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Oecologia

ISSN 0029-8549

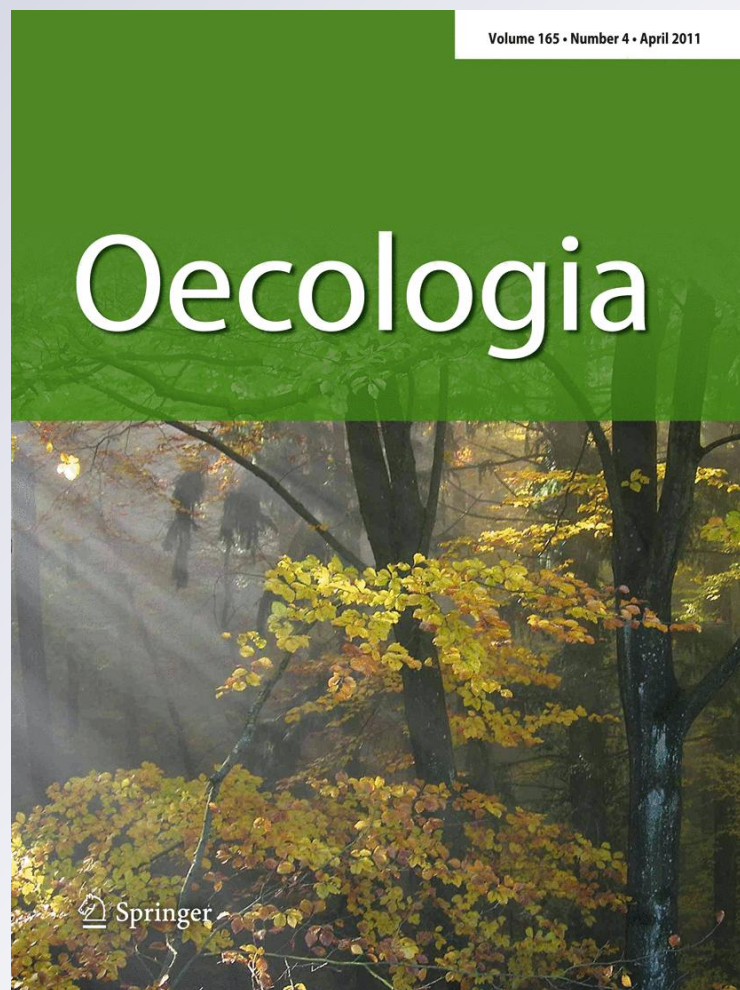
Volume 165

Number 4

Oecologia (2011) 165:827-835

DOI 10.1007/

s00442-010-1860-5



Bird population declines due to radiation exposure at Chernobyl are stronger in species with pheomelanin-based coloration

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Received: 1 July 2010 / Accepted: 15 November 2010 / Published online: 7 December 2010
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Abstract Eumelanin and pheomelanin are the most common pigments providing color to the integument of vertebrates. While pheomelanogenesis requires high levels of a key intracellular antioxidant (glutathione, GSH), eumelanogenesis is inhibited by GSH. This implies that species that possess the molecular basis to produce large amounts of pheomelanin might be more limited in coping with environmental conditions that generate oxidative stress than species that produce eumelanin. Exposure to ionizing radiation produces free radicals and depletes antioxidant resources. GSH is particularly susceptible to radiation, so that species with large proportions of pheomelanin integument may be limited by the availability of GSH to combat oxidative stress and may thus suffer more from radiation effects. We tested this hypothesis in 97 species of birds censused in areas with varying levels of radioactive contamination around Chernobyl. After controlling for the effects of carotenoid-based color, body mass and similarity among taxa due to common

phylogenetic descent, the proportion of pheomelanin plumage was strongly negatively related to the slope estimates of the relationship between abundance and radiation levels, while no effect of eumelanin color proportion was found. This represents the first report of an effect of the expression of melanin-based coloration on the capacity to resist the effects of ionizing radiation. Population declines were also stronger in species that exhibit carotenoid-based coloration and have large body mass. The magnitude of population declines had a relatively high phylogenetic signal, indicating that certain groups of birds, especially non-corvid passeriforms, are particularly susceptible to suffer from the effects of radioactive contamination due to phylogenetic inertia.

Keywords Eumelanin · Glutathione · Pheomelanin · Plumage coloration · Radioactive contamination

Introduction

Melanin is the most common pigment of the vertebrate integument. It is synthesized by animals from the amino acid tyrosine in a process (i.e. melanogenesis) that is intrinsically related to oxidative stress (i.e. the imbalance between the production of reactive oxygen species and the state of the antioxidant and repair machinery; Galván and Alonso-Alvarez 2008, 2009; Galván and Solano 2009; Hřrak et al. 2010). This is because glutathione (GSH), a tripeptide thiol found in virtually all animal cells that functions as the main physiological reservoir of cysteine (Benedetto et al. 1981) and as the most important intracellular antioxidant (Anderson 1998; Wu et al. 2004), exerts a direct influence on the process. This effect occurs by directly inhibiting the action of tyrosinase (i.e. the

Communicated by Hannu Põysä.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-010-1860-5) contains supplementary material, which is available to authorized users.

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enzyme catalysing the first step of the process), by combating free radicals that stimulate the action of tyrosinase, and by increasing the ratio cysteine:dopaquinone (Galván and Alonso-Alvarez 2009).

Vertebrates synthesise two main forms of melanin: eumelanin and pheomelanin, the latter producing lighter colors than the former. It is GSH levels that determine the melanin form that is synthesised, as melanogenesis can either lead to the production of eumelanin when the activity of tyrosinase is high and the ratio cysteine:dopaquinone low, or to the production of pheomelanin or even an absence of melanin synthesis under opposing conditions (Ozeki et al. 1997; Galván and Alonso-Alvarez 2009). Therefore, eumelanogenesis takes place when the levels of GSH are low and pheomelanogenesis when the levels of GSH are high, meaning that pheomelanogenesis proceeds with higher levels of endogenous oxidative stress as compared to eumelanogenesis (Galván and Solano 2009). The evolutionary implication of this is that species in which natural selection has favoured the development of pheomelanin traits may have a decreased capacity to combat oxidative stress as compared to species with eumelanin traits, as the maintenance of high GSH levels as required by pheomelanogenesis might be metabolically costly under adverse environmental conditions that generate oxidative stress and thus use GSH resources (Galván and Alonso-Alvarez 2009; Galván and Solano 2009).

Ionising radiation produces free radicals and thus promotes the generation of oxidative stress and depletes antioxidant levels in animals (Riley 1994; Ivaniota et al. 1998; Neyfakh et al. 1998). GSH is one of the most susceptible antioxidants to radiation (Bump and Brown 1990; Navarro et al. 1997; Vartanian et al. 2004). Therefore, species that have the molecular basis to produce large amounts of pheomelanin may be more limited to use GSH to combat oxidative stress, and may thus be more susceptible to ionising radiation than species in which melanogenesis has been selectively directed towards the production of eumelanin. We test this hypothesis by examining the response of bird populations to radioactive contamination after the Chernobyl accident and relate responses to the proportion of eu- and pheomelanin-based plumage coloration exhibited by the species.

Møller and Mousseau (2007a) previously showed in a dataset of 57 bird species that the presence of melanin-based plumage coloration had no effect on population density in relation to radiation. However, they did not separately analyse colors generated by eumelanin from those generated by pheomelanin, and only prevalence (i.e. presence vs absence) of melanin-based coloration was measured. This may have obscured any relationship concerning pheomelanin species in their response to radiation levels. Here, we use a larger dataset of 97 species of birds

collected during 4 years that were censused during the main breeding season at points with varying levels of radiation around Chernobyl, and quantitatively analyse the expression of eu- and pheomelanin plumage coloration. We predicted that the slope of the regression between abundance and background radiation should be negatively correlated with the proportion of plumage colored by pheomelanin, while no correlation should be observed for the proportion of eumelanin-based plumage color. We also predicted that the slope of the regression between abundance and radiation should be more negative in species with plumage color patches generated by carotenoids, as these pigments act as antioxidants and are also depleted by radiation (Møller and Mousseau 2007a).

Møller and Mousseau (2007a) found that declines in bird abundance with background radiation levels were stronger not only in species that exhibit carotenoid-based coloration but also in species with long migration and dispersal distances, and large egg and body mass. From these variables, body mass may also be related to the expression of eu- and pheomelanin coloration, as the production of diverse plumage color patches has been found to be constrained in large species (Galván et al. 2010). Thus, body size may be related to both slope estimates of the regression between abundance and background radiation, and to the expression of melanin-based coloration, thereby constituting a potentially confounding variable. Therefore, we estimated the relationship between slope estimates and proportion of pheomelanin coloration while simultaneously controlling for the effects of body mass.

Materials and methods

Study sites

A.P.M. conducted standard point counts of breeding birds during 29 May–9 June 2006, 1–11 June 2007, 29 May–5 June 2008 and 1–6 June 2009, with each count lasting 5 min during which birds seen or heard were identified and recorded (Møller 1983; Bibby et al. 2005; Voříšek et al. 2010). A radiation protection suit was worn in the most contaminated parts of the Red Forest to keep dust particles from contact with the body. The legs and torso, but not the head, were covered by the radiation protection suit ensuring that observing and hearing birds were not affected by the suit. The census was conducted within the Chernobyl Exclusion Zone, Ukraine, or in areas adjacent on the southern and western borders with a permit from the Ukrainian authorities, and in areas in Southern Belarus around Gomel during the 2006–2009 breeding seasons. The most distant census points were several hundred kilometers apart [A total of 254 points (breeding season

2006), 235 points (breeding season 2007), 237 points (breeding season 2008) and 159 points (breeding season 2009) were located at ca. 100-m intervals within forested areas (excluding successional stages of secondary forest due to abandoned farming (these areas are still almost exclusively open grassland)). Only birds recorded less than a distance of 50 m from the observation point were included in the census, thus avoiding the risk of inclusion of the same individual multiple times. Most census points were sampled each year to allow for analysis of species turnover. There was no significant difference in radiation level at census points among years ($F_{3,894} = 1.25$, $P = 0.29$).

We censused birds mainly during mornings before 1000 hours at the end of May and the beginning of June when most individuals reach their annual maximum of singing activity, making censuses of breeding birds highly reliable (Møller 1983; Bibby et al. 2005; Voříšek et al. 2010). We directly tested the reliability of our counts by letting two persons independently perform counts. The degree of consistency was high for both species richness and abundance (Møller and Mousseau 2007a).

Confounding habitat and weather variables

Abundance estimates can be affected by numerous confounding variables (Møller 1983; Bibby et al. 2005; Voříšek et al. 2010), and, therefore, it is crucial to control such variables statistically to assess the underlying relationship between radiation and abundance. We classified habitats [agricultural habitats with grassland or shrub (either currently or previously cultivated), deciduous forest, or coniferous forest] and estimated to the nearest 10% ground coverage by these different habitat types within a distance of 50 m from the observation point. Agricultural habitat included edges between forest and open areas, and the agricultural habitat variable thus also reflected the amount of edge habitat between forest and open areas. Maximum height of trees was estimated to the nearest 5 m and soil type was recorded as loam/clay or sand. The presence of open water within a distance of 50 m was also recorded. Weather conditions can affect animal activity and hence census results (Møller 1983; Bibby et al. 2005; Voříšek et al. 2010), and we recorded cloud cover at the start of each point count (to the nearest eighth, range 0–1 during the censuses), temperature (°C, range 12–25°C), and wind force (Beaufort, range 0–4 during the censuses). For each census point, we recorded time of day when the count was started (to the nearest min). Because activity may show a curvilinear relationship with time of day, for example, with high levels of activity in the morning and to a lesser extent in the evening for birds (Møller 1983; Bibby et al. 2005; Voříšek et al. 2010), we also included time squared as an explanatory variable.

Measuring background radiation levels

We measured radiation levels in the field and cross-validated these with measurements by the Ukrainian Ministry of Emergencies. We measured α , β , and γ radiation at ground level at each census point after having conducted the census (thus making the census blind with respect to radiation level) using a hand-held dosimeter (Inspector; SE International, Summertown, TN, USA). We measured levels 2–3 times at each site and averaged the measurements. Our data were validated with correlation against data from the governmental measurements published by Shestopalov (1996), estimated as the mid-point of the ranges published, with analyses showing a high degree of consistency between methods (Møller and Mousseau 2007a). Radiation levels vary greatly at a local scale due to heterogeneity in deposition of radioactive material after the Chernobyl accident (Shestopalov 1996). Our measurements at the census points ranged from 0.01 to 135.89 mSv/h. Background radiation levels for ^{90}Sr and ^{137}Cs are strongly positively correlated with internal doses in Chernobyl birds (Gaschak et al. 2008).

Melanin-based plumage coloration

Information on melanin-based plumage coloration for bird species censused at Chernobyl was obtained by examining illustrations in Cramp and Simmons (1977–1992) and Cramp and Perrins (1993–1994). Eumelanic and pheomelanic traits are generally of distinctive colors, the former being responsible for black and grey colors and the latter for yellowish, reddish, chestnut and brown colors (Toral et al. 2008). Eumelanin and pheomelanin normally occur simultaneously in the tissues (Ozeki et al. 1997), but the darker colors conferred by eumelanin (Toral et al. 2008) make evident the lower content of this pigment in chestnut and brown colors as compared to black and grey colors (Galván and Alonso-Alvarez 2009). Furthermore, many bird species have feather melanin contents of high purity (>90% of either eumelanin or pheomelanin; McGraw and Wakamatsu 2004; J.J. Negro, personal communication). Therefore, we considered that black and grey plumage colors were predominantly generated by eumelanin, while chestnut and brown colors were predominantly generated by pheomelanin. We did not consider conspicuous yellow or red colorations which are assumed to be generated by other pigments (i.e. carotenoids), unless chemically identified as melanin-based by Toral et al. (2008). Although only a rough approximation to the real proportion of eumelanic and pheomelanic plumage, the assumption that black-grey colors are eumelanic and brown-chestnut colors are pheomelanic should be adequate for comparative purposes (Owens and Hartley 1998). Thus, we quantified the

proportion of melanic plumage parts by examining illustrations in Cramp and Simmons (1977–1992) and Cramp and Perrins (1993–1994). Illustrations of both resting and flying adult birds in breeding plumage were examined. The method used by Beauchamp and Heeb (2001) and Galván (2008) was followed to obtain estimates of the proportion of eu- and pheomelanic color present in the plumage of each species, assigning scores that ranged from 0 (total lack of melanic color) to 5 (all melanic). When a species was sexually dichromatic regarding melanin-based coloration, eumelanic and pheomelanic scores were the average obtained for males and females. When a species had different subspecies differing in extent or type of melanin-based coloration, we used the nominate subspecies. It must be noted that eu- and pheomelanic color patches can coexist in the same feathers, and thus the sum of both color scores, in a species that presents both color types, is not always necessarily limited to five, and higher values are also possible. Information on eu- and pheomelanic plumage color scores for the species used in the study is provided in Online Resource 1.

Carotenoid-based plumage coloration

Prevalence of plumage patches colored by carotenoids was determined by examining illustrations of adult breeding birds in Cramp and Simmons (1977–1992) and Cramp and Perrins (1993–1994). We considered colors that were yellow, orange and red to be caused by carotenoids (Tella et al. 2004; Olson and Owens 2005). Information on carotenoid-based plumage color for the species used in the study is provided in Online Resource 1.

Body mass

We extracted mean body mass of males and females during the breeding season from Cramp and Simmons (1977–1992) and Cramp and Perrins (1993–1994) and Lislevand et al. (2007). Body mass was estimated as the mean value of the means for males and females. Body mass values for the species used in the study are provided in Online Resource 1.

Data analyses

We quantified the relationship between abundance of birds and level of background radiation by estimating the coefficient of determination and by calculating the slope of the relationship between abundance and \log_{10} -transformed background radiation, while including the potentially confounding variables. Radiation level and abundance were \log_{10} -transformed, while ground coverage with farmland and deciduous forest was square root arcsine-

transformed (coniferous forest was not included as an explanatory variable, because it simply represents the ground coverage not attributed to farmland and deciduous forest). We also included radiation level squared to account for non-linear relationships between species richness and abundance, respectively, and radiation. Furthermore, population density often varies considerably among years. Therefore, we included year as a factor in the analysis of population density in relation to background radiation level. In any case, estimates of relationships between abundance and background radiation levels were very similar when including and excluding year as a factor (results not shown). It must be noted that the relationship between abundance and background radiation level is highly repeatable among years (Møller and Mousseau 2010). These slopes were used for subsequent analyses.

Bird species are evolutionarily related as reflected by phylogeny, and, therefore, they should not be treated as independent sample units (Felsenstein 1985; Harvey and Purvis 1991). Thus, the effect of common ancestry among taxa can lead to an overestimation of degrees of freedom if phylogenetic relationships are not taken into account. We used phylogenetic eigenvector regression (PVR) to quantify the amount of phylogenetic signal and to correct for it in the analysis of the relationship between the expression of melanin-based coloration and bird responses to radiation levels (Diniz-Filho et al. 1998). Diniz-Filho and Torres (2002) and Martins et al. (2002) have tested several comparative methods (Felsenstein's independent contrasts, autoregressive method, PVR, and phylogenetic generalised least squares) and have found that PVR yields good statistical performance regardless of the details of the evolutionary mode used to generate the data and provides similar results to other methods, with very good (i.e. low) error types I and II. Moreover, PVR does not assume any a priori evolutionary model (an advantage if the true evolutionary model is unknown, or if it is too complex) and has similar statistical performance even under evolutionary processes distinct from Brownian motion.

We first performed a principal coordinates analysis (PCORD) on the matrix of pairwise phylogenetic distances between the 97 bird species (after a double-centre transformation). In a second step, we selected the first nine eigenvectors obtained by the broken-stick rule as the most parsimonious accounting of the phylogenetic signal. Eigenvectors extracted from double-centred phylogenetic distance matrices are able to detect the main topological features of the cladogram under different sample sizes or number of taxa used in the analyses (Diniz-Filho et al. 1998). We found that the original matrix of phylogenetic distances between the 97 bird species and the reproduced matrix of distances estimated based on the first nine eigenvectors were very similar (Mantel test with 999

randomised matrices to estimate significance: $r = 0.840$, $P < 0.0001$; test carried out using PopTools 3.2.3; Hood 2010). These eigenvectors were used as additional predictor variables in a general linear model (see below) in order to control for phylogeny.

The phylogenetic hypothesis (see Online Resource 2) was taken from the species-level supertree constructed by Davis (2008), with additional information from other sources for some species not covered by Davis (2008), Grosso et al. (2006), Voelker et al. (2007), Alström et al. (2008) and the phylogeny compiled by Møller (2006). Since we used different phylogenies that employed different methods, we set all branch lengths equal to unity in our compiled phylogeny, thus assuming a speciation model of evolution.

We used a general linear model to regress the slope estimates of the regression between bird abundance and radiation levels on the proportion of eu- and pheomelanin plumage color, prevalence of carotenoid-based plumage color, body mass (all \log_{10} -transformed) and the first nine phylogenetic eigenvectors (EV1–EV9 hereafter). All predictor variables were added as covariates to the model. Carotenoid-based plumage color score was added as a dummy variable with two levels: no = 0, yes = 1. We included the interaction between melanin- and carotenoid-based color scores to investigate whether its effect on slope estimates differed from the effect of each color type separately. The phylogenetic signal [i.e. amount of variance (i.e. R^2) exclusively explained by phylogeny] in slope estimates was calculated by regressing this variable on EV1–ssEV9. The models were weighted by number of individuals recorded to estimate the slope of the relationship between abundance and radiation, thereby placing more weight on species with many individuals (slope estimates were negatively related to sample size; $r = -0.30$, $n = 97$, $P = 0.003$). The assumption of normality was checked by exploring the distribution of residuals.

Results

A total of 64 of the 97 species considered (66%) had negative slope estimates for the regression between abundance and radiation levels, while 33 species (34%) had positive slopes (Online Resource 1). Among the 97 slopes, 30 (30.9%) were statistically significant at the 5% level, while 4.85 would be expected by chance (0.05×97). Among negative slopes, 25 were significant, while only 5 of the positive slopes were significant. The relationship between eumelanin color score and slope estimates for the regression between abundance and radiation levels was not significant either alone ($F_{1,84} = 0.05$, $P = 0.818$) or when including pheomelanin color score in the model ($F_{1,83} = 2.96$, $P = 0.089$). Since both color variables were

strongly negatively correlated ($b = -0.48$, $F_{1,95} = 19.15$, $P < 0.0001$), eumelanin color score was not included in subsequent analyses to avoid problems of collinearity. The interaction between pheomelanin- and carotenoid-based color scores was not significant ($F_{1,83} = 2.50$, $P = 0.117$), so it was also removed from the model.

The full model was significant ($F_{12,84} = 7.66$, $P < 0.0001$) and explained 52.3% of variance in slope estimates. As predicted, the slope decreased with the proportion of pheomelanin plumage color (Table 1; Fig. 1), indicating that the effect of radioactive contamination on abundance was more negative in species that produce large amounts of pheomelanin. The presence of carotenoid-based color was also negatively related to slope estimates, as expected (Table 1). Body mass also had a significant effect on slope estimates (Table 1). Pheomelanin plumage color score was significantly related to slope estimates in the previous model (Table 1) even without weighting by number of individuals ($b = -0.03$, $F_{1,84} = 4.27$, $P = 0.042$).

The phylogenetic signal in slope estimates was significant ($F_{9,87} = 5.83$, $P < 0.0001$) and relatively high (37.6%). The influence of phylogeny on slope values was mainly reflected through the significant positive effects of EV1 and EV4 (especially EV1; Table 1). EV1 discriminated non-passerines and corvid passerines (families Oriolidae, Laniidae and Corvidae), on the positive part of the axis, from the rest of passerines on the negative part. The negative part of EV4 comprised the species of the non-passerine families Phasianidae, Columbidae, Rallidae, Accipitridae, Ciconiidae, Ardeidae, Charadriidae, Scolopacidae and Lariidae, and of the passerine families Hirundinidae, Sylviidae, Motacillidae, Passeridae, Emberizidae and Fringillidae.

Discussion

The proportion of pheomelanin-based plumage coloration was negatively related to the slope of the regression between abundance and radiation levels in 97 species of birds censused at Chernobyl, as predicted. This was independent of the presence of carotenoid-based coloration and body mass, two factors that were also related to population responses to radiation (Møller and Mousseau 2007a). Thus, the negative effects of radiation exposure on bird populations (Møller and Mousseau 2007a, b, 2009) are stronger in species that have the molecular basis to produce large amounts of pheomelanin as reflected by their plumage color. Thus, pheomelanin birds are constrained to adapt to and overcome environmental changes that increase environmental oxidative stress such as radioactive contamination following the Chernobyl accident. This is the first study reporting an effect of melanin-based coloration on sensitivity to ionising radiation.

Table 1 Results of the general linear model testing the effects of melanin- and carotenoid-based plumage coloration, body mass and phylogenetic effects on the slope of the regression between abundance and radioactive levels in 97 bird species at Chernobyl

Effect	<i>b</i>	<i>df</i>	Sum of squares	<i>F</i>	<i>P</i>
Pheomelanin-based plumage color score	-0.063	1	0.80	6.29	0.014
Carotenoid-based plumage color score	-0.043	1	1.02	7.96	0.006
Body mass	-0.064	1	1.00	7.85	0.006
EV1	0.014	1	1.69	13.21	<0.001
EV2	-0.002	1	0.08	0.64	0.426
EV3	0.002	1	0.03	0.22	0.639
EV4	0.006	1	0.58	4.58	0.035
EV5	0.007	1	0.44	3.46	0.066
EV6	0.000	1	0.00	0.00	0.945
EV7	-0.001	1	0.00	0.01	0.924
EV8	0.004	1	0.15	1.16	0.283
EV9	-0.002	1	0.03	0.27	0.601
Error		84	10.73		

For each variable, we show the fitted regression coefficient (*b*) and its corresponding significance. For carotenoid-based plumage color score the regression coefficient refers to a dummy variable with two levels: no = 0, yes = 1. Phylogenetic effects are computed from the first nine eigenvectors (*EV1–EV9*) obtained from a principal coordinates analysis applied to the matrix of pairwise phylogenetic distances between the 97 species. Significant effects are shown in *bold*

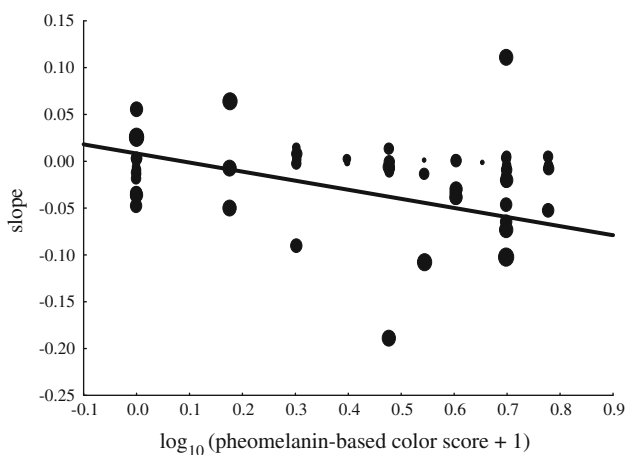


Fig. 1 Relationship between pheomelanin-based plumage color score and slope estimate of the regression between abundance and radiation levels in 97 species of birds at Chernobyl. The size of the data points increases logarithmically with the number of individuals used to estimate the slopes for illustrative purposes only. The *line* is the regression line

The increased susceptibility of pheomelanin species to radioactive contamination might be due to the physiological costs of meeting the GSH demands of pheomelanogenesis (Ozeki et al. 1997; Galván and Alonso-Alvarez 2009; Galván and Solano 2009), as this probably limits levels of GSH available to combat free radicals. Indeed, GSH is one of the antioxidants most susceptible to ionising radiation (Bump and Brown 1990; Navarro et al. 1997; Vartanian et al. 2004). Although GSH is endogenously synthesised by organisms, its production depends on the

availability of the amino acid cysteine and its precursor methionine (Lu 1999). Thus, although GSH levels are under genetic control (Soltaninassab et al. 2000), they are also influenced by environmental factors.

Similarly, the hypothesised trade-off between protection against free radicals and coloration likely also explains the negative slopes of the regression between abundance and radiation that were found in species with carotenoid-based plumage coloration, as carotenoids that are used to color the integument can no longer fulfil their role as circulating antioxidants (reviewed in Møller et al. 2000). This latter result reaffirms the previous findings by Møller and Mousseau (2007a) that had been detected with a smaller sample of species.

Since GSH, which is a key intracellular antioxidant, inhibits the synthesis of eumelanin and directs the process toward the synthesis of pheomelanin, eumelanogenesis requires lower antioxidant levels to proceed than pheomelanogenesis (Galván and Alonso-Alvarez 2008, 2009; Galván and Solano 2009; Hōrak et al. 2010). This means that eumelanin synthesis should more readily proceed than pheomelanin synthesis under environmental conditions that generate oxidative stress such as radiation exposure at Chernobyl (see Almasi et al. 2008; Dauwe and Eens 2008 and Roulin et al. 2008 for intraspecific studies suggesting positive selection on eumelanism by different factors that generate oxidative stress). However, the slope of the regression between abundance and radiation was not significantly related to the proportion of plumage colored by eumelanin, even when eu- and pheomelanin color scores were negatively correlated. The reason for this is probably

related to the fact that, while the synthesis of pheomelanin (for which GSH is a substrate) is traded against other processes that demand GSH resources to combat free radicals, the synthesis of eumelanin does not necessarily improve the performance of these processes. In other words, pheomelanin seems to act as a limiting factor, while eumelanin does not improve the antioxidant machinery.

The fact that species with a large proportion of pheomelanic plumage were particularly affected by radiation, while species with a large proportion of eumelanic plumage were unaffected, suggests that the characteristics of melanin-based plumage coloration may be changing the competitive interactions between species inhabiting the Chernobyl area. This is because the negative effect on the abundance of pheomelanic species may favour other species that thrive or even occupy the niche space that may have been abandoned or modified in width by pheomelanic species (Ricklefs 2010). Indeed, 34% of the species considered here had a positive slope estimate for the regression between abundance and radiation level, indicating that some species were favoured or little affected by radioactive contamination. Although the activity of free radicals at certain levels is essential for development of certain physiological processes, particularly those related to immunity (Knight 2000), to our knowledge there are no published reports of positive effects of free radicals generated by radioactive contamination in any living organism, certainly because the levels of free radicals that this produces are far higher than those needed for physiological processes (Knight 2000). Therefore, it is probable that the positive slope estimates for the regression between abundance and radiation levels that we obtained for some species of birds are due to these species benefiting from relaxation of competitive interactions with other species that suffer more strongly from the effects of radiation, and a possible occupancy of new niche space by the former species.

If species with positive slope estimates benefit from relaxation of competition with species that are negatively affected by radiation, it should be expected that the former are closely phylogenetically related to the latter. This is because the most intense competitive interactions occur between closely related species, since phylogenetic similarity confers ecological similarity and thus favours niche overlap (Elton 1946; Jackson 1981). In our case, the 33 species with positive slopes were widely distributed across the phylogeny (see Online Resources 1 and 2), implying that there was no clear phylogenetic signal in the sign of slope estimates. Furthermore, with the exception of pied and collared flycatchers (*Ficedula hypoleuca* and *F. albicollis*) and Savi's and river warblers (*Locustella luscinioides* and *L. fluviatilis*), there were no congeneric sister groups in our phylogeny in which both species had positive

slopes (see Online Resource 2). Thus, the closest relatives of species with positive slopes were species with negative slopes. This may support the argument that species that have been favoured by radioactive contamination have been so due to relaxation of competition with phylogenetically similar species that were negatively affected by radiation.

Interestingly, we found a relatively strong phylogenetic signal in the slope estimates between abundance and radiation levels. This means that there are phylogenetic constraints in the capacity of species to mount an adaptive response that counteracts the negative effects of radioactive contamination. This constraint was especially marked in non-corvid passeriform birds, which corresponds to the Eurasian expansion of passerines (Davis 2008). Thus, independently of other factors that affect slope estimates (e.g. plumage coloration and body mass), collectively, this group of related species appear to be particularly sensitive to radioactive contaminants.

Finally, we found that population declines were particularly pronounced in large-sized species, in contrast to the results of Møller and Mousseau (2007a), who found a positive effect of body mass on slope estimates. These conflicting results may be due to the larger number of species considered in the present study, and the inclusion of a new variable (i.e. pheomelanin-based coloration) that is intrinsically related to oxidative stress. Møller and Mousseau (2007a) argued that population declines were more pronounced in small-sized species because their higher metabolic rates may require more antioxidant resources, which would make them more sensitive to radiation. However, smaller species may also have evolved stronger antioxidant and repair mechanisms to counteract their higher metabolic rates (Holmes et al. 2001), which may explain our results. Additionally, Møller and Mousseau (2007a) indicated that large-sized species often possess larger home ranges, which may increase the heterogeneity in radiation levels of the areas they encounter and reduce their average exposure to contaminants. For this reason, large-sized species may also have a greater propensity to encounter highly contaminated areas by chance that could be a critical factor in determining genetic and physiological consequences of radiation. Individual level dosimetry would be needed to disentangle these two hypotheses.

Responses to radioactive contaminants may seem extreme and perhaps even of little relevance to biology under natural conditions. However, natural levels of radiation vary by more than two orders of magnitude (e.g. Bristow 1978; Sanderson et al. 2001), suggesting that the effects reported here may even be discernible elsewhere. We also note that species with strongly reduced abundance in contaminated areas are more susceptible to mutagens as reflected by their higher historical DNA substitution rates

(Møller et al. 2010). Thus, the effects reported here may also have implications for understanding natural variation in mutation accumulation.

In conclusion, bird species that have a large proportion of their plumage colored by pheomelanin, have large body mass, or belong to non-corvid passeriform families, display particularly large population declines in radioactively contaminated areas. These results should be valuable for conservation purposes in areas affected by radioactive contamination.

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