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IDEAS & PERSPECTIVES

Natural radioactivity can explain clinal variation in the expression of melanin-based traits

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Abstract A recent study shows that the expression of pheomelanin-based coloration in barn owls follows a continuous gradient across Europe as a result of local adaptation. The selective pressures that promote local adaptation remain, however, unknown. Here we hypothesize and test that natural radioactivity levels follow a similar spatial gradient to that of pheomelanin-based color in Europe and thus represents a potential selective pressure. The rationale is that the production of pheomelanin consumes glutathione (GSH), a key intracellular antioxidant, and that GSH is particularly susceptible to ionizing radiation, which depletes antioxidants. As predicted, the intensity of pheomelanin-based coloration in 18 populations of barn owls was negatively associated with terrestrial γ -dose rates across Europe. Therefore, we propose that natural selection acts against barn owls that present the molecular basis to produce large amounts of pheomelanin in those populations that are exposed to high levels of natural radioactivity, as in these populations individuals would require higher antioxidant resources to combat oxidative stress. This is the first time that natural radioactivity levels are related to the expression of a phenotypic trait.

Keywords Feather melanin \cdot Glutathione \cdot Natural selection \cdot Oxidative stress \cdot Pheomelanin \cdot Terrestrial γ -dose rates

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Introduction

Responses to ionizing radiation may seem of little relevance to biology under natural conditions. However, natural levels of radiation are highly variable in space and time (e.g., Bristow 1978; Sanderson et al. 2001; Szegvary et al. 2007), suggesting that they may have important consequences for evolutionary processes (Cordeiro et al. 1973; Forster et al. 2002). Ionizing radiation produces free radicals and thus promotes the generation of oxidative stress (i.e. the imbalance between the production of reactive oxygen and nitrogen species and the state of the antioxidant and repair machinery) and depletes antioxidant levels in animals (Riley 1994; Neyfakh et al. 1998). Therefore, natural radioactivity may be involved in the evolution of phenotypes whose expression is affected by oxidative stress.

One of the types of phenotypes that may be affected by natural radioactivity are those generated by melanins, as oxidative stress is intrinsically related to the production of these pigments in vertebrates (Benedetto et al. 1981; Galván and Alonso-Alvarez 2008, 2009; Galván and Solano 2009; Hőrak 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 (Wu et al. 2004), exerts a direct influence on the process. Melanogenesis can lead to either the production of pheomelanin (a pigment generating reddish and brown colors like that present in the breast feathers of barn owls), or the production of eumelanin (a darker form of melanin). Melanocytes (i.e. cells that produce melanin) usually have the capacity to produce both pheo- and eumelanin, but pheomelanogenesis takes place when the levels of cysteine-GSH are high and eumelanogenesis when the levels of cysteine-GSH are low (Ozeki et al. 1997; Galván and Solano 2009). Although GSH metabolism is under genetic control (Soltaninassab et al. 2000), environmental influences on GSH production have been broadly reported (e.g. Congiu et al. 2000; Mateo et al. 2003; Isaksson et al. 2005; Galván and Alonso-Alvarez 2009). Thus, environmental agents that affect GSH levels are potential selective pressures shaping the evolution of melanin-based traits.

Local adaptation can result in clinal variation in the expression of phenotypic traits that are under natural selection, if selective pressures change spatially (Kawecki and Ebert 2004). In their paper "Local adaptation maintains clinal variation in melanin-based coloration of European barn owls (*Tyto alba*)", Antoniazza et al. (2010) have recently shown that the expression of the pheomelanin-based plumage coloration of barn owls varies following a continuous gradient across Europe, resulting in white birds in the populations of the westernmost parts of the continent, and reddish birds in the eastern populations (pheomelanin content in barn owl feathers is positively correlated with color intensity, redder feathers presenting higher amounts of pheomelanin; Roulin et al. 2008). These authors estimated the degree of neutral genetic differentiation between 18 European populations of barn owls by genotypying individual birds at seven polymorphic microsatellite loci, and concluded that the observed clinal variation in pheomelanism is not due to spatially restricted gene flow, but to local adaptation (Antoniazza et al. 2010).

Albeit comprehensive, the study of Antoniazza et al. (2010) left an important question unanswered: what is the selective force promoting local adaptation between populations regarding pheomelanin-based coloration in barn owls? Solving this question is crucial, if the results found by Antoniazza et al. (2010) are wanted to be converted from a spatial pattern into an ecological pattern.

Here we propose natural radioactivity as a factor explaining variability in barn owl pheomelanism across Europe. Importantly, GSH is one of the most susceptible antioxidants to radiation (Bump and Brown 1990; Navarro et al. 1997). Therefore, individual birds from populations exposed to high levels of radiation should present lower GSH levels than individuals from populations exposed to lower radiation levels, which would result in a lower production of pheomelanin in the former as compared to the latter, as pheomelanogenesis requires high GSH levels to proceed (Ozeki et al. 1997). Indeed, a recent study shows that populations of bird species that produce large amounts of pheomelanin suffer more the negative effects of radioactivity at Chernobyl than populations of species producing lower amounts of pheomelanin (Galván et al. 2011). Thus, the intensity of the expression of pheomelanin-based color in barn owls (Antoniazza et al. 2010) should be negatively correlated with natural radiation levels between populations. We test this prediction by using the high-resolution map of terrestrial radiation levels in Europe made by Szegvary et al. (2007), and values of pheomelanin-based plumage color in barn owl populations provided by Antoniazza et al. (2010).

Materials and methods

As a proxy for the expression of pheomelanin-based coloration in barn owls, Antoniazza et al. (2010) obtained the reflectance spectra of breast feathers and calculated the brown chroma, a physical metric that quantifies the contribution of the red part of the reflectance spectrum (600–700 nm) to the entire visible spectrum (300–700 nm). Brown chroma is thus calculated as mean reflectance in the 600–700 nm interval divided by mean reflectance in the entire spectrum. We obtained the mean chroma values per population that were shown by Antoniazza et al. (2010) in the Fig. 3 of their study. For this, we divided the Y-axis of their Fig. 3 in millimetric portions with Adobe Photoshop CS, which allowed us to obtain brown chroma values for males and females in each of the 18 European populations considered by Antoniazza et al. (2010).

To obtain natural radiation levels in the 18 populations of barn owls, we used the maps provided in Fig. 6 of Szegvary et al. (2007). These maps show terrestrial γ -dose rates for summer and winter for Europe obtained from routinely collected data by the EUropean Radiological Data Exchange Platform (EURDEP, http://eurdep.jrc.it) in 2006. Natural terrestrial radiation originates from the γ radiating progenies of the ²³⁸U and ²³²Th decay series and from ⁴⁰K, and its patterns of variation largely correspond to the location of magmatic rocks and to certain characteristics of soils (Asch 2005; Szegvary et al. 2007). Although, to our knowledge, possible effects of terrestrial γ -radiation on physiological parameters have never been investigated in wild populations of animals, high γ -radiation levels have been associated with oxidative damage (Sperati et al. 1999) and increased mutations in humans (Forster et al. 2002). In Europe, terrestrial γ -dose rates vary from more than 170 nSv/h in Spain to less than 30 nSv/h in eastern parts of the continent (Szegvary et al. 2007).

We plotted the 18 locations where barn owl samples were obtained (Figure 1 in Antoniazza et al. 2010) on the Europe maps of terrestrial γ -dose rates made by Szegvary et al. (2007). As barn owl feather samples were collected in nestlings during the breeding season (Antoniazza et al. 2010), we used the γ -dose rate map made by Szegvary et al. (2007) for the summer season. Since coordinates in Szegvary et al. (2007)'s map are in GISCO Lambert, we converted the geographical coordinate of the 18 locations into GISCO Lambert coordinates by using Eye4Software Coordinate Calculator (Eye4Software B.V. 2009–2010), which allowed us to directly plot the 18 locations on Szegvary et al. (2007)'s γ -dose rate map and obtain γ -dose levels for them with a precision of 5 nSv/h from the color scale provided with the map (scale values ranged from 0 to 180 nSv/h). The



Fig. 1 Map showing the average terrestrial γ -dose rates for summer in Europe (coordinates in GISCO Lambert, units in m; redrawn from Szegvary et al. 2007), and the location of the 18 barn owl populations (biggest city in an 80 km radius around the actual sampling area) used by Antoniazza et al. (2010)

plot showing the 18 European populations of barn owls in the map of terrestrial γ -dose rates is provided in Fig. 1.

Possible correlations between barn owl pheomelanin-based plumage color and terrestrial γ -dose rates were tested by means of spatial generalized least squares (GLS) models that incorporate spatial structure in the error term (Selmi and Boulinier 2001), thus accounting for spatial autocorrelation in the variables. Latitude and longitude were used to account for the spatial variation. Different models of spatial structure (spherical, linear, exponential, Gaussian and rational) and the model without spatial structure were tested, and the best fitting model was defined using the Akaike's second order information criterion (AICc) and the model weight. These models were implemented in R using the "nlme" library. Separate models were tested for color values of males, females and the mean color of males and females. The results of the models with all spatial structures are shown in Supplementary Appendix 1.

Results

As predicted, mean pheomelanin-based plumage color for male and female barn owls was negatively correlated with terrestrial γ -dose rates between populations (exponential spatial structure: $b = -3.66 \times 10^{-4}$, t = -2.25, P = 0.039; Fig. 2). The negative correlation



Fig. 2 Relationship between the expression of pheomelanin-based plumage color and terrestrial γ -dose rates in 18 European populations of barn owls. *Solid symbols, continuous line:* females; *hollow symbols, dashed line:* males. The *lines* are the regression lines. Data were obtained from Szegvary et al. (2007) and Antoniazza et al. (2010)

remained when pheomelanin-based color for males and females was analyzed separately, although the association with terrestrial γ -dose rates was marginally non-significant in males (females: exponential spatial structure: $b = -5.62 \times 10^{-4}$, t = -2.76, P = 0.014; males: linear spatial structure: $b = -3.37 \times 10^{-4}$, t = -1.91, P = 0.074; Fig. 2).

A point presented an exceptionally high γ -dose rate (>110 nSv/h; Fig. 1), corresponding to the barn owl population located at Évora, Portugal (Antoniazza et al. 2010). When this point was removed, the negative correlation between pheomelanin-based color and terrestrial γ -dose rates kept significant in the case of females ($b = -5.79 \times 10^{-4}$, t = -2.18, P = 0.045), a non-significant tendency remained in the case of the mean color of males and females ($b = -3.04 \times 10^{-4}$, t = -1.59, P = 0.132), and the effect was not significant in the case of males ($b = 3.33 \times 10^{-5}$, t = 0.17, P = 0.865). However, this point did not represent an statistical outlier on the basis of Cook's distances and leverage values.

Discussion

The geographic cline in pheomelanin-based color expression in barn owls is associated with the geographic cline in terrestrial γ -dose rates. As predicted on the basis that pheomelanogenesis consumes GSH (Ozeki et al. 1997; Meyskens et al. 1999; Galván and Alonso-Alvarez 2009), and that GSH is one of the most susceptible antioxidants to radiation (Bump and Brown 1990; Navarro et al. 1997), the expression of pheomelanin-based coloration was negatively correlated with terrestrial γ -dose rates between populations of barn owls. Therefore, there may be selection against individual barn owls that have the molecular basis to produce large amounts of pheomelanin in those populations located in areas exposed to high doses of natural radioactivity, as in these populations a higher antioxidant capacity would be needed to combat oxidative stress.

Although the heritability of the pheomelanin-based color of barn owls can be high in some populations (Roulin and Dijkstra 2003), the expression of melanin-based coloration has been shown to be highly sensitive to environmental factors such as exposure to prooxidant agents, food abundance and habitat quality in other species of birds (Poston et al. 2005; Fargallo et al. 2007; Dauwe and Eens 2008; Galván and Alonso-Alvarez 2009; Galván et al. 2010). Moreover, the genetic determination of between-population differences in the expression of pheomelanin-based coloration could be modulated by natural selection acting through environmental factors across historical times, even if the permeability of this trait to environmental influences is low (e.g. Price et al. 2003). Thus, future studies should determine whether variation in the expression of pheomelanin-based color in barn owls (Antoniazza et al. 2010) is due to phenotypic plasticity modulated by ionizing radiation, or to genetic differences resulted from evolutionary adaptation to ionizing radiation levels.

Our correlational test will also require measuring GSH levels in barn owls from the 18 European populations considered by Antoniazza et al. (2010), and controlling for other potentially covarying environmental variables, to establish a causational pattern, but the fact that the relationship between pheomelanin-based color expression and terrestrial γ -dose rates was significant and negative as predicted suggests that natural radioactivity has the potential to be an environmental agent that allows to understand which selective pressures are leading to the pattern found by Antoniazza et al. (2010). Natural radioactivity is ubiquitous and has affected to all life forms since the beginning of evolution, so it is possible that natural radioactivity is an overlooked agent determining the course of evolutionary processes (Forster et al. 2002). As far as we know, this is the first time that natural radioactivity levels are related to variability in the expression of a phenotypic trait in any living organism.

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