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# Determinants of interspecific variation in population declines of birds after exposure to radiation at Chernobyl

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**Summary**

**1.** Radiation can reduce antioxidant levels dramatically because of the use of antioxidants to eliminate free radicals produced in the presence of radiation. Antioxidants are crucial biochemicals for elimination of free radicals, which can cause permanent damage to DNA and other molecules. If antioxidants are a limiting resource, we would expect individuals of species with a high expenditure of antioxidants to suffer the most from radiation. We tested this hypothesis by investigating interspecific variation in the relationship between abundance and level of radiation in breeding birds inhabiting forests around Chernobyl, Ukraine. We used bird point counts to estimate abundance of 57 species of birds at 254 locations where background radiation levels were quantified.

**2.** Migratory birds use large amounts of antioxidants during their annual migrations to neutralize free radicals, and migrants have depleted antioxidant levels upon arrival at their breeding grounds. Consistently, abundance decreased with increasing levels of radiation in species that migrated the longest distances.

**3.** Bird species with long dispersal distances may experience deficiencies in antioxidant levels because of physical activity but also because of exposure to novel antigens, implying that species with long dispersal distances should suffer the most from exposure to radiation. Indeed, the slope of the relationship between abundance and radiation decreased with increasing dispersal distance.

**4.** Female birds deposit large amounts of antioxidants in their eggs, with the total amount deposited often exceeding the total amount in a female's. Accordingly, the decrease in abundance with radiation level increased with relative egg size in different species.

**5.** Many bird species have plumage that is coloured by carotenoids, which cannot be recovered once deposited in feathers. Therefore, bird species with carotenoid-based plumage should show stronger declines with increasing levels of radiation than species with melanin-based or structural coloration. In accordance with this prediction, the decline in abundance with radiation was the strongest in species of birds with carotenoid-based plumage.

**6.** *Synthesis and applications*. These findings highlight the importance of antioxidants for understanding the ecological consequences of radiation on the abundance of free-living animals, showing that species using large amounts of antioxidants will be particularly susceptible to the effects of low-level radiation.

*Key-words*: carotenoids, dispersal, maternal effects, migration, sexual coloration

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## Introduction

World-wide, thousands of square kilometres of land are radioactively contaminated, mainly as a result of the Chernobyl disaster but also as a result of tests with nuclear bombs. Furthermore, there is considerable natural variation in background radiation, with many areas reaching non-negligible levels. Yet the ecological and evolutionary consequences of human-induced and natural variation in radiation remain poorly known (Zakharov & Krysanov 1996; Møller & Mousseau 2006).

Radiation and antioxidants interact in a number of different ways, making them key biochemicals for understanding the interactions between radiation on one hand and ecology and evolution on the other. First, a deficiency of antioxidants such as carotenoids and vitamins A and E can increase the DNA damage caused by free radicals (Ames 1983; Edge, McGarvey & Truscott 1997; Rice-Evans *et al.* 1997; Bast *et al.* 1998; Krinsky 1998; Møller *et al.* 2000), which might in particular be the case in the presence of high levels of radiation. Secondly, radiation produces free radicals that are removed by antioxidants, resulting in depletion of antioxidants in the presence of high levels of radiation, leaving few reserves for scavenging the free radicals produced by ordinary metabolic activity (Ben-Amotz *et al.* 1998; Ivaniota, Dubchak & Tyshchenko 1998; Neyfakh, Alimbekova & Ivanenko 1998a,b; Kumerova *et al.* 2000). This reduction in antioxidants may be one factor responsible for the increased levels of mutation in species in radioactively contaminated areas such an those around Chernobyl in Ukraine, while the alternative factor is the direct effects of radiation on mutations (Ellegren *et al.* 1997; Møller & Mousseau 2003).

Antioxidants are important biochemicals that provide protection against the damaging effects of free radicals on DNA and other molecules (reviewed by Halliwell & Gutteridge 1990; Yu 1994; Møller *et al.* 2000; Surai 2002). Elevated levels of radiation, as experienced by recovery workers and other people exposed to the Chernobyl accident, are associated with a diminished antioxidant defence as a result of the use of antioxidants for free radical scavenging (Ivaniota, Dubchak & Tyshchenko 1998; Neyfakh, Alimbekova & Ivanenko 1998a; Kumerova *et al.* 2000). Likewise, Møller, Surai & Mousseau (2005) showed various changes in barn swallows *Hirundo rustica* exposed to high levels of radiation in Chernobyl. In particular, circulating plasma levels of carotenoids and vitamins A and E were dramatically reduced, as were levels of these antioxidants stored in the liver and eggs. However, such effects of radiation on antioxidant levels are reversible because supplementation with b-carotene and vitamins A and E can reduce the effects of the lipo-peroxidative cascade among individuals subject to radiation (Clavere *et al.* 1996; Ben-Amotz *et al.* 1998; Neyfakh, Alimbekova & Ivanenko 1998b). Administration of antioxidants such as vitamin E to subjects reduced the effects of radiation on immunity (Rana & Malhotra 1995; Moriguchi *et al.* 1996) and the intestine (Empey *et al.* 1992; Felemovicius *et al.* 1995). While these studies provide important information regarding the links between radiation exposure and antioxidant defence, there has been no study that links the effects of radiation on population size of different species to antioxidant defence. We have carried out a preliminary test of the links between effects of radiation on population density and antioxidant use, by studying a community of free-living birds in areas in Ukraine varying by three orders of magnitude in levels of radioactive contamination (0·04–135·89 mR $h^{-1}$).

All organisms must cope with the detrimental effects of free radicals in order to transfer undamaged DNA to offspring, and a number of different defence mechanisms have evolved to cope with such effects. Although all species must cope with the potentially detrimental effects of free radicals, because of their use of antioxidants, certain species are predisposed to suffer particularly from these negative effects. We hypothesized that, while all species of higher organisms use antioxidants to cope with free radicals, some species use disproportionate amounts but also have to maintain disproportionately large margins to ensure survival. A migratory species, with individuals weighing 10 g, annually travelling 10 000 km, would produce vast amounts of free radicals (Costantini, Cardinale & Carere 2007). These free radicals have to be neutralized, thereby reducing antioxidant stores during and after migration (Ninni *et al.* 2004). The amounts of antioxidants needed for free radical scavenging should exceed, by a large margin, what is available in favourable years, because poor environmental conditions before the start of migration, head winds and other factors may increase the need for antioxidants considerably. This argument is based on geometric rather than arithmetic means, emphasizing the importance of selection arising from rare events of adverse conditions, thus implying that, on average, migratory species will suffer from low levels of antioxidants more often than resident species. We argue that four different activities account for reductions in antioxidant levels in free-living organisms, and that each of these factors will increase the susceptibility of a species to the negative effects of radiation. These factors are (i) extreme physical activity, such as that associated with long-distance migration; (ii) dispersal; (iii) deposition of antioxidants in eggs; and (iv) deposition of antioxidants in signals used for social or sexual communication.

We will briefly review the empirical basis for considering these four factors as sinks for antioxidants. First, twice annually long-distance migration constitutes a level of extreme physical activity (Berthold 2001) that may deplete antioxidant levels. Direct physiological evidence suggests that migrants arrive at their breeding grounds with depleted levels of antioxidants, with early-arriving migrants differing in levels of carotenoid depletion from late-arriving individuals (Ninni *et al.* 2004). Similarly, there is experimental evidence from salmon showing that oxidative stress is an important cost of migration (Welker & Congleton 2005). This implies that migrants will differ from residents in antioxidant levels, but also that long-distance migrants will differ from short-distance migrants in antioxidant levels.

Secondly, migrants disperse over long distances but there is also movement to areas with novel parasite strains that have not previously been encountered by a host. This raises the prospect of induction of immune responses (Møller & Erritzøe 2001), which are known to be potent sources of free radicals (Costantini & Dell'Omo 2006) because of rapid cell proliferation (Roitt, Brostoff & Male 1998; Møller *et al*. 2000). Immune cells use free radicals as a powerful weapon to kill bacteria and other infections by stimulating free radical production by macrophages (Chapple 1997). Enhanced immune cell activity can overproduce free radicals, and antioxidant protection in this case could be vital in avoiding damage to DNA and other molecules (Gille & Sigler 1995; Jaeschke 1995). Given that there are interspecific differences in dispersal propensity (Paradis *et al*. 1998), we suggest that there are interspecific differences in the use of antioxidants associated with dispersal, causing a negative relationship between dispersal distance and population decline caused by radiation.

Thirdly, females of invertebrates, fish and birds deposit large amounts of antioxidants in their eggs, providing an important source of maternal effects (Blount, Houston & Møller 2000). Antioxidants play a central role in reproduction during embryo development and at hatching (reviewed by Surai, Speake & Sparks 2001). In particular, this period of intense growth is associated with increased oxidative stress, to which embryos are very susceptible (Surai *et al*. 1999; Surai 2002). Female birds allocate large amounts of lipid-soluble antioxidants, such as carotenoids and vitamin A and E, to their eggs compared with their circulating levels and body stores (Blount, Houston & Møller 2000), and experimental provisioning with carotenoids has shown that the egg-laying ability of female gulls is limited by carotenoid availability (Blount *et al*. 2003). Finally, an extensive study of dose rates of free-living birds in Chernobyl has shown that residual dose rate, after accounting for background radiation level, is significantly explained by the relative size of an egg (A. P. Møller, S. Gaschak & T. A. Mousseau, unpublished data). This implies that females of bird species with relatively large eggs for their body size suffer proportionately more from radiation exposure.

Fourthly, the colour of many sexual signals is based on carotenoids. Because carotenoids can only be synthesized by algae, bacteria, fungi and plants (Fox 1979; Brush 1981; Goodwin 1984; Latscha 1990; Stradi 1998), higher animals must ingest them in order to produce carotenoid-based sexual signals. Animals differ in their rate of carotenoid absorption (Scheidt 1989) and rate of carotenoid deposition in signals (Brush & Power 1976; Hudon 1991) but also in their ability to convert ingested carotenoids into other pigments (Fox 1979; Goodwin 1984; Putnam 1992; Stradi 1998). Furthermore, availability of one kind of antioxidants can reduce the use of other kinds (Møller *et al*. 2000; Surai 2002) and conversion between different kinds of antioxidants may depend on the relative availability of one or more kinds (Møller *et al*. 2000; Surai 2002). Given that a relatively large amount of the total body content of carotenoids can be deposited in the plumage, we hypothesized that carotenoid-based plumage can affect the scarcity of carotenoids and other antioxidants.

The objective of this study was to investigate interspecific variation in response to spatial variation in background radiation around Chernobyl and the ecological factors accounting for such interspecific variation. More specifically, we tested whether (i) bird species with longer distance migrations suffer stronger declines in population size with radiation because of the reductions in levels of antioxidants caused by annual migrations; (ii) dispersal is associated with greater reductions in abundance during exposure to radiation; (iii) species that produce relatively large eggs for their body size suffer stronger declines in population size with radiation because of the reductions in levels of antioxidants caused by maternal allocation to eggs; (iv) species with carotenoid-based plumage, and hence allocation of carotenoids to feather coloration suffer greater declines in population size with radiation because of the reductions in levels of antioxidants caused by production of carotenoid-rich plumage.

We tested these four predictions by determining whether declines in abundance estimates of breeding birds in relation to radiation based on results from standardized point counts could be accounted for by migration distance, dispersal, relative egg mass and presence of carotenoid-based plumage, using a data set of 57 species of birds. Finally, different species may show similar responses to radiation as a result of the effects of common descent rather than convergent evolution, and we controlled for such effects by analysing the data using standardized linear contrasts.

## Materials and methods

### BIRD CENSUS

We used standardized point counts to census birds (Møller 1983; Bibby *et al*. 2005). Standard point counts of breeding birds were conducted from 29 May to 9 June 2006, with each count lasting 5 min, during which time all individual birds seen or heard were recorded. Only one person performed the counts, to avoid problems of interobserver variability. The points were located at 100-m intervals within the Chernobyl exclusion zone, or in areas adjacent to the exclusion zone on the southern and western borders. A total of 254 point counts was positioned in forested areas across a range of radiation levels spanning more than three orders of magnitude. Census points were not selected based on radiation level, because this was recorded when the census had already been made. The high degree of spatial heterogeneity in background radiation level (Fig. 1; Shestopalov 1996) ensured that the census points

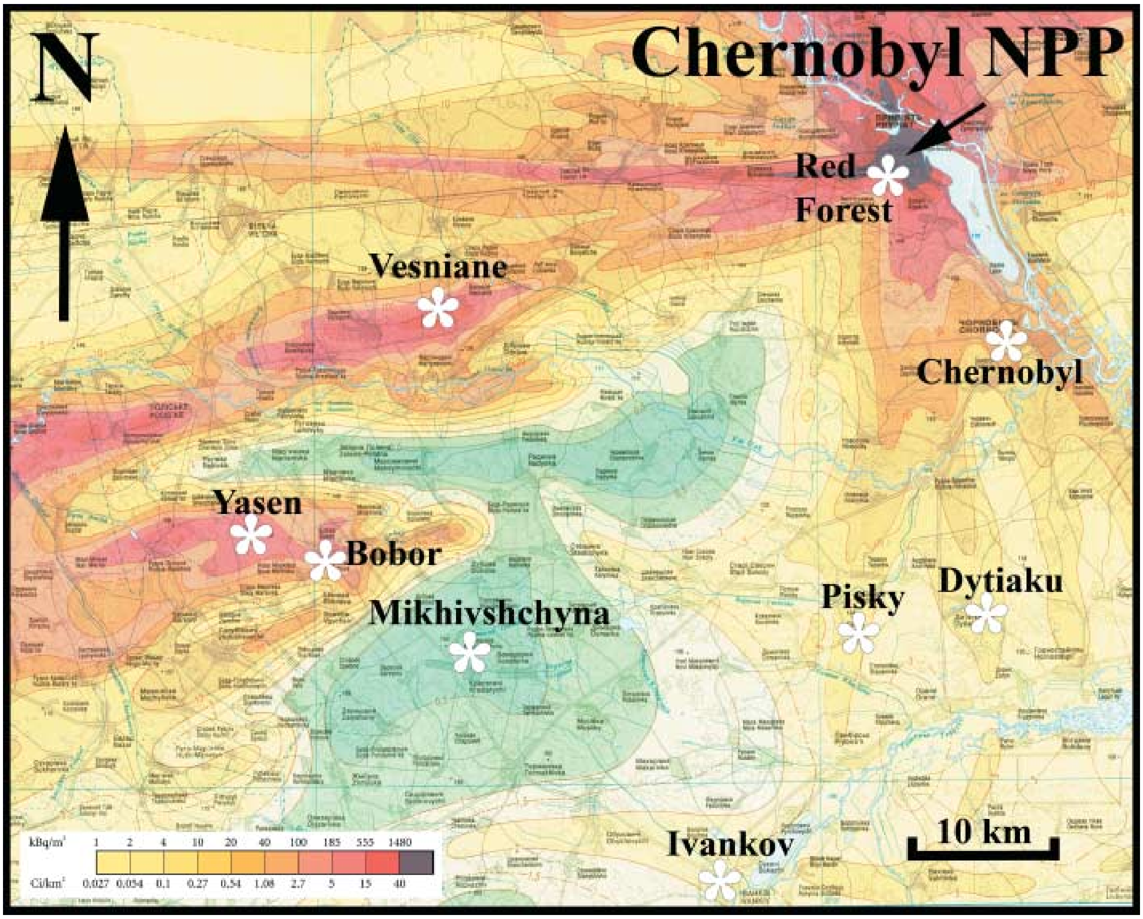


**Fig. 1.** Locations of sampling areas in the Chernobyl area of northern Ukraine. Modified from Shestopalov (1996).

varied by more than three orders of magnitude in radiation level.

The point count method to census birds provides highly reliable estimates of species richness and abundance, as shown by numerous methodological studies (Møller 1983; Bibby *et al*. 2005). We tested the reliability of our counts directly for a sample of 10 points, with two people independently performing the counts. To strengthen the power of this test and to ensure that the outcome would not be determined by greater carefulness or dedication, the second person performing the counts was unaware of the purpose of the task and that the counts would be used for assessing the quality of the census. The Pearson product–moment correlation between species richness in these two series of counts was 0·99, $t = 42{\cdot}06$, d.f. = 8, $P < 0{\cdot}0001$, and for abundance it was equally high, $r = 0{\cdot}99$, $t = 12{\cdot}47$, d.f. = 8, $P < 0{\cdot}0001$.

### MEASURING BACKGROUND RADIATION LEVELS IN THE FIELD

We obtained radiation estimates from our own field measurements and cross-validated these with measurements made by the Ministry of Emergencies. We measured α, β and γ radiation levels at ground level directly in the field at each point where birds were censused, using a hand-held dosimeter (Model Inspector, SE International Inc., Summertown, TN). We measured levels two to three times at each site and averaged the measurements. Our data were validated by correlation with data from the government measurements published by Shestopalov (1996), estimated as the mid-point of the ranges published. This analysis revealed a very strong positive relationship [linear regression on log-log transformed data, $F = 1546{\cdot}49$, d.f. = 1,252, $r^2 = 0{\cdot}86$, $P < 0{\cdot}0001$, slope (SE) = 1·28 (0·10)], suggesting that our field estimates of radiation provided the same ranking of levels of radiation among sites as the published estimates. The measurements by the Ministry of Emergencies were obtained by repeated standardized measurement of radiation at the ground level in a large number of different localities in Ukraine. Radiation levels vary considerably at very short geographical distances because of the heterogeneity in the deposition of radiation after the Chernobyl accident (Fig. 1; Shestopalov 1996) and our measurements at the census points ranged from 0·04 to >135 mR $h^{-1}$.

### VARIABLES REFLECTING CONDITIONS DURING CENSUSES

Bird abundance estimates can be affected by numerous potentially confounding variables other than the actual number of individual birds present (Møller 1983;

Bibby *et al.* 2005). Therefore it is crucial to control such variables statistically to assess the underlying relationship between radiation and abundance. We quantified three aspects of habitat directly at each of the points (agricultural habitats with grassland or shrub, deciduous forest, and coniferous forest estimated to the nearest 10% of ground coverage within a distance of 50 m from the observation point). Furthermore, the maximum height of trees was estimated to the nearest 5 m, while soil type was recorded as loam/clay or sand. Finally, the presence of open water within a distance of 50 m was recorded because access to water can increase abund-ance of birds.

A second category of variables with an important impact on bird censuses is weather conditions, which can affect animal activity and hence census results (Møller 1983; Bibby *et al.* 2005). Therefore we recorded three different aspects of weather at each point: (i) cloud cover at the start of each point count (to the nearest eighth, range 0–1 during the censuses), (ii) temperature (degrees Celsius, range 12–22 °C during the censuses) and (iii) wind force (Beaufort, range 0–4 during the censuses). For each point count we recorded the time of day when the count was started (to the nearest minute). Because bird activity may show a curvilinear relationship with time of day, with high levels of activity in the morning and lower levels in the evening (Møller 1983; Bibby *et al.* 2005), we also included time squared as an additional variable.

## EXPLANATORY VARIABLES

### *Plumage coloration*

We scored the breeding plumage of all species as sexually monochromatic if males and females did not differ in coloration according to information provided by the descriptions in Cramp & Perrins 1977–1994), and otherwise as sexually dichromatic, separately for carotenoid- and melanin-based coloration. We considered sexually dichromatic colours that were yellow, orange and red to be caused by carotenoids (Tella *et al.* 2004; Olson & Owens 2005). For melanin-based coloration we included all colours that were brown, black or reddish brown as typical for coloration based on phaeo- and eu-melanin (Gray 1996; Tella *et al.* 2004; Olson & Owens 2005).

### *Migration distance*

We recorded the northernmost and southernmost breeding and wintering latitude to the nearest 0·1 degrees latitude based on the distribution maps in Cramp & Perrins (1977–1994). Migration distance was the obvious difference in the mean of the two breeding latitudes and the mean of the two wintering latitudes.

### *Dispersal distance*

We estimated maximum dispersal distance as the minimum distance from the mainland to an island with a permanent breeding population, using information in Cramp & Perrins (1977–1994). We achieved this by considering the distance from the mainland to all islands closer than the furthest island from the mainland, including those that did not hold permanent populations. Therefore, the estimate of maximum dispersal distance was a minimum estimate because many populations on islands are not likely to have taken the shortest route from the mainland to an island, and islands may have been colonized directly rather than by using intermediate islands as stepping-stones.

### *Life history*

We recorded egg mass (g), age at first reproduction, clutch size, maximum number of clutches per season and adult survival rate from Cramp & Perrins (1977–1994). If multiple estimates were provided, we extracted the information from the UK data because those estimates were generally based on the largest sample sizes.

### *Body mass*

We extracted mean body mass of males and females during the breeding season from Cramp & Perrins (1977–1994), again generally preferring estimates from the UK because of larger sample sizes. Body mass was estimated as the mean value of the means for males and females.

The entire data set is reported in Appendix S1 in the Supplementary material.

## PHYLOGENETIC ANALYSES

Because of common ancestry, comparative analyses based on species-specific data overestimate the number of independent observations, thus increasing the risk of statistical type I errors (Harvey & Pagel 1991). In order to identify evolutionary independent comparisons, we used the method of independent contrasts (Felsenstein 1985) as implemented in the Macintosh-based software CAIC, using the CRUNCH algorithm (Purvis & Rambaut 1995). To do this we constructed a composite phylogeny of all species in our database (see Fig. S1 in the supplementary material), based on Sibley & Ahlquist (1990) and updated with recent phylogenies of a more limited range of taxa (Blondel, Catzeflis & Perret 1996; Slikas, Sheldon & Gill 1996; Badyaev 1997; Barker, Barrowclough & Groth 2001). All branches were assigned the same length, and analyses performed assuming uneven branch lengths produced similar results. Deleting contrasts with extreme residuals to test the robustness of the analysis did not change the results (Purvis & Rambaut 1995). Contrasts were analysed by forcing regressions through the origin, because the dependent variable is expected not to have changed if there is no change in the independent variable (Harvey & Pagel 1991). In order to weight regressions for sample size in the analysis of contrasts, we calculated weights for each contrast by calculating the mean sample size for the taxa immediately subtended by that node, and log-transforming this value to achieve normality.

### STATISTICS

All analyses were made with the statistical software JMP (SAS 2000). Radiation level, migration distance, dispersal distance, egg mass and body mass were $\log_{10}$-transformed, while coverage with farmland, deciduous forest and coniferous forest was square root arcsine-transformed before analysis. For each species we used the number of individuals at each of the 254 points as the dependent variable and the habitat variables, weather variables, time of day, time of day squared and radiation level as independent variables, using a Poisson distribution. We extracted the slope of the relationship between abundance and radiation level from the partial regression, and we subsequently used these slope estimates for the 57 species for further analyses. First, we estimated mean slope weighted by sample size and tested whether the mean differed significantly from the null expectation of a slope of zero (under the assumption that radiation had no effect on abundance). Secondly, we used stepwise regression analyses weighted by sample size to assess whether interspecific differences in slope could be accounted for by the potential explanatory variables. We used stepwise regression models with a backward elimination procedure to assess the relationship between slope (dependent variable) and the potential predictor variables. We used a probability of $P < 0{\cdot}25$ for variables to enter and $P > 0{\cdot}10$ for variables to leave the model, as implemented in the statistical software JMP (SAS 2000). The models developed were later confirmed in forward stepwise regression models, and in no case did the forward and backward models differ.

**Table 2.** Best-fit stepwise regression model weighted by sample size of the relationship between slope of the relationship between abundance and background radiation level (dependent variable) and presence of carotenoid-based plumage, migration distance, dispersal distance, egg mass and body mass. The model for species had the statistics $F = 9{\cdot}67$, d.f. = 5, 51, $r^2 = 0{\cdot}49$, $P < 0{\cdot}0001$, while the model for contrasts had the statistics $F = 7{\cdot}77$, d.f. = 4, 52, $r^2 = 0{\cdot}12$, $P < 0{\cdot}0001$

| Variable | Sum of squares | *F* | *P* | Slope (SE) |
|---|---|---|---|---|
| Species | | | | |
| Carotenoid-based plumage | 1·12 | 12·02 | 0·0011 | –0·077 (0·022) |
| Migration distance | 1·20 | 12·87 | 0·0007 | –0·060 (0·017) |
| Dispersal distance | 0·50 | 5·31 | 0·025 | –0·032 (0·014) |
| Egg mass | 1·65 | 17·64 | 0·0001 | –0·295 (0·070) |
| Body mass | 1·17 | 12·52 | 0·0009 | 0·169 (0·048) |
| Error | 4·77 | | | |
| Contrasts | | | | |
| Carotenoid-based plumage | 0·011 | 11·39 | 0·0014 | –0·069 (0·021) |
| Dispersal distance | 0·011 | 11·23 | 0·0015 | –0·040 (0·012) |
| Egg mass | 0·003 | 3·27 | 0·077 | –0·172 (0·095) |
| Body mass | 0·004 | 4·28 | 0·044 | 0·145 (0·070) |
| Error | 0·052 | | | |

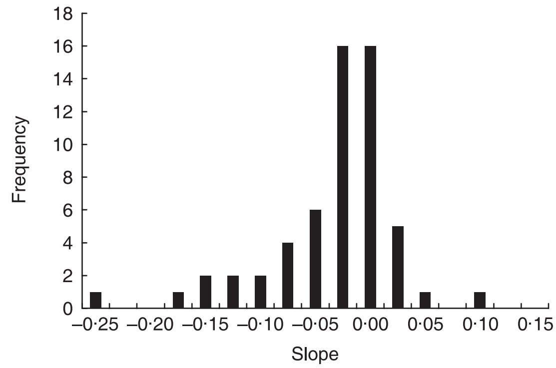


**Fig. 2.** Slopes of the relationship between abundance and background radiation level for 57 breeding bird species near Chernobyl, Ukraine.

**Table 1.** Model of the relationship between abundance at 254 points in relation to background level of radiation, species and radiation by species interaction. The model had the statistics $F = 60{\cdot}47$, d.f. = 113, 14364, $r^2 = 0{\cdot}32$, $P < 0{\cdot}0001$

| Variable | *F* | *P* | Slope (SE) |
|---|---|---|---|
| Radiation | 151·61 | < 0·0001 | –0·028 (0·002) |
| Species | 107·70 | < 0·0001 | |
| Radiation × species | 11·63 | < 0·0001 | |

## Results

We recorded a total of 1570 individuals of 57 species during the censuses of the forested areas around Chernobyl (and thus no species was excluded from the subsequent analyses). The slope of abundance in relation to radiation ranged from –0·25 to 0·11, with a mean weighted by sample size of –0·022 (SE = 0·008) that differed significantly from zero (one-sample *t*-test, $t = 2{\cdot}90$, d.f. = 56, $P < 0{\cdot}0001$). Eighteen of the 57 slopes were statistically significant, compared with the 2·85 that would be expected by chance. Thirteen of these significant slopes were negative, while only five were positive. There was considerable variation among species in slope of the relationship between abundance and radiation level (Fig. 2). In fact, a model revealed a significant relationship between abundance and radiation, an important effect of species and significant heterogeneity in response to radiation among species (Table 1). The significant heterogeneity implied that species responded differently in terms of abundance to radiation.

Interspecific variation in the effect of radiation on abundance was further analysed in a stepwise model that weighted the slope of each species by sample size to account for differences in sampling effort. The best-fit stepwise overall model included five variables and accounted for almost half of the variance (Table 2). First, species with carotenoid-based plumage coloration had stronger decreases in abundance as a result of radiation than other species (Fig. 3a and Table 2). This factor accounted for 10% of the variance. Secondly, migratory bird species showed stronger decreases in

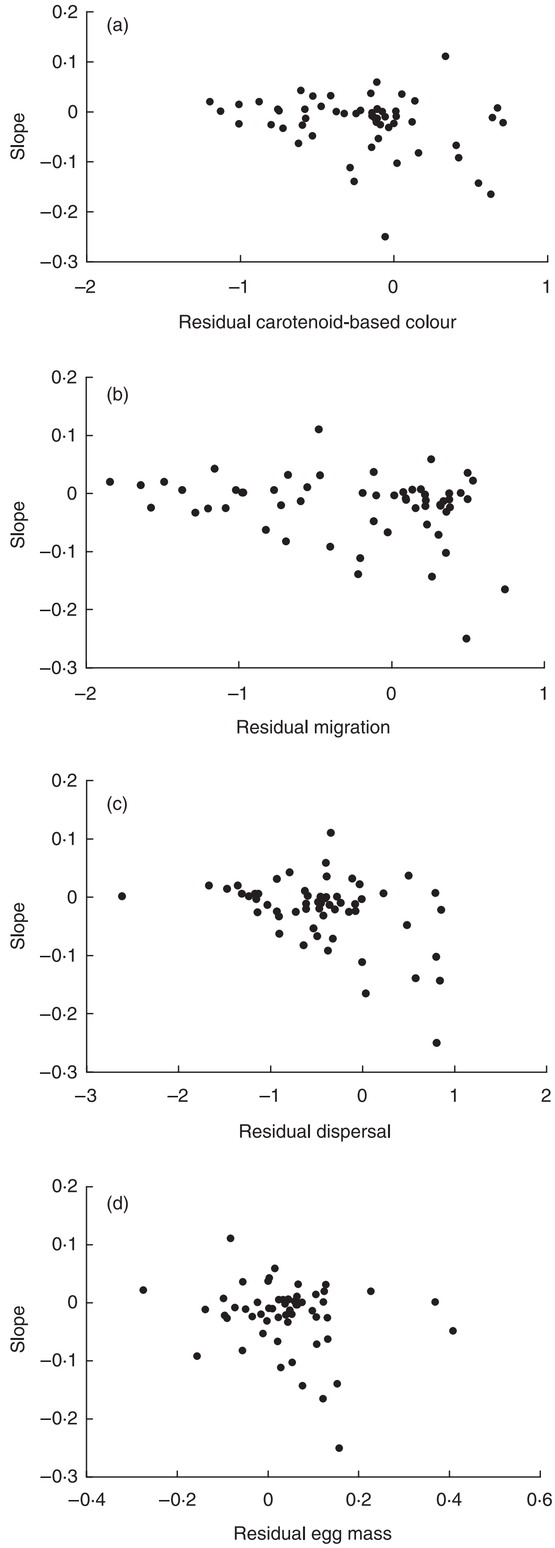


**Fig. 3.** Population trends of different species of breeding birds near Chernobyl, Ukraine, in relation to (a) presence or absence of carotenoid-based plumage, (b) migration distance (degree latitude), (c) dispersal distance (km) and (d) relative egg mass (g), after adjusting for body mass. All relationships are based on species-specific data, while the phylogenetically controlled analyses are reported in Table 2. All independent variables were expressed as residuals after adjusting for the three other independent variables, although this was only done for illustrative purposes. The full analysis is reported in Table 2.

abundance with radiation than resident species (Fig. 3b and Table 2), accounting for 11% of the variance. Thirdly, dispersal distance accounted for 4% of the variance in slope of the relationship between abundance and radiation (Fig. 3c and Table 2), implying that species with long dispersal distances suffered the strongest declines associated with radiation. Fourthly, large relative egg mass was associated with stronger declines in abundance associated with radiation (Fig. 3d and Table 2), accounting for 15% of the variance. Finally, bird species with large body mass suffered less from the effects of radiation on abundance (Fig. 3c and Table 2), accounting for 10% of the variance. Melanin-based plumage and a range of other life-history traits, such as adult survival rate, age at first reproduction, clutch size, number of clutches, incubation period, nestling period and annual fecundity, did not enter the model. An analysis of standardized linear contrasts revealed a model that included carotenoid-based plumage, dispersal distance and body mass as significant predictors of slope (Table 2). The effects for egg mass and migration distance did not reach statistical significance, implying that the relationships for these two variables in the analyses of species-specific data were the result of similarity in response among species because of the effects of common descent.

## Discussion

The main findings of this study were that (i) bird species differed in their response to radiation from Chernobyl but with, on average, a decrease in population density with increasing level of background radiation, and (ii) the strongest declines in population density with radiation level were documented for species with carotenoid-based plumage, long-distance migration and dispersal, and large eggs for their body size. All four of these factors are associated with antioxidant levels, suggesting that reduced antioxidant levels may precipitate population declines when species are exposed to radiation. A phylogenetic analysis of standardized linear contrasts confirmed the significant effects of carotenoid-based plumage and dispersal distance. We will briefly discuss the evidence linking these four factors to antioxidants and radiation.

Point counts provide robust estimates of abundance of birds because of their clearly defined procedures, simple assumptions and ability to record a wide range of species (Møller 1983; Bibby *et al.* 2005). All 254 points used for the censuses were assessed for the presence and abundance of all 57 species of birds, making comparisons among species feasible. Although it is well known that bird species differ in their detection probability because of differences in behaviour and conspicuousness (Møller 1983; Bibby *et al.* 2005), such differences should be similar among census locations, rendering estimates of the relationship between abundance and radiation unbiased. The overall abundance of species across all 254 points ranged from one to 369 individuals, causing heterogeneity in sampling effort among species. We took that fact into account by weighting the slope of the relationship between abundance and radiation by number of individuals recorded, thereby placing more weight on species with many individuals. We believe that this provided a sound, stringent and coherent way of analysing the effect of radiation on abundance of different species of birds.

Carotenoid-based coloration is common in numerous animals, including birds. Because animals cannot synthesize carotenoids, any carotenoids used for coloration or physiological processes must be ingested (Fox 1979; Brush 1981; Goodwin 1984; Latscha 1990; Stradi 1998). In contrast, animals can produce other kinds of colour, such as those based on melanins and structure (Fox 1979; Brush 1981; Goodwin 1984; Stradi 1998). Birds with carotenoid-based plumage allocate large amounts of carotenoids to coloration, potentially facing a trade-off with other uses of carotenoids (reviewed by Møller *et al*. 2000). We found that bird species with carotenoid-based plumage colour, but not those with melanin-based colour, suffered the strongest declines in abundance with increasing levels of radiation. This finding suggests that something specific to carotenoids accounts for the difference in relationships between the two kinds of coloration and the effect of radiation on abundance. We have suggested that the simplest explanation is that birds in highly contaminated areas suffer from a deficiency of antioxidants, including carotenoids. Use of carotenoids for plumage coloration may further reduce levels of antioxidants, with the consequence that such species experience the strongest declines in abundance. Previous studies of barn swallows from Chernobyl have shown circulating and stored levels of antioxidants, including carotenoids, to be suppressed by up to more than half of their levels in uncontaminated control areas (Møller, Surai & Mousseau 2005), and this has consequences for the plumage coloration of migratory barn swallows developed several months after leaving the breeding grounds (Camplani, Saino & Møller 1999). A difference in antioxidant levels between contaminated and control areas may arise from a difference in use of antioxidants, availability or both. Given that there was a sex difference in liver carotenoid levels, but not in levels of other antioxidants, between Chernobyl and control areas (Møller, Surai & Mousseau 2005), we suggest that the alternative explanation, that availability differs among areas, is less likely. If availability had differed, we would expect a difference in antioxidant levels of both sexes.

Both migration distance and dispersal distance independently explained variation in population size of breeding birds with level of radiation, although only dispersal distance remained a significant predictor in analyses of standardized linear contrasts. Møller *et al*. (2006) analysed source–sink dynamics of barn swallows in Ukraine before and after the Chernobyl disaster. Because rates of reproduction and adult survival of barn swallows in contaminated areas do not allow the maintenance of a stable population (Møller *et al*. 2005), the population can only be maintained by immigration from source populations from neighbouring areas that are not contaminated. Consistent with this scenario, Møller *et al*. (2006) found that the variance in stable isotope profiles increased significantly for birds from Chernobyl after the disaster in 1986, while there was no difference between Chernobyl and the control areas before 1986. These findings suggest that Chernobyl populations after 1986 contain more individuals that have immigrated from far away than the same population before 1986. Such a difference is not present in uncontaminated control populations. These differences may arise from the source–sink dynamics of the populations, as suggested by Møller *et al*. (2006). Dispersal may be costly for different reasons, but first and foremost because an individual enters a novel site where enemies including parasites are unknown. The ability to fend off parasites will depend on the ability to produce efficient immune responses to novel antigens (Møller & Erritzøe 2001), and this ability will depend on the availability of immunostimulants such as carotenoids (Møller *et al*. 2000) but also antioxidants such as carotenoids and vitamins A and E (Møller *et al*. 2000). Such biochemicals are in short supply in the Chernobyl region (Møller, Surai & Mousseau 2005; A. P. Møller *et al*., unpublished data), suggesting that any individual from Chernobyl will be at a selective disadvantage during dispersal. The negative relationship between susceptibility to radiation and dispersal distance may arise as a consequence of this effect.

Bird eggs contain large amounts of antioxidants such as vitamin A and E, but also carotenoids that give egg yolks their characteristic colour (Blount, Houston & Møller 2000). Such antioxidants of maternal origin provide important protection against free radicals of developing embryos before, during and after hatching (Surai, Speake & Sparks 2001; Surai 2002). We found that the relative egg mass of different bird species accounted for a significant amount of interspecific variation in the relationship between abundance and level of radiation, although that effect did not reach the level of statistical significance in a phylogenetic analysis of standardized linear contrasts. In contrast, there was no similar effect observed for age at first reproduction, clutch size, maximum number of clutches per year or adult survival rate. Therefore something specific about relative egg mass, reflecting the rate of production of eggs, rather than the total production of eggs, as reflected by clutch size or the total number of clutches produced per year, accounts for this observation. Birds typically invest large amounts of antioxidants into egg production, with the total amount of antioxidants allocated to the eggs of a clutch exceeding the total body store of these biochemicals several times (Blount *et al*. 2003; Biard, Surai & Møller 2006). Thus females have to ingest sufficient amounts of high-quality food to be able to produce eggs with normal levels of antioxidants. If levels of circulating and stored antioxidants are severely depressed in birds exposed to radiation, as shown for the barn swallow (Møller, Surai & Mousseau 2005), production of relatively large eggs for a given body size will impose even greater restrictions on antioxidant availability. For example, Blount *et al*. (2003) showed that access to carotenoids by gulls, even in uncontaminated areas, improved egg-laying ability and, by implication, female birds in heavily contaminated areas will suffer even greater carotenoid depletion, especially if laying relatively large eggs for their body size.

Body mass predicted decline in abundance as a result of radiation, with large-sized species showing smaller declines than small species. Small species of birds have relatively higher rates of metabolism than large species (Schmidt-Nielsen 1984), implying greater production of free radicals during ordinary metabolism. Relatively smaller storage of antioxidants by small species will render such species more susceptible to the effects of antioxidant shortage, particularly if encountering radiation levels that will increase the use of antioxidants. Furthermore, large species generally have much greater home ranges than smaller species, and this implies that large species will encounter a greater heterogeneity in levels of radiation than small species. Given that there is enormous heterogeneity in levels of radiation around Chernobyl (Shestopalov 1996), large species will more often have relatively uncontaminated areas within their home range than small species.

The slight differences in conclusions from analyses of species-specific data and standardized linear contrasts merit some discussion. For example, we found that migration distance explained interspecific variation in the relationship between abundance and background radiation level, while that was not the case in an analysis of contrasts (Table 2). This implies that migratory birds, in particular long-distance migrants, showed the strongest declines in abundance with increasing levels of radiation. However, the analysis of contrasts revealed that this relationship was not caused by evolutionary differences in migration distance among taxa, but by specific clades having long-distance migration and declines in population size with increasing level of radiation. This implies that migration distance is indeed a predictor of susceptibility to radiation, albeit not an evolutionary one.

There are several implications of this study. First, we would expect individuals of bird species with carotenoid-based plumage to have paler plumage colour in Chernobyl than individuals from control sites in Ukraine. Secondly, we would predict species of birds with long-distance dispersal to have severely depleted antioxidant levels in Chernobyl, but not in control sites, after the period when antioxidant levels are usually replenished for migrants. Thirdly, we would expect antioxidant levels of eggs laid by birds in Chernobyl to be reduced compared with eggs from control sites. Indeed, Møller, Surai & Mousseau (2005) found dramatically reduced antioxidant levels in barn swallow eggs from Chernobyl. Alternatively, because antioxidant availability can limit egg production in birds (Blount *et al*. 2003), we suggest that a reduction in egg size is one way by which birds from highly contaminated areas can maintain clutch size at a lower availability of antioxidants. Another way is to reduce clutch size, as already reported for the barn swallow from Chernobyl (Møller *et al*. 2005).

While the present study has implications for the study of animals living in radioactively contaminated areas such as Chernobyl and several test sites used for exploding nuclear bombs, it may also have important implications for animals elsewhere. There is large variation in natural levels of radioactivity as a result of variation in abundance of radioactive isotopes, mainly in mountain regions where the underlying rock reaches the surface. There are no studies of the biological consequences of such variation in natural levels of radioactivity, but we suggest that some of the consequences can be predicted from the present study.

In conclusion, we have shown that bird species are affected by radiation to a different extent, as reflected by their relationship between abundance and the level of radiation. Different characteristics of birds linked to the use of antioxidants accounted for this variation: carotenoid-based plumage, dispersal distance and, to some extent, egg mass. These findings suggest that antioxidants play a key role in determining interspecific variation in the ecological consequences of radiation.

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## Supplementary material

The following supplementary material is available for this article.

**Appendix S1.** Information on slope of the relationship between abundance and radiation, total abundance, dispersal distance, migration distance, carotenoid- and melanin-based coloration, egg mass, and body mass of 57 species of breeding birds from forests near Chernobyl.

**Fig. S1.** Phylogenetic relationships among species breeding in the Chernobyl region and used for analyses of factors predicting the association between abundance and level of background radiation.

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