



Barn owls display larger black feather spots in cooler regions of the British Isles

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Although, in many organisms, genotypes are adapted to specific environmental conditions, the identification of the ecological factors explaining patterns of local adaptation is not a trivial task. In relation to the cosmopolitan barn owl (*Tyto alba*), its plumage varies from white to dark pheomelanic and shows a difference in the number and size of black spots located at the tip of ventral feathers. The expression of these traits is strongly heritable and weakly sensitive to variation in body condition. Therefore, if owls located in cold or rainy regions are differently plumaged compared to owls living in warm or dry regions, this may not be a result of climate affecting the expression of plumage traits. Instead, different plumages might be selected under different environmental conditions. We have found that, on the British Isles, comparatively larger spots are present on barn owls found in regions that are cooler in summer. This is similar to the findings of a previous study performed in North America and on continental Europe, raising the possibility that larger-spotted barn owls better cope in cold temperatures during the rearing period or that they are better adapted to some environmental factors prevailing in cooler summers. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 445–454.

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INTRODUCTION

The study of geographical variation in phenotypic traits is useful for clarifying the potential adaptive function of phenotypic variation (Laaksonen *et al.*, 2015). Different analytical approaches have shown that heterogeneity in environmental factors can generate geographical variation in fitness-related traits, with alternative phenotypes being adapted to specific local condition (Brommer, 2011), as shown in many different organisms, such as fish (Herczeg, Turtiainen & Merila, 2010; Lamichhaney *et al.*, 2012) or birds (Amar *et al.*, 2014; Antoniazza *et al.*, 2014).

Spatial variation in environmental factors, and hence in spatially selective regimes, is a potent mechanism for promoting the evolution of genetic variation (Hedrick, 1986). Studies about local adaptation to spatially varying selection (e.g. a study on trees by Savolainen, Pyhajarvi & Knurr, 2007) have shown that the identification of the environmental factors to which alternative phenotypes or genotypes

are adapted is not an easy exercise. Another level of the complexity scale is the possibility that a given phenotype such as melanin-based coloration is adapted to many stressful environmental conditions (Ducrest *et al.*, 2008) so that the source of stress promoting the evolution of this phenotype can differ between regions. For example, the high cost paid to resist parasites in equatorial ecosystems as shown in humans (Guernier, Hochberg & Ghégan, 2004) and resistance to harsh winters in regions poleward may select for similar phenotypic signals of individual quality. Developing a thorough knowledge about the adaptive function of phenotypic variation necessitates the study of biological systems in multiple populations at large geographical scales.

The barn owl (*Tyto alba*) is widespread worldwide and shows pronounced variation in melanin-based plumage traits. The ventral body side of owls varies from white to dark reddish and from immaculate to heavily marked with black feather spots; traits that are strongly heritable ($0.57 < h^2 < 0.84$; Roulin & Jensen, 2015). Studies on the European continent have shown that clinal variation in pheomelanin-

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based reddish coloration, a trait that is strongly genetically correlated with number and size of black feather spots (redder owls are more heavily spotted), is maintained by natural selection (Antoniazza *et al.*, 2010, 2014) without, however, pointing out the environmental factors to which each colour morph is adapted.

In a first move towards resolving this issue, we performed a study on the North American continent where we measured plumage traits in 1369 barn owls collected by natural history museums throughout the USA, Mexico, and southern Canada. This work showed that, at the continental scale, variation in plumage traits is associated with climatic factors. For example, barn owls display larger black feather spots in regions where ambient temperatures are colder (Roulin & Randin, 2015). The same applies to the European continent, with barn owls having larger spots in the north-eastern regions and being smaller-spotted in the southern parts of the continent (Roulin, 2003). These observations suggest that, compared to small-spotted owls, large-spotted individuals are better able to cope with cooler temperatures or with some other correlated environmental factors. A potential reason explaining these patterns lies in the fact that the genes encoding the size of black spots pleiotropically regulate the ability to cope with stressful factors, as suggested by several studies performed on the barn owl (Roulin & Ducrest, 2011).

The aim of the present study is to examine whether, on the British Isles, geographical variation in melanin-based coloration covaries with climatic variables in the same way as in North America and on the European continent. Compared to barn owls found on the latter two continents, British and Irish barn owls are, on average, whiter and with fewer spots (Roulin, 2003). England is sufficiently far from the European continent to limit gene flow from the continent (Wernham *et al.*, 2002) and therefore we do not expect barn owls from southern England to be darker and more spotted than those from the rest of the British Isles, as a consequence of gene flow from France, Belgium or the Netherlands where owls are darker and spottier (Roulin, 2003).

During the present study, we visited 37 natural history museums where we obtained data on plumage traits in 410 British and 59 Irish barn owls of known sex (see Appendix, Table A1).

MATERIAL AND METHODS

GENERAL METHOD

We measured 208 males and 202 females from Great Britain and 33 males and 26 females from Ireland.

Birds were collected between 1830 and 2014 (mean year is 1931). The degree of reddish pheomelanin-based coloration and the number and size of black spots were identified on the breast and belly of all individuals and assessed using the same method as that described previously (Roulin & Randin, 2015). In short, number of spots were counted within a $60 \times 40\text{-mm}^2$ frame and their diameter measured to the nearest 0.1 mm and coloration was scored on a scale from -8 for white to -1 for dark reddish.

As shown in another study, reflectance spectra measured with a spectrophotometer is strongly correlated with colour scores assessed visually (Pearson's correlation: $r = 0.78$, $N = 1107$, $P < 0.0001$; Dreiss & Roulin, 2010). For all plumage traits, a mean value was calculated over the two body parts (note that, if the statistical analyses are performed on the breast and belly separately, we obtain qualitatively similar results). To compare plumage traits, we standardized their values using the formula (values $-$ mean value)/SD. This transformation ensured that the different plumage traits had the same mean value (0) and SD (1).

In the present study, we first compared white vs. non-white individuals and spotted vs. unspotted individuals, and then considered only the pigmented individuals in further analyses. We decided to do so for three reasons; first, the proportion of white and unspotted individuals is very high on the British Isles (particularly in males) (Fig. 1) and much higher than in most places around the globe, with the notable exception of Sardinia. Second, removing nonpigmented individuals from the analyses allowed us to compare individuals that express melanogenic genes at different levels without the case where some individuals do not express the genes at all. Third, the distributions of plumage traits strongly departed from normality (Fig. 1).

For the above reasons, we first compared climatic variables between white and non-white individuals, as well as between immaculate and spotted individuals, using logistic regressions. By considering only non-white or only spotted individuals, we then examined the relationships between climatic variables and Box-Cox transformed reddish coloration, number, and size of black spots. In logistic regression analyses, we did not find any difference in the mean year of collection between white and non-white individuals ($\chi^2 = 0.15$, d.f. = 1, $P = 0.70$) or between immaculate and spotted individuals ($\chi^2 = 0.82$, d.f. = 1, $P = 0.36$).

CLIMATIC VARIABLES

The locations where specimens were collected were indicated on the labels, thus allowing us to

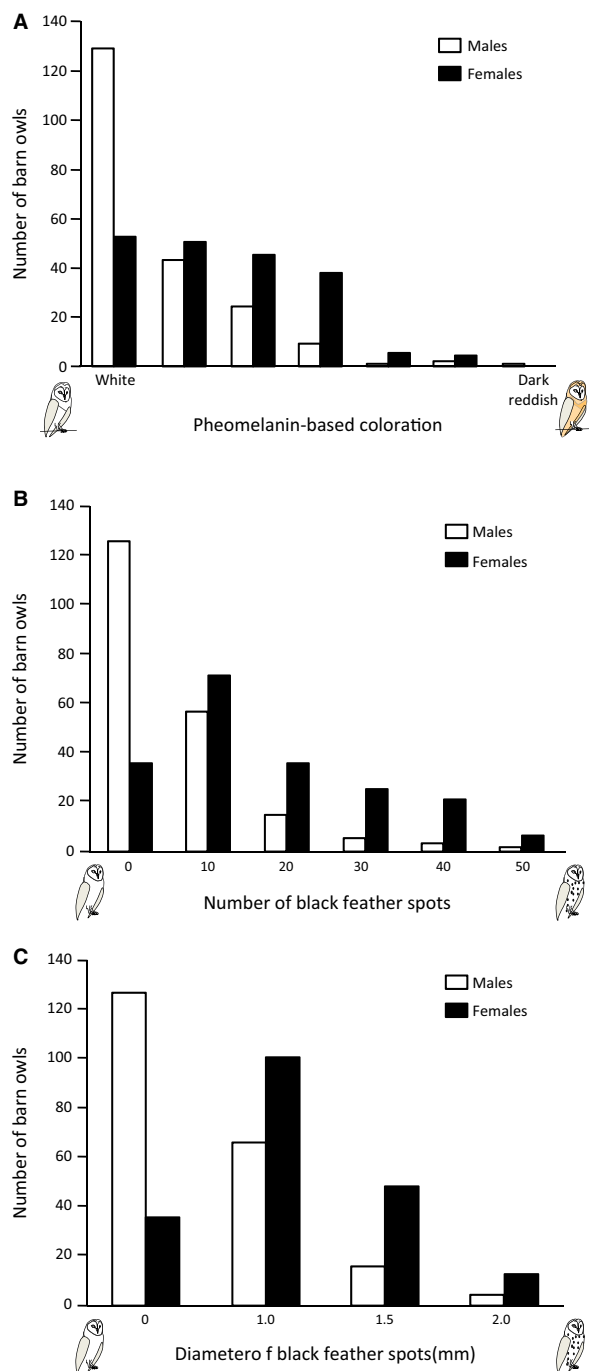


Figure 1. Frequency distribution of reddish plumage coloration (A), number (B), and size of black feather spots (C) in male and female barn owls on the British Isles.

determine the geographical coordinates. We selected specimen records with a horizontal uncertainty of the geographical coordinates of < 1 km to match with the spatial resolution of geographical climate layers. The accuracy of the geographical coordinates, as defined in Chapman (2005), was estimated with

custom codes in R, version 3.2.0 (R Development Core Team, 2011) by taking into account the number of decimal digits of latitude and longitude and the position on the earth using the harvesine formula *sensu* Randin *et al.* (2013). From the Worldclim dataset and for the period 1950–2000 (<http://www.worldclim.org>) (Hijmans *et al.* 2005), we extracted long-term monthly sums of precipitation and long-term (1951–2000) monthly means of 2-m ambient daily temperature. As in the study on the North American owls (Roulin & Randin, 2015), for each individual, we had one value per day; for each year, we calculated a mean monthly value; and, for each month, we computed a mean value over the entire period 1950–2000. Therefore, for each skin specimen and each month, we had a mean precipitation level value and a mean ambient temperature value. We are aware that the period 1950–2000 is different from the period when the owls were collected (1830–2014). However, our aim is to determine whether barn owls are differently plumaged in different regions possibly as a result of climate or some correlated variables. This research goal is valid because, even if climate changed during the last 184 years, similar climatic differences should have persisted between regions.

The mean monthly ambient temperature values were normally distributed without any transformation, whereas we had to log₁₀-transform monthly precipitation values. We used JMP, version 11.0 (SAS Institute, Cary, NC, USA) to explore whether plumage traits were associated with climatic variables. We extracted the first three principal components (eigenvalue > 1.0) including the monthly values of precipitation and mean ambient temperatures (Table 1). The first component indicates high precipitation and cold temperatures in all months; the second component is mainly related to intense precipitation and warm temperatures outside the summer; the third component describes precipitation of medium intensity in winter, cold temperatures in winter, and warm temperatures in summer. These three components were used in two-tailed statistical tests and, given the loadings of each climatic variable (i.e. monthly temperature and precipitation values), we performed further analyses using monthly values. This was necessary to determine whether plumage traits are associated with weather prevailing in some particular season (note that $P < 0.05$ was considered statistically significant).

To test for spatial autocorrelation, we performed a Mantel test between the geographical distances and distances between the residuals diameter of the black spots for each observation (we extracted residuals to remove variation explained by sex). Accordingly, we calculated the two distances with the function *dist()* in R and then the *mantel.rtest()*

Table 1. Principal components analysis of monthly sums of precipitation (\log_{10} -transformed) and of monthly mean values of daily mean ambient temperatures in the UK

	Principal components of weather		
	1st	2nd	3rd
Eigenvalues	15.76	5.59	1.32
% Variance	65.66	23.30	5.50
Eigenvectors			
Precipitation			
January	0.21	0.21	0.14
February	0.21	0.21	0.004
March	0.22	0.18	0.16
April	0.21	0.19	0.11
May	0.22	0.15	0.01
June	0.22	0.12	0.22
July	0.23	0.05	0.03
August	0.23	0.06	-0.07
September	0.23	0.17	0.06
October	0.21	0.21	0.13
November	0.21	0.20	0.12
December	0.21	0.22	0.11
Temperature			
January	-0.08	0.36	-0.35
February	-0.13	0.33	-0.24
March	-0.19	0.24	-0.07
April	-0.21	0.19	0.16
May	-0.21	0.14	0.36
June	-0.21	0.10	0.39
July	-0.22	0.10	0.34
August	-0.23	0.11	0.29
September	-0.23	0.14	0.11
October	-0.21	0.21	-0.01
November	-0.18	0.28	-0.16
December	-0.10	0.34	-0.35

Only the components with eigenvalues larger than 1 are reported.

function of the *ade4* R package with 999 permutations. We used the function *correlog()* from the *nfcf* R library to estimate the spatial dependence of residual spot diameter. We calculated the interpolated *x*-intercept of Epperson (i.e. the distance at which objects are no more similar than that expected by-chance-alone across the region). We found a distance of 1055 m.

GEOGRAPHICALLY WEIGHTED REGRESSION (GWR)

To analyze spatially the relationship between weather and plumage traits, we calibrated the GWR (Brunsdon, Fotheringham & Charlton, 1996, 1998). GWR comprises an exploratory technique that can detect geographical nonstationarity (i.e. where coefficients of locally weighted regression move away from

their global values). GWR relies on the assumption that fitted coefficient values of a global model calibrated on all the data may not adequately represent local variations in the data. GWR looks for local variation in the data space but, by moving a weighted window over the data, GWR estimates one set of coefficient values at every chosen point of calibration. If the local coefficients vary in space, this can be taken as an indication of nonstationarity (Bivand, Danlin & Yu, 2014).

To assess such a spatial relationship, we only considered the climatic variable that was most strongly associated with spot diameter. To this end, we used the library *spgwr* in the R software (Bivand *et al.*, 2014) to calibrate univariate GWR with a Gaussian error distribution and with spot diameter as the response variable and this climatic variable as the predicting variable. The bandwidth of the GWR was determined by minimizing the root mean square prediction error during a drop-one cross-validation procedure. To minimize the potential effect of spatial autocorrelation and to assess the effect of the geographical distribution of sampling, GWR of spot diameter as a function of this climatic variable was calibrated 100 times and, for each repetition, 500 observations were randomly selected.

Local R^2 values, indicating the strength of the spatial relationship between spot diameter and temperature for a given spatial location, were classified into three categories with the Jenks natural break optimization. We assessed whether high local R^2 could be associated with a high spatial heterogeneity of temperature. Here, we hypothesized that a high spatial variability of temperature will create a higher selective pressure on spot diameter. To this end, we used elevation from the 90-m SRTM Digital Elevation Database, version 4.1 (<http://srtm.csi.cgiar.org>) as the best available measured proxy for temperature variability and calculated the range of elevation within each of the three categories of local R^2 .

RESULTS

SEXUAL DIMORPHISM AND GEOGRAPHICAL VARIATION

In Britain, a higher percentage of male barn owls are immaculate (60.7%) compared to females (19.2%) ($\chi^2 = 82.46$, d.f. = 1, $P < 0.0001$). Males had a significantly lighter reddish colour than females (Wilcoxon test: $Z = 7.93$, $P < 0.0001$) and displayed fewer ($Z = 9.49$, $P < 0.0001$) and smaller black spots ($Z = 9.11$, $P < 0.0001$) (Fig. 1). We tested whether sexual dimorphism differed between the three plumage traits by considering only individuals displaying at least one black spot and using Box-Cox transformed standardized plumage trait values. The

degree of sexual dimorphism differed significantly between plumage traits (linear mixed model with individual identity as random variable; sex: $F_{1,273} = 5.54$, $P = 0.019$; plumage trait: $F_{2,546} = 1.17$, $P = 0.31$; interaction: $F_{2,546} = 8.23$, $P = 0.0003$) (Fig. 2). Pairwise comparisons showed that sexual dimorphism was more pronounced with respect to spot size than reddish coloration (linear mixed model with individual identity as random variable; sex: $F_{1,273} = 0.10$, $P = 0.75$; plumage trait: $F_{1,273} = 1.48$, $P = 0.23$; interaction: $F_{1,273} = 7.70$, $P = 0.0059$) and number of spots than reddish coloration (sex: $F_{1,273} = 1.78$, $P = 0.18$; plumage trait: $F_{1,273} = 1.05$, $P = 0.31$; $F_{1,273} = 9.66$, $P = 0.0021$), whereas sexual dimorphism in spot size did not differ significantly from sexual dimorphism in number of spots (sex: $F_{1,273} = 9.80$, $P = 0.0019$; plumage trait: $F_{1,273} = 0.11$, $P = 0.74$; interaction: $F_{1,273} = 1.60$, $P = 0.21$) (Fig. 2).

If immigration from the continent is assumed to be frequent, we would predict a south–north cline variation in plumage trait in Great Britain. However, this was not the case with respect to the three plumage traits within both males (Spearman's correlation between plumage traits and latitude, all P -values > 0.81) and females (all P -values > 0.11). Irish males are whiter coloured (Wilcoxon test: $Z = 2.49$, $P = 0.013$) and display fewer black spots ($Z = 2.07$, $P = 0.038$) but have spots of a similar size ($Z = 1.67$, $P = 0.10$) compared to those found in Great Britain. Similarly, Irish females have a significantly larger amount of white colour than British females ($Z = 3.57$, $P = 0.0004$) and display fewer black spots ($Z = 2.25$, $P = 0.024$) but have spots of a similar size ($Z = 1.79$, $P = 0.07$).

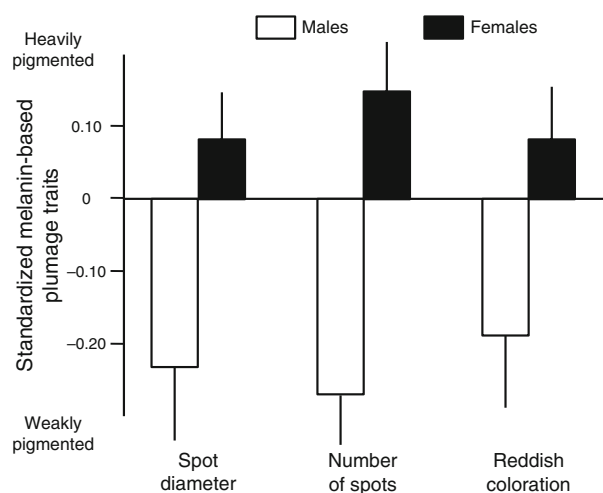


Figure 2. Mean standardized plumage trait values in male and female British and Irish barn owls, based on birds displaying at least one black feather spot on the ventral body side.

CLIMATE

Because a large proportion of the birds are white (46.3%) and immaculate (53.7%) (Fig. 1), we first examined whether climate predicts the distribution of white vs. non-white birds and of immaculate vs. spotted owls. This was not the case for both coloration and spottiness in logistic regression analyses with sex as factor (P -values for the three principal components of weather > 0.07).

When considering only birds displaying at least one black spot, the Box-Cox transformed number of black spots was not significantly associated with the three principal components of weather [analysis of covariance (ANCOVA), sex: $F_{1,241} = 16.32$, $P < 0.0001$; 1st component: $F_{1,241} = 0.10$, $P = 0.75$; 2nd component: $F_{1,241} = 0.09$, $P = 0.77$; 3rd component: $F_{1,241} = 1.95$, $P = 0.16$]. In non-white individuals, we also found no significant association between box-cox transformed reddish coloration and the three components of weather (ANCOVA, sex: $F_{1,220} = 9.48$, $P = 0.002$; 1st component: $F_{1,220} = 1.30$, $P = 0.25$; 2nd component: $F_{1,220} = 1.30$, $P = 0.25$; 3rd component: $F_{1,220} = 1.33$, $P = 0.25$).

When considering only individuals displaying at least one black spot, Box-Cox transformed spot size was significantly associated with the third principal component of weather (ANCOVA, sex: $F_{1,241} = 9.95$, $P = 0.0018$; 1st component: $F_{1,241} = 3.13$, $P = 0.078$; 2nd component: $F_{1,241} = 0.42$, $P = 0.52$; 3rd component: $F_{1,241} = 11.27$, $P = 0.0009$) (Fig. 3A). Adding latitude and longitude, as well as museum identity where birds were measured as random variable, did not modify our conclusion (not shown). In a preliminary model, interaction between the principal components of weather and sex was not significant and hence was removed from the final model.

Finally, if we restrict the analysis to birds collected in Great Britain (i.e. by excluding Irish birds), we obtain a similar result (ANCOVA, sex: $F_{1,220} = 13.48$, $P = 0.0003$; 1st component: $F_{1,220} = 2.24$, $P = 0.14$; 2nd component: $F_{1,220} = 0.34$, $P = 0.56$; 3rd component: $F_{1,220} = 4.75$, $P = 0.03$). The 3rd component is mainly associated with mean ambient temperatures in summer (mainly June) and winter (mainly December) (Table 1).

We thus performed two separate ANCOVAs with sex as factor and mean June ambient temperature (sex: $F_{1,243} = 10.08$, $P = 0.0017$; temperature: $F_{1,243} = 8.00$, $P = 0.005$) (Fig. 3B) or mean December ambient temperature (sex: $F_{1,243} = 9.41$, $P = 0.002$; temperature: $F_{1,243} = 0.01$, $P = 0.91$). These results indicate that owls are larger-spotted in regions where mean ambient temperatures are lower in June (Fig. 4). Because these results could still be inflated by spatial autocorrelation, we

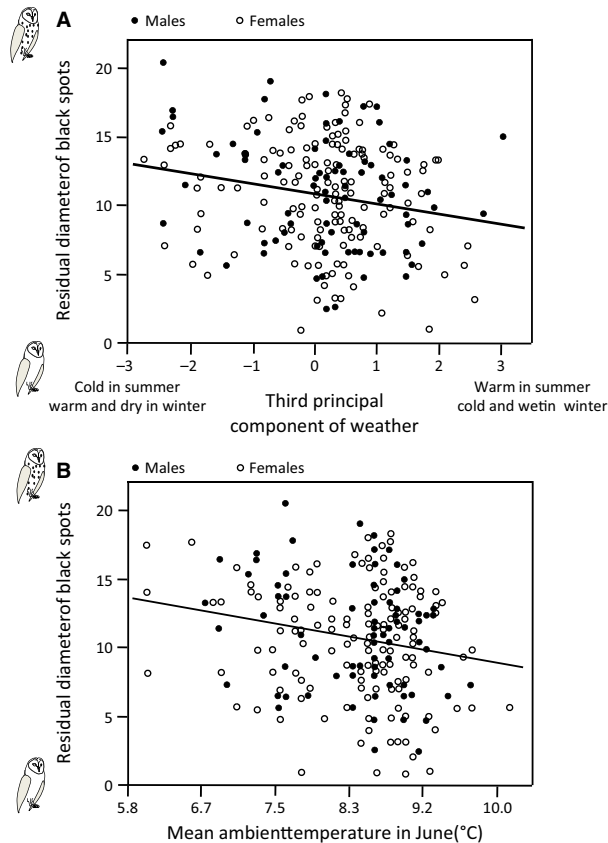


Figure 3. Diameter of black feather spots in relation to an index of weather (A) and mean ambient temperatures in June (B) in barn owls located on the British Isles. Only individuals displaying at least one black spot are considered. Full circles, males; open circles, females.

performed a Mantel test. This analysis showed that greater but nonsignificant differences in black spot diameter were generally seen among pairs of observations that were close to each other rather than far from each other ($r = -0.03$; $P = 0.76$). The x -intercept of Epperson was 1055 m. We thus removed observations that were closer than 1055 m from each other with the *ecospat.occ.desaggregation* function of the *ecospat* R package. One hundred and forty-six observations were kept in the new matrix used to assess the relationships between residual spot diameter (after removing variation explained by sex) and mean ambient temperature in June. This relationship was still significant ($r^2 = 0.03$ with $P = 0.028$).

GWR ANALYSES

By using GWR analyses, the relationship between spot diameter and mean June temperature was the highest (i.e. high values of local R^2) in the centre of

England, in the north of Scotland, and in the western part of Ireland (Fig. 5A). This was also where the SD of local R^2 was the most important among the 100 repetitions of GWR (Fig. 5B). Interestingly, the mean (all $P < 0.001$; Wilcoxon signed-rank tests) and range of elevation (i.e. distribution of elevation of 90-m pixels of the landscape; all $P < 0.001$; Levene's tests) increased significantly from geographical regions with low to high local R^2 . In other words, the strength of the association between spot diameter and mean ambient temperature in June was stronger in regions located at higher elevation (Fig. 5C).

DISCUSSION

The present study shows that, on the British Isles, barn owls display comparatively larger black feather spots on the ventral body side in cooler regions in summer. This result is consistent with what we found on the North American (Roulin & Randin, 2015) and European continents (Roulin, 2003). However, in another study considering the *T. alba* taxa as the unit of our statistics (instead of North-American or European barn owl individuals), we found that, on the northern hemisphere, taxa located near the equator display larger black spots than taxa located towards the poles (Roulin, Wink & Salamin, 2009). This suggests that several selective factors influence the evolution of spot sizes in the *Tyto* genus.

At a worldwide scale, selective agents not related to ambient temperature, such as parasitism, may favour the evolution of large black feather spots in the equatorial region. At a more local geographical scale, other ecological factors may contribute to explaining the nonrandom distribution of barn owls with respect to spot size, with one of them being summer ambient temperature or some variables correlated with it. Assuming that, in the UK, as in Switzerland, the expression of black feather spots is under strong genetic control and not (or only weakly) influenced by the environment (Roulin *et al.*, 2010), we suggest that the reported relationship between ambient temperature and spot size takes place not because ambient temperature (or a correlated factor) influences the expression of black spots but because large-spotted birds have a selective advantage in cooler regions. Obviously, we cannot discard the possibility that, on the British Isles, there are environmental effects on the expression of spots. Still, the relationship between spot size and June ambient temperatures is not inflated by gene flow from continental Europe where owls are larger-spotted because, in such a case, we should have observed larger-spotted and redder barn owls mainly in southern England, which was not the case. In this context, we

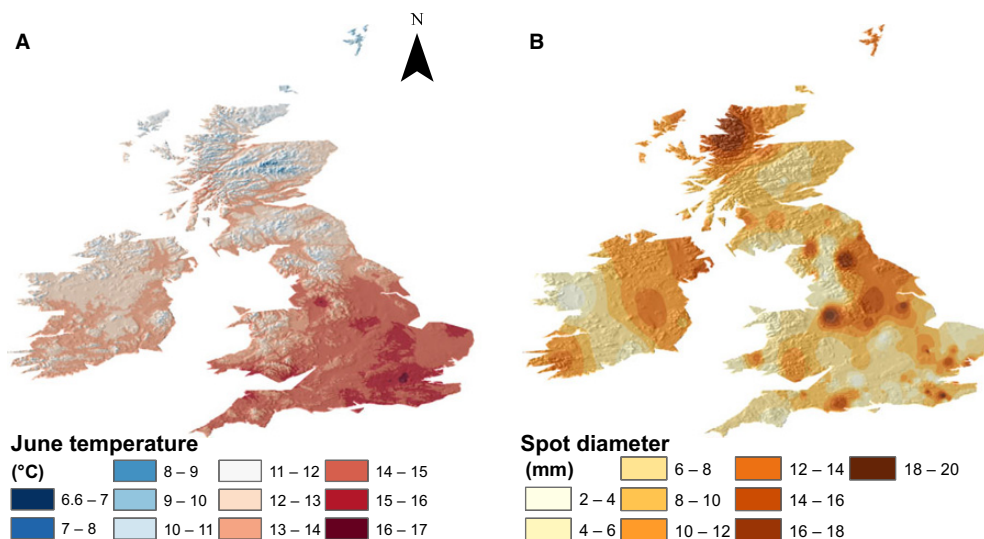


Figure 4. Long-term mean ambient temperatures in June on the British Isles (A) and interpolated distribution of the diameter of black feather spots in the barn owl (B). The interpolation was performed with an inverse distance weighting algorithm of power three with a variable search radius based on twelve points.

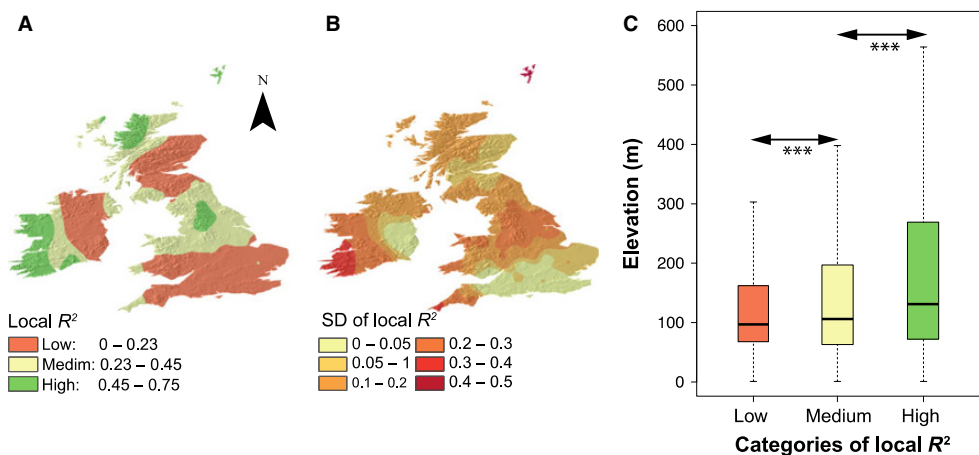


Figure 5. Spatial distribution of three categories (low, medium, and high) of the mean local R^2 from 100 repetitions of geographically weighted regression (GWR) of the diameter of black feather spots in the barn owl as a function of the long-term mean ambient temperatures in June on the British Isles (A) and the associated SD of the local R^2 obtained from the 100 repetitions (B). The three categories of local R^2 (C) were determined by the Jenks natural breaks optimization. Values of local R^2 and SDs on points of observations were interpolated using the inverse distance weighting algorithm of power three with a variable search radius based on twelve points. Box plots of the range of elevation of pixels at a 90-m spatial resolution within each of the three categories of (A) are shown in (C). Arrows indicate the pairs in Wilcoxon signed-rank tests and in Leven's tests. Asterisks indicate the level of significance for the two tests (all $P < 0.001$).

could show that the association between spot diameter and summer temperature was stronger in regions where the geographical variability of elevation was high rather than low. This further suggests that the structure of the regional landscape could promote local adaptation, with larger-spotted barn owls being adapted to regions where it is cooler in summer. The present study raises the interesting possibility that,

as a result of the ongoing climate warming, barn owls may become less spotted with time; a phenomenon that might not only be restricted to the barn owl (Roulin, 2014).

The finding that spot size was associated with summer rather than winter ambient temperatures suggests that, during harsh winters, larger-spotted owls may not have a survival advantage over

smaller-spotted conspecifics. Rather, it appears that the selective advantage accrued by larger-spotted barn owls is mainly associated with reproductive activities. Cold temperatures may indeed negatively impact rearing conditions, as shown in Switzerland, where brood size is positively correlated with ambient temperatures prevailing during rearing (Chausson *et al.*, 2014). Therefore, larger-spotted owls may better be able to cope with cooler temperatures during the rearing period. Spot size has been repeatedly shown to be associated with the ability to resist stressful situations (Roulin & Ducrest, 2011). Therefore, adults and/or their offspring may be better able to cope with the stress associated with cooler summers. For example, individuals displaying more black feather spots (a trait that is strongly genetically correlated with spot diameter; Roulin & Jensen, 2015) have a colder body temperature and a higher metabolic rate and, under cooler temperatures, they more often socially huddle to thermoregulate (Dreiss *et al.*, 2016); as noted by Dreiss *et al.* (2016) 'if melanin spots themselves helped thermoregulation, we would have expected the opposite results'. Plumage spottiness therefore appears to be associated with physiological and behavioural adaptations to cold temperatures because low temperatures select for a higher metabolic rate to maximize heat production (White & Kearney, 2013). A subsequent step will be to investigate the relationship between ambient temperatures and reproductive success in interaction with spot size. In addition, we need to confirm whether ambient temperatures are associated with melanin-based plumage traits in other parts of the world.

The British Isles are also particularly interesting because many barn owls are nonpigmented, offering the possibility of comparing pigmented vs. nonpigmented individuals and performing other analyses where we relate the degree of pigmentation to other variables by considering only the pigmented individuals. Such analyses showed that there were no difference in the climate where the skins of pigmented vs. nonpigmented individuals were collected, whereas we detected a relationship between spot size and climate when considering only the pigmented individuals. Assuming that our results are robust and biologically sound, this suggests that immaculateness is a peculiar state in the barn owl. The absence of black feather spots might be associated with a very different physiological state compared to individuals that produce at least a few small spots. We speculate that the molecules triggering the production of these spots epistatically prevent other molecules from regulating physiological processes. This speculation could lead to further research.

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APPENDIX

Table A1. LIST OF MUSEUMS WHERE BARN OWLS (*TYTO ALBA ALBA/GUTTATA*) OF KNOWN SEX WERE COLLECTED ON THE BRITISH ISLES

Country (State)	City	Museum	Number of barn owl skins
Netherlands	Amsterdam	Zoological Museum	6
USA (Michigan)	Ann Arbor	University of Michigan, Museum of Zoology	1
Northern Ireland	Belfast	National Museums Northern Ireland	13
USA (California)	Berkely	The Museum of Vertebrate Zoology	1
Germany	Bonn	Alexander Koenig Research Museum	5
Great Britain	Bristol	Bristol Museum and Art Gallery	17
Great Britain	Cambridge	University Museum of Zoology	13
Great Britain	Cardiff	National Museum Cardiff	16
USA (Illinois)	Chicago	The Field Museum of Natural History	1
Denmark	Copenhagen	Natural History Museum of Denmark	3
USA (New York)	Cornell	Cornell University	2

Table A1. *Continued*

Country (State)	City	Museum	Number of barn owl skins
Germany	Dresden	Senckenberg Natural History Museum	2
Ireland	Dublin	National Museum of Ireland	30
Great Britain	Edinburgh	National Museum of Scotland	63
Germany	Frankfurt	Senckenberg Naturmuseum Frankfurt	4
Great Britain	Sevenoaks	Harrisson Institute	38
Finland	Helsinki	Finnish Museum of Natural History	6
Japan	Hyogo	Museum of Nature and Human activities	2
Great Britain	Inverness	Inverness Museum and Art Gallery	1
USA (Kansas)	Lawrence	Natural History Museum & Biodiversity Research Center	1
Netherlands	Leiden	National Museum of Natural History	4
Great Britain	Liverpool	World Museum Liverpool	26
Great Britain	Manchester	Manchester Museum	5
Australia	Melbourne	Museum Victoria	1
USA (Connecticut)	New Haven	Peabody Museum of Natural History at Yale University	1
USA (New York)	New York	American Museum of Natural History	68
Great Britain	Newcastle	Great North Museum	26
Great Britain	Perth	Perth Museum and Art Gallery	3
USA (Pennsylvania)	Philadelphia	Academy of Natural Sciences	2
USA (California)	San Francisco	The California Academy of Sciences	1
Sweden	Stockholm	Naturhistoriska riksmuseet	1
Canada (Ontario)	Toronto	Royal Ontario Museum, Department of Natural History	8
Great Britain	Tring	Natural History Museum at Tring	86
USA (District Columbia)	Washington DC	Smithsonian Institution, Natural Museum of Natural History	4
USA (Delaware)	Wilmington	Delaware Museum of Natural History	1
Japan	Abiko City	Yamashina Institute for Ornithology	4
USA (Massachusetts)	Cambridge	Harvard Museum of Natural History	1
Total			469