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# High frequency of albinism and tumours in free-living birds around Chernobyl

### 3 Q1 A.P. Møller<sup>a,\*</sup>, A. Bonisoli-Alquati<sup>b</sup>, T.A. Mousseau<sup>b</sup>

<sup>a</sup> Laboratoire d'Ecologie, Systématique et Evolution, CNRS UMR 8079, Université Paris-Sud, Bâtiment 362, F-91405 Orsay Cedex, France
<sup>b</sup> Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

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#### ABSTRACT

The effects of radioactive contamination on the phenotype of free-living organisms are poorly understood, mainly because of the difficulty of capturing the large numbers of individual specimens that are required to quantify rare events such as albinism and tumour formation. We hypothesized that the frequency of abnormalities like albinism and the frequency of radiation-induced diseases like cancer would increase with the level of background radiation, that the two markers of radiation would be positively correlated, and that the reduction in abundance of animals would be greater in species with a higher frequency of albinism and tumour formation, if these markers reliably reflected poor viability. Here we analyzed the frequency of albinistic feathers and tumours in a sample of 1669 birds captured during 2010–2012 at eight sites around Chernobyl that varied in level of background radiation from 0.02 to more than 200 µSv/h. We recorded 111 cases of partial albinism and 25 cases of tumour formation. Nominal logistic models were used to partition the variance into components due to species and background radiation. Radiation was a strong predictor of the two markers in birds, with a small, but significant effect of species for albinism. The slope of the relationship between abundance and radiation in different bird species was significantly inversely correlated with the frequency of albinism and tumours, as was to be expected if a common underlying cause (i.e. radiation) affects both variables. These findings are consistent with the hypothesis that background radiation is a cause of albinism and tumours, that albinism and tumours are biomarkers of radiation exposure, and that high frequencies of albinism and tumours were present despite the low viability of birds with these conditions.

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#### 21 **1. Introduction**

Radioactive contamination is known to increase mutation rates 22 with significant effects on phenotype [1,2]. A recent review sug-23 gests that natural populations may be almost an order of magnitude 24 more sensitive to ionizing radiation than previously predicted by 25 laboratory models [3], implying that natural variation in back-26 ground radiation [4], but also low-dose radioactive contamination 27 from Chernobyl and other nuclear accidents may cause significant 28 changes in the appearance of animals and other organisms [2]. 29 Abnormalities are disproportionately frequent in radioactively con-30 taminated areas [2,5–10]. For example, for barn swallows Hirundo 31 rustica from Chernobyl, Møller [7] reported abnormal barbs in 32 33 feathers, which prevented these feathers from fusing normally. This novel condition was associated with a significant delay in repro-34 duction, suggesting that the condition had significant fitness costs. 35 Likewise Hesse-Honegger and Walliman [10] observed elevated

1383-5718/\$ - see front matter © 2013 Elsevier B.V. All rights reserved. http://dx.doi.org/10.1016/j.mrgentox.2013.04.019 levels of abnormalities in bugs from contaminated areas, including the vicinity nuclear power plants. We have documented this abnormality in several bird species in 2012, more than 25 years after the Chernobyl disaster. Furthermore, Møller et al. [8] reported elevated frequencies of ten different kinds of abnormalities in barn swallows from Chernobyl compared with frequencies in four local and more distant control populations. These abnormalities included changes in patterns of colouration, morphology and shape of feathers, and malformed or missing digits, beaks and eyes. Such abnormalities were rare or completely absent in control populations despite very large sample sizes, nor have they been described in the extensive literature on this species anywhere throughout its range [8,9].

Albinism, the result of absence of melanin pigments from tissue, occurs with a frequency that is typically extremely low in free-living organisms (*e.g.*, [11,12]). In particular small and inbred populations show an increased frequency of albinism in accordance with the expectation that albinism is caused by a recessive allele [13–17]. Barn swallows from around Chernobyl show a highly elevated frequency of partial albinism reaching 13–15%. This albinism is of germ-line origin as determined from a significant parentoffspring resemblance, and barn swallows with this condition

<sup>\*</sup> Corresponding author. Tel.: +33 1 69 15 56 88; fax: +33 1 69 15 56 96. *E-mail address:* anders.moller@u-psud.fr (A.P. Møller).

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Fig. 1. Location of the eight study sites in relation to background radiation level (Cu/km<sup>2</sup>) around Chernobyl, Ukraine.

suffer from reduced survival prospects [18]. This was also found 58 in another study of birds [19]. A disproportionate fraction of partial 59 albinism in the barn swallow occurs in the facial red plumage, but 60 hardly any is seen in the dark blue plumage of the neck, back, wings 61 and tail [20,21]. Individual barn swallows with partially albinistic 62 plumage had on average lower mean phenotypic values than other 63 64 individuals [21], as also reported for the hooded crow, another bird species [19]. 65

A fraction of invasive cancers in humans is caused by radiation 66 exposure, which includes non-ionizing radiation and in particular 67 ionizing radiation (e.g., [22,23]). Natural variation in background 68 radiation due to radon and other radioactive elements has a sig-69 nificant impact on the incidence of cancer in humans [4,24]. The 70 incidence of all cancers caused by ionizing radiation increases with 71 effective dose [25]. Many kinds of cancer often take a long time to 72 develop (i.e. they have a long latency period), implying that long-73 term effects on cancer incidence from radioactive contamination 74 from Chernobyl are not yet detectable. Serdiuk et al. [26] and Cardis 75 and Hatch [27] reviewed the extensive evidence for other can-76 cers being linked to radiation due to the Chernobyl catastrophe. 77 However, next to no information exists on cancers in free-living 78 vertebrates, although their short generation time would provide 79 insights into long-term effects that cannot yet be studied in humans 80 81**Q2** (Fig. 1).

The objectives of this study were to determine (1) the relationship between the incidence of albinism and tumours, respectively, and background radiation, using a large database of free-living birds captured around Chernobyl during 2010–2012. In other words, we intended to assess the reliability of the rate of albinism and the incidence of tumours as biomarkers of species decline due to radiation exposure. Because the lifespan of most free-living animals with abnormalities is short [8,18,21] due to elevated risk of predation, field estimates of prevalence of abnormalities are by definition conservative. If mortality related to abnormalities tumours were a major cause of disappearance of individual animals from free-living populations, we should expect that a smaller number of individuals remained in contaminated areas in species with a high frequency of abnormalities. Therefore, the second objective of this study was (2) to investigate the relationship between the slope of the relationship between abundance and background radiation for different species of birds and the relative frequency of albinism and tumours.

#### 2. Materials and methods

#### 2.1. Study areas

We captured birds in mist nets at eight sites around Chernobyl in the period 25 May–5 June during the years 2010–2012 [28,29]. We used a total of 35–45 mist nets each 12 m long during two consecutive days at each of the study sites (i.e. one

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evening and one morning capture session). All birds were marked with a numbered
aluminium band for individual identification, and they were subsequently sexed and
aged according to standard criteria [30], measured, sampled for blood and released
after sperm collection. As a control sample from uncontaminated areas in Europe,
the first author (APM) captured 35,578 birds in mist nets near Brønderslev, Denmark,
during 1969–2012.

#### 110 2.2. Frequency of albinism and tumours in birds

Upon capture all individual birds were examined by APM for the presence of deviations from the normal phenotype by careful examination, while recording twenty morphological measurements and three measures of abundance of parasites on each individual [31]. The overall procedure lasted 5–6 min per bird and was similar at all study sites.

*Partial albinism* refers to the presence of single or a few white feathers in the
otherwise reddish brown or dark blue plumage [21]. This trait is maintained across
moults in adults [21].

*Tumours* were recorded as externally clearly visible lumps of hard tissue (with a diameter of more than 0.5 cm) on the outside of the body. These structures included papillomas on the legs as shown in Fig. 2.

APM likewise recorded cases of albinism and tumours in the sample of birds from Denmark with exactly the same procedures. We provide summary statistics for the frequency of abnormalities and tumours in each species, and the slopes of the relationships between abundance and background radiation for each species is given in Electronic Supplementary Material Table S1.

Supplementary data associated with this article can be found, in the online
version, at http://dx.doi.org/10.1016/j.mrgentox.2013.04.019.

#### 129 2.3. Breeding bird censuses

The point-count census method provides reliable information on relative abun-130 131 dance of birds [32–35]. It consists of counts over a 5-min period during which the number of birds seen or heard is recorded. APM conducted these standard point-132 counts during May-June in the years 2006-2009 in the Chernobyl area. The fact that 133 one person made all counts eliminates any variance in results due to inter-observer 134 variability. The counts were made approximately on the same dates and at the same 135 136 time of the day during each of the four years. Møller and Mousseau [34] provide further information on the methods and potentially confounding variables that were 137 taken into account in the analyses. We have previously tested the reliability of our 138 139 counts by having two persons independently perform the counts, and the degree of 140 consistency was high, both for species richness and abundance (details are reported 141 by Møller and Mousseau [34]).

#### 142 2.4. Background radiation

We measured radiation in the field and cross-validated these measurements 143 with those reported by the Ukrainian Ministry of Emergencies. Once having finished 144 the 5-min point-count we measured radiation levels with a hand-held dosimeter 145 (Model: Inspector, SE International, Inc., Summertown, TN, USA), directly in the 146 147 field at ground level at each point where we censused birds. We measured the radiation 2-3 times at each site and averaged the measurements. We cross-validated 148 our measurements against governmental measurements published by Shestopalov 149 150 [36], estimated as the mid-point of the ranges published. This analysis revealed a very strong positive relationship (linear regression on log-log transformed data: 151 152 F = 1546.49, d.f. = 1,252,  $r^2 = 0.86$ , P < 0.0001, slope (SE) = 1.28 (0.10)), suggesting that 153 our field estimates of radiation provided reliable measurements of levels of radiation among sites. 154

Handheld Geiger-counters are likely to provide reliable measures of background
contamination levels of radionuclides for gamma sources, and to a lesser degree for
beta emitters when the Geiger detector is in close proximity to the source. However,
characterization of alpha emitters usually requires more complex methods that are
usually only tractable in a laboratory setting due to the short transmission distance
of alpha particles in air.

Given the different characteristics of radionuclides in the environment around 161 Chernobyl, field measurements of contaminant levels are likely to underestimate 162 biologically relevant radiation levels when the main exposure pathway is via 163 ingestion. Similarly, measurements of background radiation in the areas around 164 Chernobyl closest to the reactor (e.g., the Red Forest) very likely underestimate 165 biologically relevant doses, given the abundance of alpha-emitting actinides (e.g., 166 plutonium isotopes) that were differentially deposited in this area. That said, 167 168 Gashchak et al. [37] previously reported that the internal dose of small birds 169 captured in Chernobyl is strongly positively correlated with the external dose, accounting for more than 50% of the variance in internal dose. This would sug-170 171 gest that measurements of the gamma radiation are a reasonable proxy for total 172 exposure, at least for the type of comparative analysis reported here, and that 173 results will be conservative with respect to any reported association with radiation 174 levels.

#### 2.5. Statistical analyses

Using Generalized Linear Models, we previously estimated the slope of the relationship between abundance and background radiation with log10-transformed abundance for each species at each observation point and log<sub>10</sub>-transformed radiation level for each species [38]. We used nominal logistic regression to relate the incidence of albinism and tumours to background radiation level after inclusion of species as a factor. These two models showed no significant lack of fit. We subsequently related the slope of the relationship between abundance and background radiation to the squareroot-arcsine-transformed prevalence of albinism and tumours in different species of birds, weighing each species estimate by sample size. Models were weighed by sample size (the number of individuals) to account for the fact that sample sizes differed and hence the precision of the estimate differed among species. Most statistical analyses assume that data points provide equally precise information about the deterministic part of total process variation, i.e. the standard deviation of the error term is constant over all values of the predictor variable [39]. Garamszegi and Møller [40] showed that bias due to variation in sample size is a serious problem in comparative analyses, similar to considering species as statistically independent observation. If this assumption of even sampling effort is violated, weighing each observation by sampling effort allows the use of all data. giving each data point a weight that reflects its degree of precision due to the sampling effort [40-42]. This procedure will also allow both rare and common species to be included and hence avoid any bias in sampling due to rarity. All analyses were made with IMP version 10.0 [43].

#### 3. Results

We recorded in total 111 cases of albinism and 25 cases of tumours in 1669 birds captured in the surroundings of Chernobyl, or 66.5 cases of albinism and 15.0 cases of tumours per 1000 birds. This compared with rates of 8.9 cases of albinism and no cases of tumours per 1000 birds in Denmark (N = 35,578 individual birds). The mean relative frequency of albinism across all species in Chernobyl was 0.066 (SE = 0.008), N = 63 species, while mean relative frequency of albinism was 0.102 (SE = 0.014) when only considering the 13 species with at least one case of albinism. Likewise, the relative frequency of tumours was 0.015 (SE = 0.003) for all species, but 0.025 (0.008) in the 10 species with at least one case. A Kendall rank-order correlation analysis showed a weak, but significant positive correlation between the presence of albinism and tumours in the 1669 individual birds from Chernobyl (Kendall  $\tau = 0.086, P = 0.0005$ ). There was also a significant, positive relationship between prevalence of albinism and prevalence of tumours across species of birds in an analysis with species as observations weighed by sample size (*F*=34.42, d.f.=1.61, *r*<sup>2</sup>=0.36, *P*<0.0001, slope (SE) = 1.153 (0.197)).

Therefore, we proceeded by investigating the relationship between each of the two kinds of abnormality and background radiation, while taking the non-independence of individuals among species into account by including species as a factor. There was a significant positive effect of radiation on the occurrence of both albinism and tumours (Fig. 2(A and B) and Table 1). In addition, there was a significant species effect in the analysis of albinism (Table 1). Odds ratios and their 95% confidence intervals are reported in Table 1. Unit odds ratios per unit change in radiation were 0.738 (95% CI 0.624, 0.867) for partial albinism and 0.486 (95% CI 0.310, 0.713) for tumours.

If background radiation were the underlying cause of the increase in abnormalities and the reduction in abundance, we should expect a negative relationship between the slope describing the intra-specific relationship between abundance and radiation and the relative frequency of the two kinds of abnormality (i.e. the species most affected by radiation are also more prone to abnormality and cancer). The mean slope for the 63 species was -0.062 (0.010), differing significantly from zero (one-sample *t*-test, *t* = -6.21, d.f. = 62, *P* < 0.0001). Indeed, the slopes for different species were negatively related to relative frequency of albinism in a model weighed by sample size (Fig. 3A; *F* = 11.13, d.f. = 1.61,  $r^2 = 0.15$ , *P* = 0.0015, slope (SE) = -0.124 (0.037)). Likewise, slopes for different species were negatively related to relative frequency

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**Fig. 2.** Birds with albinistic feathers and tumours from around Chernobyl as indicated by the arrows. (a) barn swallow *Hirundo rustica* with a normal plumage and no tumours; (b) barn swallow with albinistic feathers, (c)-(h) are different species of birds with albinistic feathers, while (i)-(x) are different species of birds with solid tumours clearly visible from the outside.

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#### Table 1

Nominal logistic regression models of albinism and tumours in relation to background radiation and species.  $R^2$  was 0.20 and 0.06 for the two models. Odds ratios and their 95% confidence interval are also shown.

Variable	Chi-square	d.f.	Р	Estimate (SE)	Odds ratio	95% CI for odds ratio
Albinism						
Species	146.97	61	< 0.0001			
Radiation	33.82	1	< 0.0001	0.660 (0.120)	0.309	0.162, 0.577
Tumours						
Radiation	15.06	1	0.0001	0.722 (0.210)	0.061	0.011, 0.271

of tumours in a model weighed by sample size (Fig. 3B; F = 10.92, d.f. = 1.61,  $r^2 = 0.15$ , P = 0.0016, slope (SE) = -0.923 (0.279)). Exclusion of the single species with an extremely high estimate of frequency of tumours (hoopoe *Upupa epops*) did not change the conclusion (F = 14.75, d.f. = 1.60,  $r^2 = 0.20$ , P = 0.0003, slope (SE) = -0.274 (0.071)).

#### 4. Discussion

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The main findings of this study of albinism and tumours in freeliving birds in the Chernobyl region were that (1) albinism and tumours were more common than previously reported, (2) the frequency differed among species, (3) the frequency increased with



Fig. 3. Occurrence of (A) albinism and (B) tumours in individual birds in relation to background radiation level ( $\mu$ Sv/h). The individual observations are shown with regression lines and 95% confidence intervals.

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the level of background radiation, and (4) species with the most severely depressed populations in contaminated areas were also the species that had the highest frequency of albinism and tumours. These findings have important implications for estimation of the frequency of abnormalities, but also for their consequences at the population level.

Albinism is a rare condition in free-living animals [14], the high-250 est frequencies being reported for island or marginal populations 260 [16,17]. Because it is likely that albinism has a recessive genetic 261 basis, we should expect to find more cases in populations with sig-262 nificant inbreeding. Here we have shown that the high frequency 263 of ca. 15% albinism previously reported in populations of barn 264 swallows around Chernobyl [21] can be generalized to other bird 265 species. The mean frequency was 6.6% of all individuals across all 266 species of birds, in comparison with a frequency of 0.9% in birds 267 from uncontaminated areas in Denmark. For the barn swallow we 268 have previously shown, by use of historical records, that the preva-269 lence of partial albinism was lower before the Chernobyl accident 270 [21]. Several studies have suggested that albinism is associated 271 with significantly reduced viability [18,19,21]. Therefore, albinism 272 is likely to be under-represented in our study because albinistic 273 274 animals are more prone to mortality before being captured than birds with normal plumage. We have also shown that the preva-275 lence of albinism in different bird species is a significant predictor 276 of the impact of background radiation on population size in the sur-277 roundings of Chernobyl. Møller et al. [29] reported for contamined 278 areas around Chernobyl severely reduced survival rates of the same 279 bird species that we have studied here (Fig. 4). 280

Ionizing radiation is a significant cause of cancer in humans 281 (see, e.g., [22,23,27]), and even natural variation in background 282 radiation due to radon and other isotopes has a significant impact 283 on the incidence of human cancer [4,24]. Cancer often takes long 284 time to develop, implying that long-term radiation effects of Cher-285 nobyl in terms of cancer may not yet be detectable. An exception 286 is thyroid cancer, because it appeared early after the Chernobyl 287 288 accident in 1986 due to exposure to radioactive iodine that was released in large amounts, but only has a short half-life of less 289 than ten days [27]. There is almost no information available on 290 radiation-induced cancers in free-living organisms, although their 291 short generation time would provide insights into long-term effects 292 293 that are not yet detectable for humans. Tumours need less time to reach a detectable or a lethal size in smaller organisms [44]. 294 Here we have shown a frequency of 1.5% of birds having tumours 295 in the neighbourhood of Chernobyl, while not one single tumour 296 case was recorded in a much larger sample of birds captured in 297 uncontaminated areas in Denmark. Thus the frequency of tumours 298 in birds is elevated in Chernobyl. The true frequency in the popula-299 tion will depend on the viability cost of the tumours. If we assume 300 that tumours result in premature death in wild birds, then the true 301 prevalence must be significantly higher than the 1.5% recorded in 302 our study. We can also expect that the relationship between the 303 slope of the relation between abundance and radiation in differ-304 ent species of birds and the prevalence of tumours constitutes an 305 under-estimate of the true relationship due to differential mortal-306 ity of individual birds with tumours. Finally, we have shown that 307 the frequency of albinism and the frequency of tumours were pos-308 itively correlated across individual animals but also across species. 309 Again, this is what would be expected if the two conditions have a 310 common underlying cause. 311

There was evidence of an increase in the frequency of albinism and tumours with increasing background radiation in birds. We have previously reported that the prevalence of albinism in the barn swallow increases with the level of background radiation, and that uncontaminated control populations of birds in Ukraine and elsewhere in Europe have low frequencies of albinism [7,8,18]. The frequency of tumours increased with the level of background



**Fig. 4.** Slope of the relationship between abundance and radiation ( $\mu$ Sv/h) and frequency of (A) albinism and (B) tumours in different species of birds. The estimates for different species are shown with circles proportional to the sample size.

radiation, accounting for a small, but significant amount of variance. Small birds typically have a generation time of two years or less, implying that the 26 years since the Chernobyl disaster equals 13 generations or more. In contrast, this period only equals a single generation in humans. The high frequencies of albinism and tumours reported here for birds may be attributed to accumulation of mutations over time, high metabolic rates resulting in high rates of ingestion of radionuclides, genomic instability or a combination of these factors. Mutations may accumulate across generations with consequences for the development of diseases, including cancer [26,45]. Likewise, genomic instability caused by radiation can render genomes unstable across generations with accumulating effects [45-47]. Either way, the findings reported in this study suggest that the accumulation of effects of radiation recorded in birds is significant, and they suggest that similar accumulation across generations may cause an increase in the frequency of tumours and other medical conditions in other classes of animals including humans.

Ellegren et al. [18] reported that the survival prospects of barn swallows with albinism were severely reduced compared with those of birds with normal plumage. If it turned out that the probability of survival is linked to albinism in birds in general, then we may consider the field estimates of albinism to be conservative. Greater risk of mortality in albinistic birds should translate into an under-estimation of the prevalence of albinism. It should also imply that the reduction in abundance of birds at high levels of background radiation should be greater in species with a higher prevalence of albinism (and tumours). Indeed, slopes of the

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relationship between abundance and level of background radiation 346 were significantly negative, as previously reported for birds breed-347 ing around Chernobyl [34,38]. Interestingly, the slopes for different 348 species were inversely related to the relative frequency of albinism 349 and tumours. These relationships must be considered conserva-350 tive due to the disappearance of individual birds with albinism and 351 tumours. 352

Biological indicators of radioactive contamination under field 353 conditions include the abundance of many different taxa of animals 354 [48], the frequency of albinism [5,8,21] and other abnormalities 355 [8], the amount of genetic damage [50], the frequency of abnormal 356 sperm [51], impaired sperm movement [52] and reduced repro-357 ductive variables and survival rate of birds [53]. It is important to 358 cross-validate such indicators as a test of their reliability, as already 359 provided for censuses of different animal taxa [48]. Here we have 360 shown that the occurrence of albinism and tumours in birds are 361 weakly positively correlated among individuals, but also among 362 species. In addition, we have shown that the frequency of albinism 363 and tumours in different bird species is inversely correlated with 364 the reduction in abundance of different species of birds at high lev-365 els of radiation. These findings provide important cross-validation 366 for different indicators of radioactive contamination. Future studies 367 will investigate whether particular ecological and life-history traits 368 predict variation across species in frequency of tumours under 369 conditions of radioactive contamination, similarly to what has pre-370 viously been done for data on population trends of different bird 371 species [34]. For example, rapid growth rate and large body size can 372 373 positively predict cancer incidence, and selection of the capacity to fight cancer - also called cancer selection - may well vary with the 374 life history of different animal species [44]. 375

In conclusion, we have shown that albinism and tumour for-376 mation are frequent in many bird species in the surroundings of 377 Chernobyl; that the frequency increases with background radia-378 tion level; and that species showing the most severe decrease in 379 abundance are also the species with higher frequency of albinism 380 and tumours. These estimates exceed the frequencies reported for 381 humans and suggest that albinism and tumours may accumulate 382 in frequency over time. 383

#### **Conflict of interest statement** 384

The authors declare that there is no conflict of interest. 385

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- [49]. 387

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