RESEARCH ARTICLE

Island volcanism predicts pheomelanin-based plumage colouration in a cosmopolitan raptor

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Abstract

Aim: Pheomelanin is a pigment responsible for yellowish-to-reddish colours of vertebrate teguments. Its biosynthesis is favoured under high concentration of intracellular thiols, which, in turn, can depend on the environmental exposure to sulphur. Thus, pheomelanin production should be more intense and frequent in environments characterized by high level of sulphur, such as volcanic regions. In this study, we aimed at addressing this hypothesis by investigating variation in plumage colour of insular populations of the cosmopolitan barn owl (*Tyto alba* species complex) according to the presence of soils of volcanic origin (i.e. andosols) and recent volcanic activity.

Location: World.

Taxon: Barn owl species complex.

Methods: We measured plumage colouration of more than 2000 museum specimens from 50 islands and archipelagos worldwide. We then compared plumage colouration between populations living on volcanic (i.e. where andosols and/or recent volcanic activity are present) and non-volcanic islands/archipelagos.

Results: Consistently with the prediction, plumage colouration is significantly darker (i.e. pheomelanic) on islands/archipelagos where andosols and/or recent volcanic activity are present than absent, although this environmental factor explains a small fraction of plumage colour variability across islands (<10%). Similar results were obtained when specimens' sex and climatic predictors were included in the analyses.

Main Conclusions: Because excessive intracellular levels of thiols can be toxic, pheomelanin synthesis may function as a mechanism keeping these compounds below the toxicity threshold and limiting their detrimental effects on physiology. Darker plumage may also be favoured because it promotes background matching against the dark environment typical of volcanic islands (dense vegetation cover and/ or dark soil). Our results add to the little evidence that the environmental exposure of compounds that are involved in melanin biosynthesis can affect animal pigmentation and suggest that soil composition may be a factor that affects melanogenesis, possibly contributing to generate spatial variation in pheomelanin-based traits in animals.

1 | INTRODUCTION

Colouration can have multiple functions, spanning from conspicuous visual signals evolving under sexual selection regimes to camouflage resulting from predator–prey interactions (Cuthill et al., 2017). Variation in animal colour patterns is therefore the consequences of the action of various, maybe conflicting, selective pressures, often resulting in compromises between such opposing forces (Cuthill et al., 2017). One of the possible consequences of the presence of multiple selection pressures within the same population is the evolution of colour polymorphism as alternative phenotypes can be simultaneously favoured (Cuthill et al., 2017; Roulin, 2004). When distinct selective pressures act in different populations, but also when the same selective pressures vary spatially due to differences in climate, environmental conditions and community composition as well as in breeding behaviour and sexual selection, the presence and/or distribution of alternative colour morphs may differ among populations (Cuthill et al., 2017; Hill & McGraw, 2006). Under such circumstances, polymorphism may vary spatially with populations being differently pigmented along geographical gradients, as shown in several model systems at different scales (Cerezer et al., 2020; Delhey et al., 2019, 2020; Passarotto et al., 2022; Romano et al., 2021b; Romano, Séchaud, et al., 2019).

Such a complex evolutionary scenario is further complicated when the genes responsible for the synthesis of pigments have pleiotropic effects on other behavioural and physiological traits. This is the case of melanins, the pigments conferring reddish-to-black colourations in animals (Ducrest et al., 2008; Roulin & Ducrest, 2011). Indeed, melanin-based traits can have multiple functions, spanning from protection against ultraviolet radiation and mechanical damage to thermoregulation and socio-sexual signalling (Ducrest et al., 2008; Roulin & Ducrest, 2011), but they are also related to other various traits because of pleiotropic effects of genes regulating melanogenesis (Ducrest et al., 2008; Roulin & Ducrest, 2011), possibly resulting in an indirect selection of different pigmentation. A given colour morph (or a given proportion of the colour morphs) could therefore locally emerge as the consequence of selection in favour of other adaptive physiological and or/behavioural traits that are genetically linked to colouration (San-Jose & Roulin, 2018), thus possibly contributing to generate spatial polymorphism.

In a recent study on the cosmopolitan barn owl species complex (*Tyto alba* group), we showed that insular populations display a generally lighter plumage colouration than they mainland counterparts (Romano et al., 2021a), as repeatedly shown in other species (e.g. Fabre et al., 2012; Figuerola & Green, 2000; but see Doutrelant et al., 2016). In addition, we also observed that such a difference between insular and continental plumage colour is particularly marked in large islands/archipelagos, while it almost fully

disappears in small and remote archipelagos (Romano et al., 2021a). We interpreted this finding as a possible by-product of selection of phenotypes that can be adaptive on islands and are concomitantly linked to melanin-based colouration. In the model system studied here, dispersal propensity (Van den Brink et al., 2012) and resistance to various stressful factors (Almasi et al., 2008; Roulin et al., 2011; Roulin & Ducrest, 2011), both traits that can be crucial during island colonization, have been associated with melanin pigmentation, with more melanic individuals being more resistant and disperse further. However, such an interpretation could not explain alone why some very isolated barn owl populations show darker plumage than the corresponding continental one, thus leaving open the possibility that plumage darkening might also have occurred in situ, according to the environmental conditions experienced by single populations.

There are several climatic and environmental factors that are known to potentially select for a given level of plumage pigmentation. Traditionally, darker plumage (but also pelage in mammals) is expected to be more common in humid climates, according to Gloger's rule (e.g. Cerezer et al., 2020; Delhey, 2018; Delhey et al., 2019, 2020; Passarotto et al., 2018; Romano, Séchaud, et al., 2019), because darker individuals can better match the shady background typical of highly vegetated, cloudy and rainy environments and/or because darker feathers are more resistant to the effects of ectoparasites, which are more abundant under humid climatic conditions, and abrasion, which is larger where vegetation cover is dense (Barrowclough & Sibley, 1980; Burtt & Ichida, 2004). In addition, it has been recently suggested that darker colouration may also help endotherms in thermoregulation in cold environments, and this is especially the case for birds (Angelier, 2020; Delhey, 2018; Delhey et al., 2020; Galván et al., 2018; Romano, Séchaud, et al., 2019). However, increasing evidence in different species showed that melanin-based colouration can also result from environmental exposure of compounds that are involved in the biochemical synthesis of melanins (Galván, Jorge, Nielsen, & Møller, 2019; Galván, Jorge, Sánchez-Murillo, & Gutiérrez-Espeleta, 2019; McGraw, 2007; Rodríguez-Martínez et al., 2019).

Melanin pigments are synthesized in specific organelles, the melanosomes, contained in melanocytes. The two main types of melanin pigments present in the soft tissues of vertebrates are called eumelanin, which is responsible for the production of dark colours such as black, dark brown and grey, and pheomelanin, producing yellowish-to-reddish colour shades (Ducrest et al., 2008; Hill & McGraw, 2006; Ito & Wakamatsu, 2008). Differently from eumelanin, pheomelanin contains sulfhydryl groups, which are transferred into the polymer by thiol compounds (Ito & Wakamatsu, 2008; Rodríguez-Martínez et al., 2019). Because pheomelanin production is enhanced under high concentration of thiols (Ito & Wakamatsu, 2008; Rodríguez-Martínez et al., 2019), its biosynthesis may be favoured under high

exposure of environmental sulphur. Environmental sulphur could be adsorbed by organisms in different ways, such as through food and water consumption but also air inhalation and direct skin contact, with possible effects on individual physiology (reviewed by Kuklińska et al., 2013). Therefore, the exposure of high environmental sulphur, occurring for example in volcanic areas, has the potential to promote the production of pheomelanin.

The aim of this study is to specifically test for this hypothesis using insular populations of the barn owl as a model system. The barn owl is a cosmopolitan species complex inhabiting most of the globe, with the exception of very high latitudes (see e.g. Romano et al., 2020; Romano, Séchaud, et al., 2019). Its distribution range includes most of the islands and archipelagos of the world, spanning from Vancouver Island to Falkland Islands in the Americas, from Great Britain to African archipelagos in the Atlantic (e.g. Sao Tomé) and in the Indian (e.g. Comoro) Oceans in the Afro-European region, from Andaman Islands to Tasmania in Australasia (see Romano et al., 2021a for details). In this study, we focused on islands because they host geographically (and genetically) well-defined populations that adapted to the local environmental conditions (Cumer et al., 2022; Machado et al., 2022; see also Losos & Ricklefs, 2009), and because many islands have a volcanic origin, and therefore high sulphur concentration in the soil. Indeed, it is well known that volcanic eruptions are the main natural sources of sulphur with massive emissions of sulphur dioxide in the air and in the soil (ATDSR, 1998). In addition, the barn owl shows a large geographical variation in the colouration of the ventral body part, with populations being dark brownish-reddish and other displaying white and immaculate plumage (see e.g. Romano, Séchaud, et al., 2019; Roulin et al., 2018). The colour of ventral plumage is mainly due to pheomelanin (Roulin et al., 2008; San-Jose et al., 2017). We therefore predicted that in volcanic islands/archipelagos, where the soil contains large sulphur levels, the pheomelanin plumage colouration should be darker (i.e. redder) that in non-volcanic ones.

2 | MATERIALS AND METHODS

2.1 | Museum skin collection and their insular origin

We recorded information on plumage colour of thousands of barn owl skins collected by several museums and private citizens (see Romano et al., 2020, 2021a, 2021b; Romano, Séchaud, et al., 2019). All measures were taken by the same measurer (A. Roulin) blindly about the origin of each individual, and collected specimens cover most of the distribution range of the taxon (see Romano et al., 2020, 2021a, 2021b; Romano, Séchaud, et al., 2019). We only focused on insular populations because islands are areas where populations are geographically (and genetically) isolated, while this is not the case for mainland where different populations can easily mix up through dispersal. This can be very problematic for soil data because, differently from climatic data that usually show a continuous variation along geographical gradients, they can be extremely discontinuous (e.g. an abrupt change in soil type may occur even at the very small spatial scale). In practice, individuals developing in sulphur-rich environments can be easily captured and measured in continental regions without sulphur in the soil (and vice versa), while this is not the case for insular populations.

Barn owls' plumage coloration is entirely due to melanins, as no carotenoids or other pigments were found in their feathers (Roulin et al., 2008). In particular, the ventral region varies from white to dark reddish for a different deposition of pheomelanin, and it can be immaculate or heavily spotted with eumelanic black spots of variable size (Romano, Séchaud, et al., 2019). The degree of red colouration was categorized on an eight-level scale ranging from 1 for dark reddish-brownish to 8 for white. This categorization was used in several previous studies (e.g. Romano et al., 2021a, 2021b; Romano, Séchaud, et al., 2019; Roulin et al., 2018) and has been shown to be highly correlated with reflectance in the visible light when compared to spectrophotometric measures (Dreiss & Roulin, 2010). Moreover, this categorization is correlated with pheomelanin, but not eumelanin (Roulin et al., 2008), thus indicating that it is a good proxy of pheomelanin content.

For each measured specimen, we collected information about its island and/or archipelago of origin. We therefore identified all the isolated islands (e.g. Sicily) or archipelagos (e.g. all the islands composing the Cape Verde archipelago were considered together thus accounting for potential dispersal between nearby islands; Doutrelant et al., 2016; Romano et al., 2021a) for which plumage colouration information was available (hereafter, isolated islands and archipelagos will be defined together as island systems). However, following previous studies (e.g. Doutrelant et al., 2016; Meiri et al., 2006; Romano et al., 2021a), Australia, Madagascar, New Guinea and Sumatra were considered as mainland because of their large size and because they are surrounded by small archipelagos, which were, in turn, considered as independent observations.

A peculiar case concerned Sunda Islands. Using the most comprehensive phylogenetic analysis available on the model system (Uva et al., 2018), such an archipelago was split into three different island systems: (1) owls living in Java and Lesser Sunda Islands (but not Timor archipelago; see below) were pooled together because of common Asian origin and close proximity between each island; (2) owls living in the Timor archipelago were considered alone (see also Romano et al., 2021a) because this population originated from the Australian lineage; and (3) owls living in Sulawesi were considered alone because they might constitute a different species (*T. rosenbergii*), which is limited to this archipelago, and because the large distance between Sulawesi and other islands where barn owls are present (Lesser Sunda at South, New Guinea at East, while the barn owl is not present in Borneo).

In addition, we did not consider as insular individuals all the owls collected on islands located at a distance smaller than 1 km from the continent (see Romano et al., 2021a for details). However, all the analyses provided qualitatively similar results when performed on islands/archipelagos further than 5 km from the coast (details

available upon request to the corresponding authors; see also Romano et al., 2021a).

Considering the huge difference in the number of specimens available for each island/archipelago (3–636 individuals; see also Figure 1), for each island system we then calculated an average score of plumage colouration. From these estimates, we included only island systems for which information of at least three individuals was available (2089 specimens on 50 island systems; Table S1). In addition, because of the large difference in plumage colouration between the sexes within single populations (Romano, Séchaud, et al., 2019; Roulin et al., 2018; San-Jose et al., 2017), but also because the level of sexual dimorphism may vary spatially (Roulin, 2003), we also calculated an average score of plumage colouration per sex per island/archipelago. In such case, we included only island systems for which information of at least two individuals of the same sex was available (1641 specimens on 94 sex-specific data for 55 island systems; Table S1). However, very similar results were obtained using different criteria of data, and therefore island systems, inclusion (e.g. at least 1 male and 1 female or at least 2 males and 2 females per island/archipelago; details not shown). Average plumage colouration and average sex-related plumage colouration were used in two different sets of analyses performed on slightly different samples of island systems (Table S1).

2.2 | Sulphur-rich soil and volcanic activity

As a proxies of sulphur environmental content of each island/archipelago, we used the presence of the macro-category of soil called andosols. Andosols are dark soils with a high humus content, and they are found in volcanic areas because they almost always form from volcanic pyroclastic products of recent age, most often from the Holocene (WRB, 2014). Therefore, andosols are often exposed to long-term volcanogenic and sulphur-rich emissions, and there is evidence that they have a higher sulphur content than less exposed soils (Delfosse et al., 2005). Such information was extracted from the harmonized world soil database of the Food and Agriculture Organization (version 3.6). The FAO Soil Map of the World was published between 1974 and 1978 at 1:5′000′000 scale and it aims to gather and unify information on soil type worldwide (FAO, 1995). For some small islands/archipelagos for which FAO data were lacking, we relied on specific scientific literature. Thus, we extracted information on the presence of andosols for each island/archipelago as a proxy for sulphur content. When the presence of andosols was found on a single island composing an archipelago (or not on all islands), we coded such archipelago as andosol rich, because of possible island hopping from nearby islands. In addition, considering that andosols are the consequences of volcanism, it is possible to assume that volcanic eruptions occurring in an island can also have environmental consequences on nearby islands by material deposition but without forming such soil type. Therefore, we recorded the presence or absence of andosols for each island/archipelago.

However, andosols take a relative long time to form, with the consequence that in some islands where volcano(s) recently erupted may not yet be composed of andosols, but their soils can anyway contain high sulphur levels. Therefore, we also collected information on the presence in each island/archipelago of volcano(s) that was/were active during the Holocene. We chose the Holocene period to ensure that we were not considering excessively old volcanoes whose activity no longer has an impact on the sulphur content of
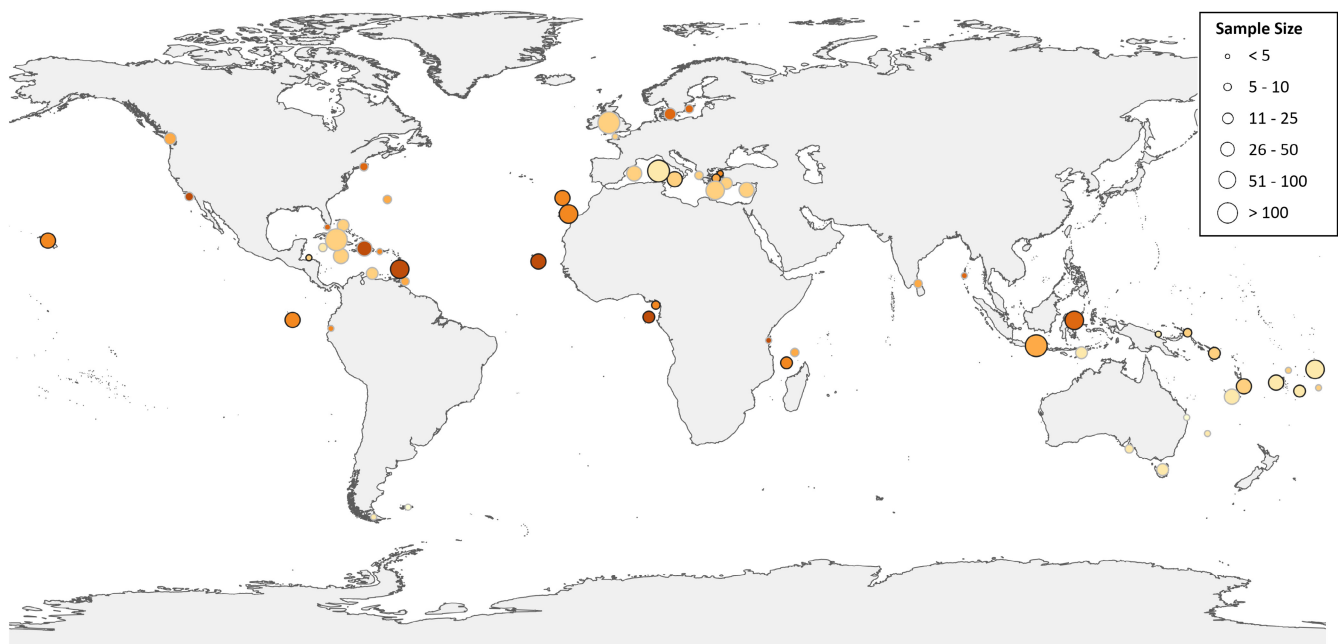

FIGURE 1 Map (equirectangular projection) showing island systems included in the analyses. Dot colours indicate the average ventral plumage colouration among all the individuals collected in each island system. Dots with black border denotes island systems where andosols and/or recent volcanic activity are present.

the soil. Information on volcanic activity was extracted from Global Volcanism Program (2013). Again, the presence of active volcano(s) was coded at the archipelago level, when proper. Considering that these two proxies of environmental sulphur represent two different phases of volcanic activity, we thus generated a novel variable pooling the information on andosols and volcanic eruptions: this variable provided information about whether island/archipelago is characterized by the presence of andosols and/or recent volcanic activity.

We opted to use presence/absence of these proxies because it is very difficult to quantify the proportion insular surface covered by andosols or volcanic dust deposition. We note that such a decision does not represent a problem for small islands/archipelagos, but it might be problematic for very large insular systems (e.g. a very small andosol coverage or a single small volcano on a very large area). However, this is not the case in the sample of islands/archipelagos used here because the very large insular systems (larger than 50′000 km^2) were either andosol free and volcano free (e.g. British Isles, Cuba, Tasmania, Sri Lanka, Hispaniola) or contained a large number of volcanos and large andosol-covered surface (e.g. 62 volcanos on Sunda Islands, 12 volcanos on Sulawesi), thus making these island systems easily includable into either category.

2.3 | Island/archipelago features and climatic variables

Considering that plumage colour traits have been suggested to vary according to different environmental variables and island features, for each island/archipelago we collected some additional information to be included in the analyses. To account for geographical variation in climatic factors, we used information of annual mean temperature and total precipitation, which were previously shown to predict plumage colouration in this model system at the global scale (Romano, Séchaud, et al., 2019). In particular, for each island/archipelago, we averaged temperature and precipitation information recorded in the locations (i.e. latitude–longitude coordinates) where each specimen was collected. Such climatic information was extracted for previous studies from the WordClim data repository (http://www.world clim.org/) for the period 1970–2000 (see Romano, Séchaud, et al., 2019; Romano et al., 2020 for details).

In addition, we also recorded information on surface area and on the shortest distance to the closest continent (including Australia, Madagascar, Sumatra and New Guinea) of each island/archipelago (see Romano et al., 2021a). In our dataset, island/archipelago area varied from 15 to 315,159 km^2, and the distances to the mainland varied between 3 and 3800 km.

2.4 | Statistical analyses

To analyse variation in plumage colouration according to the exposure of environmental sulphur, we relied on linear mixed models with the *lmer* function of the lme4 R package (Bates et al., 2015). We first analysed mean plumage colour of each island system in relation to the presence of andosols and/or recent volcanic activity (1 = present; 0 = absent). In practice, we compared islands/archipelago where at least andosols or volcano is present against those in which these two proxies of sulphur in the soil are absent. To account for possible effects of other environmental variables on ventral plumage, in the model we also included mean annual temperature and total annual precipitation, as well as island/archipelago size (log-transformed) and distance to the closest continent (log-transformed). The model also included two random factors accounting for spatial distribution of the island systems. First, we included the random factor 'evolutionary lineage', indicating if the island system is within the distribution range of *T. alba* (Europe, Middle East and Africa), *T. furcata* (Americas) or *T. javanica* (Southern Asia to Tasmania, including all the archipelagos in the Pacific Oceans). The three taxa are not formally recognized as different species, but they represent three different evolutionary lineages of the same 'common barn owl species complex' (Romano et al., 2020, 2021a, 2021b; Romano, Séchaud, et al., 2019; Uva et al., 2018). In addition, we included a further random factor, accounting for the likely common continental origin of the insular population. This random factor therefore pooled island systems likely originating from the same continental population within each barn owl lineage (e.g. Canary Islands and Madeira, Comoro and Aldabra; see Romano et al., 2021a for details).

The models described above do not account for the sex-related variation in plumage colour. We therefore repeated the model using sex-specific data (i.e. the dependent variable was the mean plumage colour of each sex per island system) as dependent variable, also including an additional random factor 'island system' to account for the non-independence of data of either sex collected on the same island/archipelago. To test for the interaction between sex and the presence of andosols (or the presence of andosols and/or volcanos), we repeated these analyses on the subsample of island systems for which information on both sexes was available.

Some insular populations might constitute different species (*T. glaucops* or *T. f. glaucops* from Hispaniola and Lesser Antilles, and *T. rosenbergii* or *T. j. rosenbergii* from Sulawesi), likely representing speciation events occurred on islands (Uva et al., 2018; see also Romano, Séchaud, et al., 2019; Romano et al., 2020). The analyses were therefore repeated excluding data of these two potential different species.

Given the large difference in sample size across islands/archipelagos included in the dataset, models were repeated by weighting each single datum by a value proportional to the number of specimens available for each island system, and also by a value proportional to the ratio between sample size and island/archipelago size. Both procedures provided very similar results compared to those reported below (details not shown for brevity).

In all the models, continuous covariates were centred to provide better model estimates and single random factors were removed from final models when their explained variance was zero. Marginal and conditional R^2 were calculated with the method implemented by Nakagawa and Schielzeth (2013), using the function *r2_nakagawa*

of the package performance in R (Lüdecke et al., 2021). We also checked for collinearity among the variables included in the models using the package performance in R (Lüdecke et al., 2021). These latter analyses prevented us from including also information of absolute latitude of island/archipelago, which was tested in preliminary explorative models, because it always highly correlated with mean annual temperature (Variance Inflation Factor always larger than 4). However, since most volcanic island systems inhabited by the barn owl are located in the tropical belt, the analyses were repeated replacing mean annual temperature with absolute latitude and hemisphere. These analyses, again, provided qualitatively similar results to those presented below (details not shown).

3 | RESULTS

Ventral plumage colouration was significantly darker in island systems where andosols and/or recent volcanic acidity are present than absent, while no effects of climatic or island variables were observed (Table 1; Figure 2).

This result was confirmed when the analyses were run using sex-specific data (Table S2). In addition, these models did not show any significant effect of the interaction between the presence of andosols and/or volcanos and sex on the sample of island systems where data of both sexes were available (estimate: 0.557; CI: −0.07 – 1.19; $p = 0.08$), thus indicating similar patterns of variation in both sexes.

Finally, results were also qualitatively similar when the analyses were limited to the subsample of insular systems excluding those inhabited by *T. glaucops* (Hispaniola and Lesser Antilles) and *T. rosenbergii* (Sulawesi) (Table S3).

4 | DISCUSSION

In this study, we showed for the first time in any organism a possible link between the environmental presence of sulphur and tegument pigmentation. Indeed, barn owl populations that inhabit island systems characterized of the presence of sulphur-rich soils and volcanic activity show a darker plumage colouration than those living in the absence of volcanic sulphur in the environment. Our results are therefore compatible with the hypothesis that the presence of environmental sulphur may promote the production on pheomelanin during melanogenesis. This may be the case because ventral plumage colouration in our model system is mainly due to the presence of pheomelanin (Roulin et al., 2008), that depends on the level of intracellular thiols (Ito & Wakamatsu, 2008), that, in turn, increases after environmental exposure to sulphur. Although correlative, our study adds to the little and scattered evidence that the environmental exposure of compounds that are involved in the biochemical synthesis of melanins can affect animal pigmentation (Galván, Jorge, Nielsen, & Møller, 2019; Galván, Jorge, Sánchez-Murillo, & Gutiérrez-Espeleta, 2019; McGraw, 2007; Rodríguez-Martínez et al., 2019). In particular, our findings indicate that spatial variation in soil composition may have the potential to promote geographical variation in melanin-based pigmentation. However, we note that the variance explained by our predictors account only for a small fraction of the variability observed in the plumage colour across islands (<10%). This observation thus confirms the complex nature of melanin-based colour polymorphism, which is often the result of many concomitant effects acting together.

Importantly, although the consequences of sulphur exposure have been poorly studied, especially in terrestrial vertebrates, there are general evidence that it can be toxic also to these organisms both per se and through an increase in thiol levels (review in Kuklińska et al., 2013). Increase in dietary elemental sulphur has been shown to provoke weight loss, reduce antioxidant enzymes and increase chromosomal damage in captive mammals, as well as to alter the property of wool fibres in sheep (review in Kuklińska et al., 2013), while an excess of thiols acquired through the diet can induce neurotoxicity and oxidative stress (Dilger & Baker, 2008; Held & Biaglow, 1994; Janáky et al., 2000). Under such circumstances, pheomelanin synthesis might maintain the concentration of thiols in the cells under the toxicity threshold. This would limit their detrimental effects on cellular functioning and thus on organismal physiology (Galván

	Estimate (SE)	CI	p
Intercept	5.588 (0.304)		
Presence of andosols and/or volcanos	−0.881 (0.280)	−1.45 to −0.32	0.003
Annual temperature	0.223 (0.262)	−0.31 to 0.75	0.40
Annual rainfall	0.163 (0.234)	−0.31 to 0.63	0.49
Surface area (log)	0.066 (0.135)	−0.21 to 0.34	0.63
Distance from continent (log)	−0.268 (0.203)	−0.68 to 0.14	0.19
Random effects			
σ^2	0.205 (0.453)		
τ_{00} Common origin	2.834 (1.684)		
ICC = 0.93			
Marginal R^2/Conditional R^2 = 0.077/0.938			

TABLE 1 Linear mixed models showing variation in mean ventral plumage colouration on island systems according to the presence of andosols and/or recent volcanic activity on islands/archipelagos. The model also included mean annual temperature, total annual rainfall, island/archipelago surface and shortest distance to the continent as fixed factors and a random factor pooling island systems originating from the same continental population within each barn owl lineage. The sample size is 50 island systems.
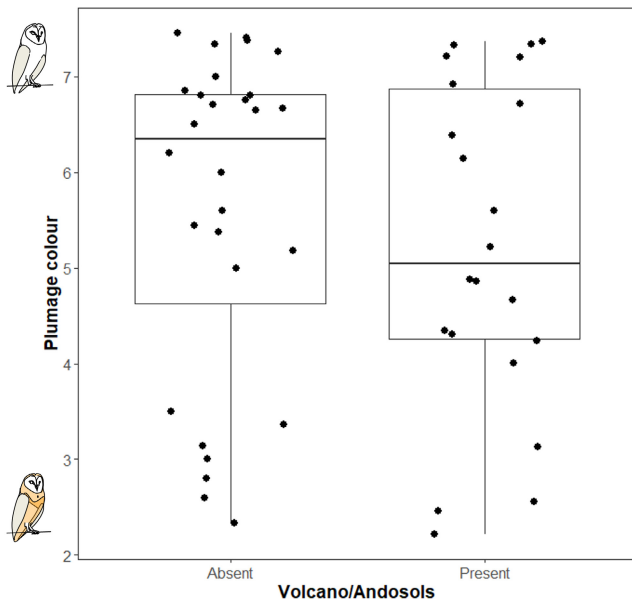
FIGURE 2 Boxplots showing variation in mean ventral plumage colouration on island systems according to the presence of andosols and/or recent volcanic activity on islands/archipelagos. Jittering was applied to show all the overlapping datapoints.

et al., 2012; Rodríguez-Martínez et al., 2019). Biochemical evidence of such a process have been provided on the amino acid cysteine, the main thiol compound involved in pheomelanin synthesis (Ito & Wakamatsu, 2008) that increases oxidative stress when above a certain concentration (Dilger & Baker, 2008; Janáky et al., 2000). For example, birds supplemented with an excess of dietary cysteine show a larger production of pheomelanin in feathers, due to a downregulation of genes involved in cysteine metabolism and transport which is mediated by DNA methylation, without paying a cost in terms of oxidative stress (Rodríguez-Martínez et al., 2019). In practice, pheomelanin may also function as an excretory, detoxifying mechanism to remove the excess of thiols when their systemic concentration is very high.

Such an increase in plumage darkness can also be adaptive because the typical dark colour of andosols would potentially favour camouflage and background matching, thus contributing to increase the fitness of darker individuals on volcanic islands. Moreover, the andosols are typically very fertile soils, thus promoting the growth of abundant and dense vegetation cover (FAO, 1995). This additional factor may, in turn, contribute to positively select for darker phenotypes that should better match the shady environment typical of dense canopy covered environments, as also suggested by Gloger's rule and shown in owls at the global scale (Passarotto et al., 2018, 2022). Though it cannot be a priori discarded, we note that such a latter scenario (i.e. background matching against dark soil and/or dense vegetation) would mainly apply to diurnal and terrestrial species, rather than to nocturnal and aerial organisms like the studied one. Overall, sulphur-rich soils may therefore act both directly, through sulphur assimilation from the environment, and indirectly, through a positive selection for darker phenotypes due to background

matching, to promote the origin and maintenance of darker plumage. Moreover, considering the well-known pleiotropy of the genes responsible for melanin production (Ducrest et al., 2008), it is possible that selection for darker phenotypes on volcanic islands may have also indirectly favoured for other physiological and behavioural traits which can be adaptive on islands and are genetically linked with pigmentation (but not linked to visual detection), such as resistance to a wide suite of stressors (Almasi et al., 2008; Barrowclough & Sibley, 1980; Burtt & Ichida, 2004; Roulin et al., 2011; Roulin & Ducrest, 2011).

Previous studies on other vertebrates showed that pheomelanic pigmentation can emerge in carnivore species as a consequence of high-protein diet (i.e. sulphur-rich diet), for example in case of prey abundance (Galván, Jorge, Nielsen, & Møller, 2019; McGraw, 2007), but also under variation in the composition of dietary amino acids underpinning melanin production (Anderson et al., 2002; Rodríguez-Martínez et al., 2019; Watson et al., 2018). Additional evidence that diet composition can affect plumage pigmentation comes from a comparative study on birds, showing that pheomelanic plumage colouration has evolved more frequently in juveniles of strict carnivore species than on species relying on other diets (Rodríguez-Martínez & Galván, 2020). Moreover, a recent shift from eumelanin (i.e. black) to pheomelanin (i.e. yellow) observed in the pelage colour of some populations of the mantled howler monkey (*Alouatta palliata*) living in Costa Rica was linked to the increasing level of sulphur-based pesticides present in the environment (Galván, Jorge, Sánchez-Murillo, & Gutiérrez-Espeleta, 2019). Thus, increasing evidence is showing that direct exposure to compounds that are directly involved in melanogenesis can affect the type and the level of individual melanin-based pigmentation. Here, we can only speculate about how environmental sulphur can accumulate in the cutaneous melanocytes of a top predator, as the barn owl, a question that remains open by the present study and should be tested using a proper experimental approach. A possibility is that sulphur could be acquired through the diet because of transmission and accumulation of this substance along the food chain (sensu McGraw, 2007; Galván, Jorge, Nielsen, & Møller, 2019). However, whether, and eventually how, sulfhydryl groups can be transferred from different trophic levels has not been investigated to date. Alternatively, but non-mutually exclusive, this compound may be directly adsorbed in other ways, such as water consumption and air inhalation as well as skin contact (Kuklińska et al., 2013).

A further interesting result is that variation in temperature and precipitation seems to have a little, if not negligible, effect on plumage pigmentation of insular barn owl populations. This result contrasts with the previous observation that the spatial distribution of plumage colouration across the globe generally follow Gloger's and Bogert's rules in the model system studied here, with populations living in humid and cold environments being darker than those inhabiting hot and dry regions (Romano, Séchaud, et al., 2019). Such a discrepancy is not fully surprising because it is well known that the evolutionary pressures moulding the phenotypes of insular populations can be very different from those promoting phenotypic variation in continental ones, as repeatedly reported in various taxa (Losos

& Ricklefs, 2009), including the studied one (Cumer et al., 2022; Machado et al., 2022). In addition, the absence of relationships with temperature and rainfall is not surprising also because departures from ecogeographical rules strongly depend on the spatial scale considered. In our analyses, island systems may represent units too small to catch any strong gradient in environmental conditions.

Intra- and inter-population variability in the expression of melanin-based pigmentation can emerge as a consequences of variation in various external variables, spanning from environmental conditions, like rainfall and solar radiation (Angelier, 2020; Cerezer et al., 2020; Delhey, 2018; Delhey et al., 2019; Galván et al., 2018; Romano, Séchaud, et al., 2019), vegetation cover (Delhey, 2018; Passarotto et al., 2022), parasite burden (Burtt & Ichida, 2004; Côte et al., 2018; Romano, Nodari, et al., 2019) and diet (Galván, Jorge, Nielsen, & Møller, 2019; Karell et al., 2021; McGraw, 2007; Rodríguez-Martínez et al., 2019; Rodríguez-Martínez & Galván, 2020; Romano et al., 2021b), to social variables, like mate choice and mate competition in socio-sexual contexts (Cuthill et al., 2017; Jawor & Breitwisch, 2003; McGraw et al., 2003; Safran et al., 2016; Vitousek et al., 2013), but also as a by-product of selection in favour to other traits because of the pleiotropy of the genes underpinning melanin production (Ducrest et al., 2008; Roulin & Ducrest, 2011). This study focused on isolated populations with little, and in most cases negligible, gene flow with the mainland, shows that soil features, in terms of chemical composition, may constitute a further external factor that contributes to enhance melanogenesis. This is the case also after taking into account for some other variables previously shown to be linked with variation in plumage colouration in several avian species (e.g. Delhey, 2018; Delhey et al., 2019; Doutrelant et al., 2016), including the one studied here (Romano et al., 2021a, 2021b; Romano, Nodari, et al., 2019), thus making our findings promising to potentially open a new research avenue.

Given the correlative nature of the data used here, however, our findings must be considered as a preliminary observation of a possible association potentially driving the evolution of melanin-based pigmentation, as well as of its spatial variation, in vertebrates. Therefore, they must be corroborated by additional observations on other model systems and at different taxonomic scales (from species to community level), as well as specific experiments are highly recommended to show the generality of this pattern across different taxa and to explain the proximate mechanisms underpinning variation in melanin-based tegument colouration according to soil composition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The dataset analysed in this study is available UNIMI Dataverse at https://doi.org/10.13130/RD_UNIMI/SE7LOY

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BIOSKETCH

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.