aitken, 2006). In turn, the capacity of penetrating the cervical mucus has been linked to the probability of in vitro fertilization in humans and cows (Sharara et al., 1995), as well as to the probability of pregnancy in vivo, particularly when controlling for the quality of the cervical mucus (Eggert-Kruse et al., 1989). Thus, contrary to our expectations, the best performance of sperm in terms of track velocity and lateral head displacement was associated with relatively high levels of radioactive contamination and highest oxidative damage and stress. A trade-off in allocation of antioxidants between blood plasma and seminal fluid could account for this effect. A number of antioxidants are known to be present in the seminal fluid of birds, including vitamins E and C, glutathione, ubiquinols, as well as antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase and catalase (Sikka, 2001; Surai et al., 2001; Rowe and McGraw, 2008). If a trade-off in scavenging ROS from different tissues is operating, relatively high levels of ROMs and a correspondingly high oxidative stress in the plasma might then be associated with low levels of ROMs and stress in the seminal fluid (and with correspondingly viable and motile sperm). This possibility, however, remains to be tested. Interestingly, a recent study of great tits (*Parus major*) has shown a negative relationship between sperm concentration of malondialdehyde (MDA), a marker of lipid peroxidation (Monaghan et al., 2009), and sperm swimming ability (Helfenstein et al., 2010). Helfenstein et al. (2010) found that an experimental increase of parental workload induced both an increase in MDA concentration and a decrease in sperm motility and swimming ability. It could be argued, however, that Helfenstein et al. (2010) induced acute, transient oxidative stress, while the individuals in our study were exposed to a mild, chronic stress. We suggest that future studies simultaneously assess oxidative status of both plasma and seminal fluid, in order to better describe the integrated response of organisms to radioactive contamination. A validation of our results through the capture and analysis of birds over a wider range of radioactive contamination levels will also be important. In fact, the range of radioactive contamination levels reported here is narrower than the range across which we have previously sampled birds (e.g. Bonisoli-Alquati et al., 2010b). However, it should be noted that all the best-fit models included both radiation and the measure of oxidative status (and most of the best-fit models also included the interaction between the two covariates; see Table 2), thus indicating that radiation level is important in explaining variation in sperm motility across individuals in our sample.

We found that the effects of radiation on sperm motility and behavior marginally depended on plasma antioxidant capacity. The percent of motile sperm was highest for the lowest level of background radiation and for highest plasma levels of antioxidants, thus suggesting that antioxidants might have a role in protecting the sperm from pro-oxidants arising as a function of radiation exposure. Conversely, slow sperm with high beat cross frequency were most frequent for relatively

low levels of plasma antioxidant capacity and relatively high background radiation, thus indicating an impairment of sperm motility (and a likely decrease in fertilizing capacity; Surai et al., 1998, 2001; Tremellen, 2008), whenever antioxidant resources are insufficient to scavenge the excess of ROS generated by ionizing radiation. Thus, a relatively worse oxidative status in terms of circulating ROMs levels was positively related to sperm velocity, particularly for higher radiation levels, while better plasmatic antioxidant defenses were associated with higher sperm motility, in particular when radiation was higher. This suggests that the antioxidant defense of some sperm traits might be traded against other sperm functions. In this case, particularly while exposed to radiation, males might have had to produce only a few, faster sperm, or more, motile but slow sperm. Similar to the hypothesis that plasma oxidative status is traded against oxidative status in the seminal fluid, this explanation is speculative and awaits further tests. Admittedly, however, differences in sperm number could have contributed to the observed pattern given that spermatogenesis entails intense metabolic cost (Parapanov et al., 2008), and could thus increase ROS production in the ejaculate. Consistent with this hypothesis, ROS have been proposed as a proximate explanation for the documented negative association between sperm traits (e.g. Levitan, 2000; Moore et al., 2004; Schulte-Hostedde and Millar, 2004), particularly between sperm numbers and indices of sperm quality (Moore et al., 2004; Schulte-Hostedde and Millar, 2004; see Dowling and Simmons, 2009 for a discussion).

Overall, these results complement previous evidence showing an increase in aberrant sperm and an impaired sperm swimming behavior with increasing background radiation levels (Møller et al., 2005a, 2008b). They also extend the finding of a negative relationship between somatic concentration of specific antioxidants and sperm aberrations under radioactive contamination to a more integrated measure of overall oxidative status (*sensu* Monaghan et al., 2009). Ultimately, rather than the level of any single antioxidant, it is the imbalance between ROS generation and available antioxidant resources that will determine the fitness outcome of oxidative insults arising from radioactive contamination (Costantini, 2008; Monaghan et al., 2009).

The finding of an impairment of sperm motility (and therefore fertilizing capacity) that simultaneously depends on individual oxidative status and background radiation level might reconcile several features of the reproductive behavior and population dynamics of barn swallows inhabiting the radioactively contaminated area around Chernobyl. Indeed, present results are consistent with a previously documented reduction in hatching success of barn swallow eggs in radioactively contaminated sites (Møller et al., 2005b), and might contribute to explaining the population decline of barn swallows breeding in the region (Ellegren et al., 1997; Møller et al., 2006).

Heritable gene mutations and chromosomal translocations (Searle et al., 1974; Russell et al., 1998), genomic instability (Dubrova et al., 1993) and cancer (Nomura et al., 2004) have all been reported as trans-generational consequences of parental exposure to ionizing radiation (Marchetti and Wyrobek, 2005). Interestingly, an increased mutation rates has also been described in the offspring of individuals heavily exposed to the Chernobyl radioactive fallout (Dubrova et al., 1996; Weinberg et al., 2001; but see Furitsu et al., 2005). Our results suggest that trans-generational effects of exposure to ionizing radiation due to sperm aberrations may vary in frequency depending on the oxidative status of the male and possibly depending on the pattern of antioxidant allocation to different body functions and tissues.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.cbpa.2011.01.018.

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