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A melanin-based trait is more strongly related to body size in the tropics than in temperate regions in the globally distributed barn owl family

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Abstract

Life history traits differ between organisms living in the tropics, northern and southern hemispheres, and sexual selection is thought to be stronger close to the equator than in temperate regions. Although birds are often supposed to be more brightly coloured in the tropics, the current evidence of geographic variation in the intensity of sexual selection and sex-specific natural selection is equivocal. Whether sex-specific traits signal aspects of individual quality better in the tropics than in the temperate regions of the northern and southern hemispheres therefore remains an open question. We examined predictions of this hypothesis in the *Tytonidae* family (barn owls and their relatives) because females, on average, display larger black spots on the tip of their ventral body feathers than males, and this trait is associated with aspects of individual quality. We measured the size of melanic spots and the wing length of 7893 *Tytonidae* skins collected worldwide and preserved in natural history museums. The covariation between spot size and wing length was stronger in females than in males, in large- than small-spotted *Tyto* taxa and close to the equator than in temperate regions. This suggests that selection for spot size, which can be used by owls as an additional cue to assess individual body size and other aspects of phenotypic quality, is stronger in females than in males, particularly near the equator.

Keywords: biogeography; colour polymorphism; honest signalling; melanism; sexual selection; sex-specific selection; *Tytonidae*.

Introduction

Identifying geographic variation in life history, morphological and behavioural traits requires the comparison of populations living in different areas. Of particular interest is the consideration of phylogenetically related organisms living across a range of latitudes on different continents, which would allow a comparison of populations occurring in more stable tropical ecosystems with those in temperate ecosystems, where there are pronounced seasonal changes in food supply. These distinct environmental conditions can profoundly alter organismal evolution in different ways across the globe: tropical birds lay smaller clutches, have a longer period of parental care and lower basal metabolic rate than observed in north-temperate species (Baker, 1938; Wiersma *et al*., 2007; McNamara *et al*., 2008). Interestingly, south-temperate birds generally exhibit life-history traits that are more similar to those of equatorial species than birds located in north-temperate regions (Martin, 1996; Russell *et al*., 2004; Jetz *et al*., 2008), likely because of the reduced seasonality of the southern hemisphere. For instance, post-fledging survival is higher in tropical species and those located in the southern hemisphere than in species located in the northern hemisphere (Remes & Matysioková, 2016).

A long-standing evolutionary question is whether sexually selected characters, as do naturally selected traits, vary clinally due to contrasting latitudinal gradients in environmental factors (Møller, 1995). Although it is often assumed that organisms are more colourful in the tropics compared to temperate regions, there is conflicting evidence; the results of some studies support this claim (Bailey, 1978; Dale *et al*., 2015), while other authors have reported no such effects in birds, butterflies and plants (Cardillo 2002; Dalrymple *et al*., 2015; Doutrelant *et al*., 2016). Clear evidence that sexual dichromatism varies with latitude is also lacking (Cardillo, 2002; Madrigal & Kelly, 2007), yet the finding that the song of the American house wren (*Troglodytes aedon*) is more complex at higher latitudes in the northern and southern hemispheres and less complex near the equator suggests that sexual selection may be more intense in strongly seasonal environments (Kaluthota *et al*., 2016). This might be linked to the short time window available for reproduction in temperate regions, which may provide more opportunities for sexual selection to operate among synchronously breeding conspecifics (Spottiswoode & Møller, 2004). This overview of the literature illustrates how little we know about the global variation in the intensity of sex-specific selection.

Assuming that individuals of one sex exert sexual selection to secure the best sexual partner, sex-linked ornaments may better signal quality such as resistance to parasitism in the tropics than in temperate regions because parasites have a greater impact on their hosts in the tropics (Møller, 1998; Guernier *et al*., 2004). Also, because clutch size, the length of the rearing period and offspring survival differ between tropical, north and south temperate species (Baker, 1938; Wiersma *et al*., 2007; McNamara *et al*., 2008; Remes & Matysioková, 2016), sex-traits that reflect the quality of parental care may be more closely associated with traits important for foraging in some regions than in others. Therefore, it would be of interest to examine if secondary sexual characters better correlate with aspects of individual quality in populations located close to the equator than those in temperate regions, and whether the intensity of such a covariation is stronger in the southern than northern hemisphere. Such a study would provide information about whether the honesty of an ornament as a signal of quality varies geographically in a predictable manner. However, measuring the covariation between secondary sexual characters and other phenotypes in a large number of species around the world is logistically difficult, so an alternative approach is to consider tractable traits, such as body size, that are often the target of sexual selection (Fairbairn, 1997) or sex-specific natural selection (Krüger, 2005). Body size could be related to secondary sexual characters for several reasons: 1) because the expression of these two traits relies on the same pool of resources; 2) because of allometry; 3) because the selective pressures exerted on body size and ornamental traits are correlated; 4) or still because ornaments have evolved to signal traits such as body size that are less variable and hence more difficult to assess by potential mates.

In this paper, we considered barn owls and their relatives (Family: *Tytonidae*; genus: *Tyto*) because this family is found on every continent except Antarctica, and except for the sooty owl group (*Tyto tenebricosa tenebricosa, T. t. arfaki* and *T. t. multipunctata*), in all populations birds exhibit variation in the size of the black spots located on the tips of the ventral body feathers. This cosmopolitan taxon is highly suitable for this kind of research because it allows for a comparison of closely related subspecies/species that are globally distributed and that all display a similar secondary sexual character. In temperate Central Europe, this trait signals multiple physiological, morphological and behavioural attributes (Roulin & Ducrest, 2011), it plays a role in male mate choice (Roulin, 1999; Roulin & Altwegg, 2007) and is directionally selected, particularly in females (Roulin *et al*., 2010). This suggests that spot size, or some genetically correlated traits, experiences sexual selection and/or sex-specific natural selection. As in most owls and raptors, females are larger and heavier than males, and males provide most of the food to their progeny. Thus, selection on body size could be sex-specific, as it is on spot size. These two traits could be differentially correlated across the globe either because spot size is a signal of body size and quality, or because the degree of sex-specific selection exerted on spot size is correlated with sex-specific selection exerted on body size or quality as a consequence of genetic correlation between these two categories of traits.

Although a previous study has shown that large *Tyto* taxa display larger black feather spots than smaller *Tytonidae* (Roulin *et al*., 2009), studies of Swiss and Israeli barn owls found that, across many individuals, the size of the black spots is not significantly associated with body size (Roulin, 2006; Charter *et al*., 2012). This suggests that the within-population association between spot size and body size might be different from that between-populations, also raising the possibility that spot size could be associated with body size in some, but not all, *Tytonidae* taxa. Because sexual selection can vary according to latitude (e.g. Møller 1995; Roulin et al. 2009; Dale *et al*., 2015), the extent to which this melanin-based trait reflects body size could also vary geographically. An interesting issue is whether the association between body size and the diameter of the black feather spots differs between the tropics and the north- and south-temperate regions, so we measured the size of the black spots and wing length of 7893 barn owl skins collected from around the world and preserved in natural history museums. This large sample of birds provided a unique opportunity to measure the strength of the covariation between individual spot size and body size in a large number of *Tytonidae* and its variation with latitude and hemisphere. The first aim of this study was to examine how melanin-based colouration and body size (i.e. wing length) vary geographically at the worldwide scale. This included measuring the coefficient of variation (CV) in these two traits among different *Tyto* taxa (see Methods). The rationale is to examine whether the size of black spots is more variable than wing length, and hence could be used by owls as an additional cue to indirectly assess variation in body size and quality between potential partners. In a second set of analyses, we investigated the geographic variation in the covariation between these two traits. To the best of our knowledge, this is the first attempt to investigate whether ornaments covary more strongly with individual quality in the tropical or temperate zones, and in the southern or northern hemispheres.

Materials and methods

Model System

The avian family of Barn Owls and relatives (*Tytonidae*) is a relatively small but diverse group of nocturnal owls, occurring in every continent except Antarctica (e.g. Gill and Donsker, 2018). They can exploit a wide range of habitats, such as open landscapes, forests and anthropogenic environments. The family comprises two genera: *Phodilus*, limited to the forests of central Africa and South-East Asia (and not included in the present analyses), and the ubiquitous *Tyto* (Gill and Donsker, 2018). The latter is majorly represented by the cosmopolitan Common Barn Owl, *Tyto alba* complex, which is characterized by recent rapid radiations, highly colour polymorphic populations, and rare endemic populations, all of which give rise to complex and controversial taxonomy and phylogenetic relationships, particularly when coupled with a generally difficult access to population-level sampling (Uva *et al.*, 2018). In this highly colour polymorphic group, variation in body size, plumage colouration, and geographical distributions, have led to an extraordinary taxonomic diversity, through the classification of more than 30 subspecies recognised worldwide.

According to the latest phylogenetic studies however, the cosmopolitan *Tyto alba* is split into three major evolutionary lineages, which entirely correspond to the three main continental regions of the world: 1) the American Barn Owl, *T. furcata*); 2) the Afro-European (or Western) Barn Owl, *T. alba*; and 3) the Australasian (or Eastern) Barn Owl, *T. javanica* (Aliabadian *et al.*, 2016; Uva *et al.*, 2018). Each of those three lineages comprises many subspecies, some of which represent several convergent morphological adaptations to insular habitats (Uva *et al.*, 2018). Body size and colour traits can both undergo rapid and significant shifts during island radiations, and in *Tytonidae* specifically, insular populations tend to be smaller and darker than their phylogenetic mainland relatives (Roulin and Salamin, 2010). Therefore, as in other cosmopolitan species (e.g. White et al., 2013), phenotypic traits will likely obscure phylogenetic relationships, and are thus not suitable diagnostic characters for species delimitations (Wink *et al.*, 2009; Uva *et al.*, 2018).

Museum skin collection and measurements

We measured the size of the black feather spots on the breasts of 7893 barn owl skins of known sex preserved in 136 natural history museums. A. Roulin visited all these museums and measured phenotypes of each specimen by hand. Information on the different *Tyto* species and subspecies (hereafter "*Tytonidae*" or "*Tyto* taxa"), the sex of the birds and collection location was obtained from labels attached to the specimens. In all the taxa included in the analyses, males and females differ in size and plumage traits. Hence, the inclusion in the dataset of a disproportionate number of males and females in different hemispheres and depending on latitude might affect the observed associations between traits. However, the proportion of male individuals in each *Tyto* taxon was not related to hemisphere ($F_{1,30} = 0.49$, $P = 0.49$), absolute latitude ($F_{1,30} = 0.01$, $P = 0.91$) or their interaction ($F_{1,30} = 0.67$, $P = 0.42$), thus preventing the possibility that our results can be affected by non-random sampling of individuals of either sex. The diameter of the most representative black spot (i.e. the spot showing an average size) on the breast of each specimen to the nearest 0.1 mm and the wing length to the nearest mm were also measured. The raw data are reported in Table 1, including the mean male and mean female spot diameter, and the mean wing length for each of the 35 *Tyto* taxa (see Tables 1-2 and Fig. S1 for specific information about the taxa included), based on the taxonomy of Aliabadian *et al.*

(2016) and Uva *et al*. (2018). The number of *Tytonidae* specimens preserved in all 136 museums is given in Appendix 1. To test whether the method of measuring the diameter of black spots is reliable, we calculated the repeatability in spot size on 199 individuals breeding in Switzerland between 1994 and 2016, which have been measured twice (range difference between the two measurements: 3-134 days) during the same breeding season. The repeatability is very high (Intra-class Correlation Coefficient = 0.85; CI 95% = 0.81-0.89).

Geographic variation in wing length and spot diameter

All the analyses described below were performed both using all the *Tyto* taxa reported in Tables 1 and 2 considered as independent observations, and, when necessary, by using phylogenetic regressions on a subsample of 28 taxa for which their phylogenetic relationships are known (see below for details).

We first examined whether *Tytonidae* living in the northern and southern hemispheres differ with respect to wing length and spot size and whether these two traits are differentially correlated with latitude. *Tyto* taxa were assigned to either hemisphere on whether the centres of their distributions are located in the northern vs. the southern hemispheres. This was also the case for the three taxa occurring on both hemispheres (*Tyto longimembris*, *Tyto alba affinis*, *Tyto furcata contempta*). We performed a linear regression with the mean of the male and female wing length means (or mean spot diameter in another model) as the dependent variable, the mean absolute latitude of the distributional range of the taxa as a covariate, and hemisphere (north vs. south) and the interaction between latitude and hemisphere as factors. We used average values within taxon rather than all the individuals as independent observations because of the very large variation in sample size among the taxa (see Table 1): an analysis including all the individuals would be mainly driven by the most represented taxon. We also note that for many specimens it was not possible to know the exact recovery location, rendering them not suitable to be used as independent observations. However, in all the analyses each datum was weighed by the relative sample size to account for heterogeneity in sampling among the taxa.

For each *Tyto* taxon, we calculated the coefficient of variation (CV) in wing length and spot diameter separately for males and females and then averaged the male and female values. A similar statistical approach was used to investigate whether the CV in these two traits covaries with latitude in both hemispheres. The CV of spot size was box-cox transformed to generate a normal distribution before the analyses. In the presence of a measurement error (ME) and constant standard deviation, the CV should decrease with increasing value of the relative measure. To check for this possible confounding factor in the analyses, we calculated the average ME in spot size using the aforementioned sample of 199 barn owls captured in Switzerland. The obtained value (ME = 0.094 mm) was used to correct the standard deviation of each taxon, and then to re-calculate the CV. This procedure takes into account for ME in the estimate of CV, because each value of standard deviation should reflect only the 'real' within taxon variability in spot size. By using such a 'weighted CV in spot diameter', the results are qualitatively identical to those ones presented below (details not show for brevity), thus indicating that they are not statistical artefacts.

Geographic variation in the covariation between wing length and spot diameter

In a second set of analyses, we carried out a linear model including mean wing length as the dependent variable, hemisphere as a factor, mean spot diameter as a covariate and their interaction.

A final set of analyses was aimed at determining the extent of the covariation between wing length and spot diameter in each of 34 *Tyto* taxa for which sufficient data were available (i.e. excluding *T. aurantia*; see Table 1 and Appendix 1). To obtain scale-independent estimates of the covariation between wing length and spot size, we centred and standardized the data within each taxon and sex with the formula [(value – mean)/SD]. We then pooled the standardized data for the males and females of each taxon and we calculated the slope of the regression of standardized wing length on standardized spot diameter. We used this approach because the sample sizes were low for some taxa and pooling the data from males and females is feasible because these phenotypes are strongly genetically correlated between the sexes (Roulin *et al.*, 2001; Roulin & Jensen, 2015). Slopes were entered as a dependent variable in a linear model that included hemisphere as a factor and mean spot diameter, mean wing length and the mean absolute latitude of the distributional range of the taxa as three covariates. Because of differences in sample size among different taxa, each datum was weighted by the number of individuals used to compute the average value.

All the linear regressions were performed using the *lm* function in the 'nlme' R package (Pinheiro *et al.*, 2014).

Relationship between spot diameter and wing length in males and females

To examine how wing length and spot diameter covary within each sex, we generated a linear mixed model by pooling all the measured individuals for which we have the information on both wing length and spot diameter (N = 7224). This model included *Tyto* taxa and the interaction between *Tyto taxa* and sex as random factors, wing length as the dependent variable, sex as a factor, spot diameter as a covariate, and their interaction. This analysis was performed with the function *lmer* in the 'lme4' R package (Bates et al. 2014).

Phylogenetic analyses

The analyses described above have the drawback of not controlling for the phylogenetic relationships between *Tyto* taxa. A comprehensive phylogeny of the *Tytonidae* family is now available (Uva *et al.* 2018). Unfortunately, because of practical limitations to obtain samples suitable for genetic analyses for all the *Tyto* taxa across the world (especially from taxa limited to small archipelagos), it is not complete. Hence, out of the 35 *Tyto* taxa included in the present study, the phylogenetic relationships of only 28 taxa are known. We have computed a molecular phylogeny with a subset of those 28 taxa (Fig. S1), using the data and methods provided by Uva *et al.* (2018).

Using this phylogenetic tree, we tested whether there was a significant phylogenetic signal in the phenotypic traits examined in our study. This was done using the function *phylosig* in the R package 'Phytools' (Revell 2012). These analyses showed a significant phylogenetic signal in spot size ($\lambda = 0.582$, $P = 0.02$) and in CV in wing length ($\lambda = 0.414$, $P = 0.033$), but not in wing length (Pagel's $\lambda = 6 \times 10^{-5}$, $P = 0.99$), in CV in spot size ($\lambda = 6 \times 10^{-5}$, $P = 0.99$) and in the slope between wing length and spot size ($\lambda = 7 \times 10^{-5}$, $P = 0.99$). However, it is important to note that the assumptions of phylogenetic independence in linear models are on the residuals not the data itself (Revell 2010). We therefore computed Pagel's λ also to estimate the phylogenetic signal in the residuals of all the regressions described above. This was done by both fitting phylogenetic regression models in which the error structure of the residuals was flexible using the 'pgls' function in the R package *caper* (Orme 2013), and also by calculating the residuals of the phylogenetic models using the function 'phyl.resid' implemented in the R package *phytools* (Revell 2010, 2012). When no phylogenetic signals in the residuals emerged, we presented in the Results section only the analyses performed without a phylogenetic correction (see Revell 2010), and included the λ value of the residuals to justify the use of a non-phylogenetic model. Conversely, when the residuals showed a significant phylogenetic dependence, we provided the results of both the linear regression model and the phylogenetic regression model because of the difference in the number of taxa included in each analysis. To account for heterogeneity in sampling among taxa, following Garamszegi & Møller (2010, 2012), the final phylogenetically-corrected models were run by using the R package 'pGLS' including a matrix framework. Such a variance-covariance matrix combined variance factors due to phylogenetic relationships and weight effects as error terms in the form of the equation Q=V+W, where V is the phylogeny matrix, W is the diagonal matrix of 1/weights (Martins & Hansen, 1997). Log_{10}-transformed sample sizes were used as weights, thus de-emphasizing observations with small sample size.

Results

Geographic variation in wing length and spot diameter

Mean wing length for each *Tyto* taxon was not associated with hemisphere ($F_{1,31}$ = 1.28, P = 0.27), absolute latitude ($F_{1,31}$ = 1.84, P = 0.18) or their interaction ($F_{1,31}$ = 3.28, P = 0.08). The residuals of the model did not show any significant phylogenetic dependence (Pagel's λ = 0.210, P = 0.44). In contrast, the interaction between hemisphere and absolute latitude significantly influenced the coefficient of variation in wing length (hemisphere: $F_{1,31}$ = 0.37, P = 0.54; absolute latitude: $F_{1,31}$ = 4.13, P = 0.06; interaction: $F_{1,31}$ = 5.49, P = 0.026; Pagel's

$\lambda = 0.571$, $P < 0.05$). In the northern hemisphere, wing length was significantly more variable close to the equator than in temperate regions ($\beta = -0.003 \pm 0.001$, $n = 23$, $P = 0.027$, Figure 1a), which was not the case in the southern hemisphere ($\beta = 0.003 \pm 0.002$, $n = 16$, $P = 0.14$). This pattern of differential variation in the coefficient of variation in wing length between hemispheres was confirmed by phylogenetic regressions (hemisphere × absolute latitude interaction: $t = 2.12$, $P = 0.044$).

As previously reported in another study (Roulin *et al.*, 2009) with a smaller within taxon sample size ($n = 4827$ vs. 7893 in the present study), there was a significant interaction between absolute latitude and hemisphere for mean spot diameter (hemisphere: $F_{1,31} = 10.13$, $P = 0.03$; absolute latitude: $F_{1,31} = 6.87$, $P = 0.013$; interaction: $F_{1,31} = 4.22$, $P = 0.048$; Pagel's $\lambda = 0.660$, $P < 0.05$). In the northern hemisphere, *Tyto* taxa display, on average, larger spots closer to the equator ($\beta = -0.28 \pm 0.07$, $n = 23$, $P = 0.001$; controlling for phylogeny: $\beta = -0.026 \pm 0.007$, $t = -3.69$, $n = 15$, $P = 0.003$), a relationship that does not prevail in the southern hemisphere ($\beta = -0.02 \pm 0.20$, $n = 16$, $P = 0.93$; controlling for phylogenetic relationships: β: -0.016 ± 0.012; $t = -1.33$; $n = 13$, $P = 0.21$). The box-cox transformed coefficient of variation of spot diameter increased with absolute latitude ($\beta = 0.038 \pm 0.009$, $F_{1,30} = 15.06$, $P < 0.001$) independently of hemisphere (hemisphere × absolute latitude interaction: $F_{1,30} = 1.10$, $P = 0.30$; Pagel's $\lambda = 0.001$). Barn owls are more variable with respect to spot size poleward than closer to the equator (Figure 1b).

The mean coefficient of variation in spot diameter was 10 times larger than the mean coefficient of variation in wing length (0.35 vs. 0.03; Wilcoxon matched pairs signed rank: $S = 297.50$, $P < 0.0001$). Among *Tyto* taxa, the two coefficients of variation were not significantly correlated ($r = -0.17$, $n = 34$, $P = 0.32$).

Geographic variation in the covariation between wing length and spot diameter

In a comparative analysis considering the mean trait values for each *Tyto* taxon with more than 10 measured individuals, a linear regression showed a significant positive covariation between wing length (dependent variable) and spot diameter ($\beta = 34.74 \pm 4.91$; $F_{1,31} = 50.08$, $P < 0.001$), but it does not differ between hemispheres ($F_{1,31} = 2.81$, $P = 0.10$; hemisphere × absolute latitude interaction: $F_{1,31} = 0.76$, $P = 0.39$; Figure 2; Pagel's $\lambda = 0.001$).

The slope of the regression of wing length against spot diameter for each *Tyto* taxon was significantly more positive in *Tytonidae* displaying larger spots on average ($F_{1,29} = 23.18$, $P < 0.001$, Figure 3a), in taxa located closer to the equator ($F_{1,29} = 16.57$, $P < 0.001$), marginally non-significantly larger in the southern than the northern hemisphere ($F_{1,29} = 3.58$, $P = 0.07$), and was not related to mean wing length ($F_{1,28} = 1.80$, $P = 0.19$). The residuals of the model did not show any significant phylogenetic dependence (Pagel's $\lambda = 0.001$).

Relationship between spot diameter and wing length in males and females

In a linear mixed model considering all 7224 individuals from 35 *Tyto* taxa, wings were longer in individuals with larger spots ($F_{1,7183} = 77.61$, $P < 0.0001$), a relationship that differed significantly between the sexes ("sex × spot diameter" interaction: $F_{1,4615} = 17.90$, $P < 0.0001$). The random variable "*Tyto* taxa" explained 89.97% of the variation and the interaction "sex × spot diameter" was significant because the relationship between wing length and spot diameter was 2.36 times stronger in females (similar linear mixed model: $F_{1,3687} = 72.16$, $P = <0.0001$; estimate: 0.248 ± 0.029) than in males ($F_{1,3484} = 14.23$, $P = 0.0002$; estimate: 0.105 ± 0.027).

Discussion

The *Tytonidae* family is globally widespread except in Antarctica, and individuals everywhere vary with respect to the size of the black spots on the tips of their ventral body feathers. Therefore, this group of birds provides an opportunity to test whether the covariation between spot size and other phenotypic traits differs between populations. While several researchers have examined whether animals are more colourful and sexually dichromatic close to the equator than in temperate regions (see Introduction), we are not aware of any study investigating whether ornaments better covary with individual quality in the tropical or temperate zones, and in the southern or northern hemispheres. In the present study, we showed that different taxa of the Tytonidae family display larger spots near the Equator if located in the northern hemisphere, and that the within-taxon variability in spot diameter increases with latitude, irrespectively of the hemisphere where each taxon live. In addition, we found that barn owls displaying large black spots had longer wings than individuals displaying smaller spots, a covariation that was stronger in equatorial regions.

Why is melanin-based plumage colouration in the form of black feather spots associated with wing length? A possibility is that barn owls assess the size of the spots on conspecifics during mate choice (Roulin, 1999; Roulin & Altwegg, 2007) or social interactions, in order to gain information about various qualities, including body size. Another possibility is that spot size is related to numerous phenotypic traits (Roulin & Ducrest 2011) including wing length. Assessing spot size instead of directly assessing body size (or other aspects of phenotype) may be easier because the degree of inter-individual variation in spot size is ten times more pronounced than the variation in wing length, which is consistent with the expectation that sexually selected traits (e.g., spot size) are phenotypically more variable than traits under strong natural selection (e.g., wing length in an aerial predator like the barn owl group) (Pomiankowski & Møller, 1995). If we would scale spot size and wing length, it would imply that a difference of one unit in wing length is associated with ten units in spot diameter. Signalling body size with a secondary sexual character may be adaptive because body size is a key morphological trait in many animals, including owls, that influence the performance of multiple activities, such as hunting (Hakkarainen & Korpimäki, 1991), egg production (Hakkarainen & Korpimäki, 1993) and social interactions (Tschumi *et al*., 2014).

In raptors and owls, a small body size can be beneficial, particularly in males, when hunting agile prey (Hakkarainen & Korpimäki, 1991; Krüger, 2005), whereas in females, a large body size may especially benefit social interactions and enable the production of large eggs (Sergio *et al*., 2007). If spot size covaries with body size, particularly in the tropics, is this trait mainly used to assess the size of males or females? The covariation between these two traits is stronger in females than in males, suggesting that spot size may more accurately reflect body size in females than in males in the tropics and, possibly, in the southern hemisphere. This is consistent with previous studies showing that positive selection on female spot size is more intense than negative selection on male spot size (Roulin *et al.*, 2010; Roulin & Ducrest, 2011). The next step will be to investigate how variation in body size correlates with fitness components in males and females as the results of the present study, and previous findings of other species (Lundberg 1986; McDonald et al. 2014), suggest that a large body size may be more important to females than a small body size is to males.

Selection to display larger spots, as a possible signal of quality, seems to be more intense in equatorial than temperate regions given that *Tyto* taxa display larger black spots at lower latitudes in the northern hemisphere (Roulin *et al.*, 2009; present study). This is consistent with the finding that variation in spot size is reduced near the equator (Figure 1b), which is potentially an outcome of intense selection eroding genetic variation. Albeit black feather spots might be indirectly linked with thermoregulatory functions (Roulin & Randin 2015, 2016) and could be associated with genes that regulate thermoregulation (Dreiss *et al.*, 2016), the main function of plumage spottiness seems to be to signal various aspects of individual quality (Roulin et al. 2000, 2001a, 2001b; Roulin & Ducrest 2011). Although this signalling function may be particularly important near the equator, studies performed in temperate Central Europe (Roulin & Ducrest, 2011) and Middle East (Peleg *et al.*, 2014) showed that spot size covaries with multiple phenotypes far from the equator and on the northern hemisphere.

The fact that spot size is not significantly correlated with body size in many *Tyto* populations indicates that the covariation between these two phenotypes is not merely a consequence of allometry. Therefore, it appears that selection on these two phenotypes is mainly correlated in tropical regions rather than in temperate ones. It now remains to identify the selective forces. Even if we cannot discard the possibility that the covariation between wing length and spot size within populations/taxa could be partly environmentally mediated (e.g., for spottier birds, being in better condition would allow them to grow longer wings), it is likely that the covariation is partly under genetic control. This is supported by the result of cross-fostering experiments showing a heritability estimate of $h^2 = 0.48 \pm 0.17$ for wing length (Roulin, 2006) and 0.67 ± 0.03 for spot size (Roulin & Jensen, 2015). Assuming that these two traits are heritable in most *Tytonidae*, the present study suggests that their genetic correlation can evolve towards stronger values such as those closer to the equator.

As a final cautionary note, we acknowledge the reader that, since phylogenetic relationships are generally positively related to the geographical distances between taxa (see Roulin *et al.*, 2009), the inclusion of geographical information in the models might have potentially obscured the effect of phylogeny. However, most of the analysed taxa are subspecies of the cosmopolitan *Tyto alba* group, which is split into three major evolutionary lineages, corresponding to distinct continental regions of the world. Therefore, very similar latitudes do not always correspond to a high phylogenetic relatedness (e.g. *T. a. thomensis* and *T. f. punctatissima* have a very similar latitudinal distribution, but they are found in two different continents), thus making this possibility as an unlikely one.

In conclusion, the finding that a melanin-based plumage trait involved in mate choice is more strongly correlated with a phenotypic trait (here wing length) near the equator suggests that this trait plays a more important signalling function in these regions. Alternatively, selection on spot size may be more strongly correlated with selection exerted on wing length in these regions. The next step will be to identify the ecological factors that account for these geographic patterns.

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References

Aliabadian, M., Alaei-Kakhki, N., Mirshamsi, O., Nijman, V. & Roulin, A. 2016. Phylogeny, biogeography, and diversification of barn owls (Aves, Strigiformes). *Biol. J. Linn. Soc.* **119:** 904-918.

Baker, J.R. 1938. The relation between latitude and breeding season in birds. *J. Zool.* **A108:** 557-582.

Bailey, S.F. 1978. Latitudinal gradients in colors and patterns of passerine birds. Condor **80:** 372-381.

Bates, D., Maechler, M., Bolker, B., & Walker, S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version, **1(7),** 1-23.

Cardillo, M. 2002. The life-history basis of latitudinal diversity gradients: how do species traits vary from the poles to the equator? *J. Anim. Ecol.* **71:** 79-87.

Charter, M., Peleg, O., Leshem, Y. & Roulin, A. 2012. Similar patterns of local barn owl adaptation in the Middle East and Europe with respect to melanic coloration. *Biol. J. Linn. Soc*. **106:** 447-454.

Dale, J., Dey, C.J., Delhey, K., Kempenaers, B., Valcu, M. 2015. The effects of life history and sexual selection on male and female plumage colouration. *Nature* **527:** 367-370.

Dalrymple, R.L., Kemp, D.J., Flores-Moreno, H., Laffan, S.W., White, T.E., Hemmings, F.A., Tindall, M.L. & Moles, A.T. 2015. Birds, butterflies and flowers in the tropics are not more colourful than those at higher latitudes. *Glob. Ecol. Biogeogr*. **24:** 1424-1432.

Doutrelant, C., Paquet, M., Renoult, J.P., Grégoire, A., Crochet, P.-A. & Covas, R. 2016. Worldwide patterns of bird colouration on islands. *Ecol. Lett.* **19:** 537-545.

Dreiss, A.N., Séchaud, R., Béziers, P., Villain, N., Genoud, M., Almasi, B., Jenni, L. & Roulin, A. 2016. Social huddling and physiological thermoregulation are related to melanism in the nocturnal barn owl. *Oecologia* **180:** 371-381.

Fairbairn, D.J. 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Ann. Rev. Ecol. Syst.* **28:** 659-687.

Garamszegi, L.Z., & Møller, A.P. 2010. Effects of sample size and intraspecific variation in phylogenetic comparative studies: a meta‐analytic review. *Biol. Rev.* **85:** 797-805.

Garamszegi, L.Z., & Møller, A.P. (2012). The interspecific relationship between prevalence of blood parasites and sexual traits in birds when considering recent methodological advancements. *Behav. Ecol. Sociobiol*. **66:** 107-119.

Gill, F. & Donsker, D. 2018. IOC World Bird List (v 8.1), DOI: 10.14344/IOC.ML.8.1.

Guernier, V., Hochberg, M.E. & Guégan, J.-F. 2004. Ecology drives the worldwide distribution of human diseases. *Plos Biol*. **2:** 0740-0746.

Hakkarainen, H. & Korpimäki, E. 1991. Reversed sexual size dimorphism in Tengmalm's owl: is small male size adaptive? *Oikos* **61:** 337-346.

Hakkarainen, H. & Korpimäki, E. 1993. The effect of female body size on clutch volume of Tengmalm's owls *Aegolius funereus* in varying food conditions. *Ornis Fenn.* **70:** 189-195.

Hamilton, W.D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science* **218:** 384-387.

Jetz, W., Sekercioglu, C.H. & Böhning-Gaese, K. 2008. The worldwide variation in avian clutch size across species and space. *Plos Biol.* **6:** e303.

Kaluthota, C, Brinkman, B.E., dos Santos, E.B. & Rendall, D. 2016. Transcontinental latitudinal variation in song performance and complexity in house wrens (*Troglodytes aedon*). *Proc. R. Soc. Lond.* **B283:** 20152765.

Krüger, O. 2005. The evolution of reversed sexual size dimorphism in hawks, falcons and owls: a comparative study. *Evol. Ecol.* **19:** 467-486.

Lundberg, A. 1986. Adaptive advantages of reversed sexual size dimorphism in European owls. *Ornis Scand*. **17**: 133-140.

Madrigal, L. & Kelly, W. 2007. Human skin-color sexual dimorphism: a test of the sexual selection hypothesis. *Am. J. Phys. Anthropol*. **132:** 470-482.

Martin, T.E. 1996. Life history evolution in tropical and south temperate birds. What do we really know? *J. Avian Biol.* **27:** 263-271.

Martins E.P., & Hansen T.F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149:** 646–667.

McDonald, P.G., Olsen, P.D., & Cockburn, A. 2004. Selection on body size in a raptor with pronounced reversed sexual size dimorphism: are bigger females better?. *Behav. Ecol.* **16**: 48-56.

McNamara, J.M., Barta, Z., Wikelski, M. & Houston, A.I. 2008. A theoretical investigation of the effect of latitude on avian life histories. *Am. Nat*. **172:** 331-345.

Møller, A.P. 1995. Sexual selection in the barn swallow (*Hirundo rustica*). V. Geographic variation in ornament size. *J. Evol. Biol.* **8:** 3-19.

Møller, A.P. 1998. Evidence of larger impact of parasites on hosts in the tropics: investment in immune function within and outside the tropics. *Oikos* **82:** 265-270.

Orme, D. 2013. The caper package: comparative analysis of phylogenetics and evolution in R. R package version, **5:** 1-36.

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. 2014. R Core Team (2014) nlme: linear and nonlinear mixed effects models. R package version **3:** 1-117.

Peleg, O., Charter, M., Leshem, Y., Izhaki, I. & Roulin, A. 2014. Conditional association between melanism and personality in Israeli barn owls. *Bird Study* **61:** 572-577.

Pomiankowski, A. & Møller, A.P. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond.* **B260:** 21-29.

Remes, V. & Matysioková, B. 2016. Survival to independence in relation to pre-fledging development and latitude in songbirds across the globe. *J. Avian Biol.* **47:** 610-618.

Revell, L. 2010. Phylogenetic signal and linear regression on species data. *Meth. Ecol. Evol.*, **1:** 319-329.

Revell L. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Meth. Ecol. Evol.* **3**: 217-223.

Roulin, A. 1999. Nonrandom pairing by male barn owls *Tyto alba* with respect to a female plumage trait. *Behav. Ecol.* **10:** 688-695.

Roulin, A. 2006. Linkage disequilibrium between a melanin-based colour polymorphism and tail length in the barn owl. *Biol. J. Linn. Soc*. **88:** 475-488.

Roulin, A. 2016. Evolutionary trade-off between naturally- and sexually-selected melanin-based colour traits in worldwide barn owls and allies. *Biol. J. Linn. Soc.* **199:** 455-476.

Roulin, A. & Altwegg, R. 2007. Breeding rate is associated with pheomelanism in male and with eumelanism in female barn owls. *Behav. Ecol.* **18:** 563-570.

Roulin, A., Altwegg, R., Jensen, H., Steinsland, I. & Schaub, M. 2010. Sex-dependent selection on an autosomal melanic female ornament promotes the evolution of sex ratio bias. *Ecol. Lett.* **13:** 616-626.

Roulin, A. & Ducrest, A.-L. 2011. Association between melanism, physiology and behaviour: a role for the melanocortin system. *Eur. J. Pharmacol.* **660:** 226-233.

Roulin, A. & Jensen, H. 2015. Sex-linked inheritance, genetic correlations and sexual dimorphism in three melanin-based color traits in the barn owl. *J. Evol. Biol.* **28:** 655-666.

Roulin, A. & Randin, C.F. 2015. Gloger's rule in North American barn owls. *Auk* **132**: 321–332.

Roulin, A. & Randin, C.F. 2016. Barn owls display larger black feather spots in cooler regions of the British Isles. *Biol. J. Linn. Soc*. **119**: 445–454.

Roulin, A., Jungi, T.W., Pfister, H. & Dijkstra, C., 2000. Female barn owls (*Tyto alba*) advertise good genes. *Proc. R. Soc. Lond. B*. **267**: 937-941.

Roulin, A., Riols, C., Dijkstra, C. & Ducrest, A.-L. 2001a. Female plumage spottiness and parasite resistance in the barn owl (*Tyto alba*). *Behav. Ecol*. **12**: 103–110.

Roulin, A., Riols, C., Dijkstra, C. & Ducrest, A.-L. 2001b. Female- and male-specific signals of quality in the barn owl. *J. Evol. Biol.* **14:** 255-267.

Roulin, A. & Salamin, N. 2010. Insularity and the evolution of melanism, sexual dichromatism and body size in the worldwide-distributed barn owl. *J. Evol. Biol.* **23:** 925-934.

Roulin, A., Wink, M. & Salamin, N. 2009. Selection on a eumelanic ornament is stronger in the tropics than in temperate zones in the worldwide-distributed barn owl. *J. Evol. Biol.* **22:** 345-354.

Russell, E., Yom-Tov, Y. & Geffen, E. 2004. Extended parental care and delayed dispersal: northern, tropical, and southern passerines compared. *Behav. Ecol.* **15:** 831-838.

Sergio, F., Blas, J., Forero, M.G., Donazar, J.A. & Hiraldo, F. 2007. Size-related advantages for reproduction in a slightly dimorphic raptor: opposite trends between the sexes. *Ethology* **113:** 1141-1150.

Spottiswoode, C, Møller, A.P. 2004. Extrapair paternity, migration, and breeding synchrony in birds. *Behav. Ecol.* **15:** 41-57.

Tschumi, M., Schaub, M. & Arlettaz, R. 2014. Territory occupancy and parental quality as proxies for spatial prioritization of conservation. *Plos One* **9:** e97679.

Uva, V.; Päckert, M., Cibois, A., Fumagalli, L. & Roulin, A. Comprehensive molecular phylogeny of barn owls and relatives (Family: Tytonidae), and their six major Pleistocene radiations. *Mol. Phylogenet. Evol*. 2018

Wiersma, P., Muñoz-Garcia, A., Walker, A. & Williams, J. B. 2007. Tropical birds have a slow pace of life. *Proc. Natl. Acad. Sci. USA* **104:** 9340-9345.

Wink, M., El-Sayed, A.-A., Sauer-Gürth, H., Gonzalez, J. 2009. Molecular Phylogeny of Owls (Strigiformes) Inferred from DNA Sequences of the Mitochondrial Cytochromeband the NuclearRAG-1gene. *Ardea* **97:** 581-591.

Table 1. Number of male and female *Tyto* taxa considered in the present study. For each taxon and sex, the geographic distribution, sample size, mean diameter of the black spots on the breast, mean wing length and coefficient of variation (CV) in wing length and spot diameter are given.

		Sample size		Spot diameter (0.1 mm)		Wing length (mm)		Mean CV	Mean CV
Tyto taxa	Distribution	Male	Female	Male	Female	Male	Female	wing length	spot diameter
Tyto alba affinis	Africa	328	356	2.05	2.19	291.2	290.9	0.033	0.261
Tyto alba alba/guttata	Europe	1223	1298	0.83	1.17	283.6	283.8	0.028	0.597
Tyto alba detorta	Cape Verde	8	17	1.81	2.29	285.8	286.5	0.030	0.258
Tyto alba erlangeri	Middle East	98	125	0.54	1.07	291.5	291.8	0.033	0.667
Tyto alba ernesti	Corsica, Sardinia	64	51	0.19	0.52	288.4	287.6	0.025	1.458
Tyto alba gracilirostris	Canary Islands	8	6	0.96	1.33	250.3	250.3	0.036	0.247
Tyto alba hypermetra	Madagascar	18	21	1.57	1.69	301.6	300.4	0.026	0.22
Tyto alba schmitzi	Madeira	10	18	1.32	1.33	270.3	273.8	0.020	0.218
Tyto alba thomensis	Sao Tomé	5	9	2.26	2.39	254.4	256.0	0.036	0.119
Tyto aurantia	New Britain Island	0	5		3.76		246.8		
Tyto capensis	Africa	72	79	2.71	2.87	325.4	325.3	0.032	0.246
Tyto furcata bargei	Curaçao	9	5	1.12	1.52	247.8	254.0	0.020	0.233
Tyto furcata contempta	South America	58	61	1.91	2.3	311.1	310.8	0.039	0.283
Tyto furcata furcata	Cuba, Jamaica	63	51	1.21	1.91	331.9	334.9	0.026	0.407
Tyto furcata glaucops	Hispaniola	17	15	1.63	1.89	256.1	263.9	0.028	0.163
Tyto furcata guatemalae	Central America	43	33	2.17	2.52	320.0	324.2	0.029	0.249
Tyto furcata hellmayri	South America	19	30	1.69	2.08	318.5	318.4	0.041	0.197
Tyto furcata insularis	Grenadines	13	25	2.52	2.66	234.1	239.4	0.034	0.179
Tyto furcata lucayana	Bahamas, Bermuda	8	6	1.45	1.62	328.1	339.8	0.029	0.391
Tyto furcata pratincola	North America	870	934	1.82	2.27	333.7	338.8	0.031	0.247

Tyto furcata punctatissima	Galapagos	12	21	2.25	2.65	223.8	225.2	0.031	0.165
Tyto furcata tuidara	South America	121	120	1.58	1.95	304.8	308.0	0.036	0.307
Tyto javanica crassirostris	Papua New Guinea	11	8	1.35	1.41	282.7	279.9	0.026	0.304
Tyto javanica delicatula	Australia	282	293	1.47	1.83	279.7	280.9	0.023	0.296
Tyto javanica everetti	Timor Island	8	10	1.26	1.29	288.8	281.3	0.037	0.386
Tyto javanica interposita	Vanuatu Island	10	10	1.39	1.32	271.0	268.8	0.023	0.479
Tyto javanica javanica	South-East Asia	65	73	1.78	1.79	303.4	303.4	0.032	0.214
Tyto javanica lifuensis	New Caledonia	17	24	0.94	1.05	266.3	265.8	0.02	0.532
Tyto javanica lulu	Fiji, Samoa, Tonga	43	54	0.88	1.35	268.9	267.4	0.022	0.453
Tyto javanica meeki	Papua New Guinea	14	0	0.74		278.9		0.027	0.563
Tyto javanica rosenbergii	Celebes	18	14	2.62	2.72	337.0	337.6	0.031	0.192
Tyto javanica stertens	India	61	41	1.58	1.57	293.3	292.7	0.031	0.252
Tyto longimembris	Asia	72	84	1.60	2.26	328.9	340.4	0.051	0.374
Tyto novaehollandiae castanops	Tasmania	89	69	3.09	3.49	324.3	350.1	0.045	0.252
Tyto novaehollandiae novaehollandiae	Australia	74	96	2.59	3.26	306.3	329.4	0.047	0.352
Total		3831	4062						

Table 2. Relationship between wing length and plumage spot diameter in barn owls (*Tyto alba*, *T. javanica*, *T. furcata*), grass owls (*T. capensis* and *T. longimembris*) and masked owls (*T. novaehollandiae*). All values were centred for each sex and *Tyto* taxon to compare estimates.

	F	*df*	*P*	Estimated slope
Tyto alba affinis	37.67	1,628	<0.0001	0.2322
Tyto alba alba/guttata	0.01	1,2224	0.92	-0.0021
Tyto alba detorta	0.58	1,23	0.45	0.1573
Tyto alba erlangeri	0.71	1,207	0.40	-0.0582
Tyto alba ernesti	4.31	1,108	0.04	-0.1991
Tyto alba gracilirostris	2.32	1,12	0.15	-0.4022
Tyto alba hypermetra	0.23	1,30	0.63	-0.0808
Tyto alba schmitzi	0.28	1,26	0.60	0.1031
Tyto alba thomensis	0.22	1,12	0.65	0.1343
Tyto capensis	30.58	1,136	<0.0001	0.4237
Tyto javanica crassirostris	0.55	1,16	0.47	0.1795
Tyto javanica delicatula	0.49	1,561	0.49	-0.0293
Tyto javanica everetti	1.21	1,7	0.31	0.3615
Tyto javanica interposita	4.00	1,18	0.06	0.4264
Tyto javanica javanica	7.08	1,119	0.009	0.2353
Tyto javanica lifuensis	0.27	1,36	0.61	0.0848
Tyto javanica lulu	0.02	1,90	0.89	-0.0144
Tyto javanica meeki	0.22	1,12	0.65	0.1330
Tyto javanica rosenbergii	2.97	1,29	0.10	0.3049
Tyto javanica stertens	0.05	1,96	0.83	0.0225

Tyto furcata bargei	0.23	1,10	0.65	0.1544
Tyto furcata contempta	3.18	1,100	0.08	0.1669
Tyto furcata furcata	1.78	1,109	0.18	0.1267
Tyto furcata glaucops	2.54	1,30	0.12	0.2795
Tyto furcata guatemalae	0.13	1,68	0.72	0.0421
Tyto furcata hellmayri	2.33	1,43	0.13	0.2217
Tyto furcata insularis	1.05	1,36	0.31	0.1684
Tyto furcata lucayana	2.10	1,11	0.17	-0.3845
Tyto furcata pratincola	16.37	1,1671	<0.0001	0.0985
Tyto furcata punctatissima	2.99	1,30	0.09	0.3030
Tyto furcata tuidara	0.38	1,201	0.54	0.0415
Tyto longimembris	1.02	1,140	0.31	-0.0842
Tyto novaehollandiae castanops	8.90	1,151	0.0033	0.2342
Tyto novaehollandiae novaehollandiae	33.95	1,162	<0.0001	0.4106

Figure legends

Figure 1. Coefficient of variation in (a) wing length in the northern hemisphere and (b) the size of black breast spots in relation to mean absolute latitude in *Tytonidae*. In panel (b), open and closed circles represent *Tyto* taxa for which the centres of their distributions are located in the southern **and northern** hemispheres, respectively.

Figure 2. Relationship between mean wing length and mean spot diameter in *Tyto* taxa for which the centres of their distributions are in the southern hemisphere (open circles) and northern hemisphere (closed circles).

Figure 3. Slope of the regression of the size of the black breast spots against wing length in *Tyto* taxa in relation to (a) mean black breast spot size, and (b) mean absolute latitude. Panel (a) shows that, in *Tyto* taxa with larger black spots, individuals with large spots have longer wings than individuals with smaller spots. In contrast, there was no association between individual spot size and wing length in *Tyto* taxa displaying relatively small spots (slopes are close to zero, on average). Panel (b) shows that the relationship between individual spot size and wing length was stronger in *Tyto* taxa located near the equator. Closed and open circles represent *Tyto* taxa for which the centres of their distributions are located in the northern or southern hemisphere, respectively.

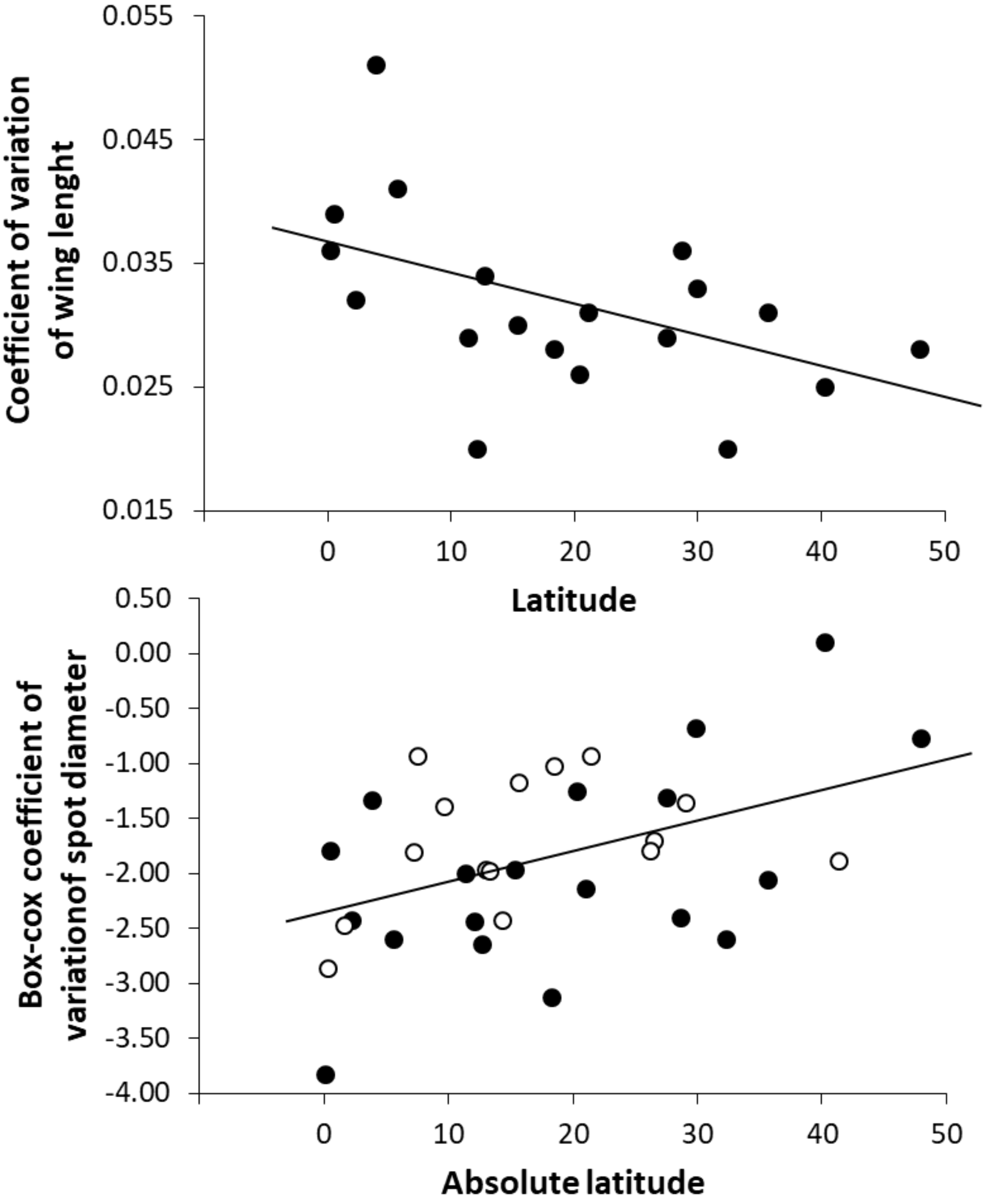
Coefficient of variation of wing lenght
0.055
0.045
0.035
0.025
0.015
0
10
20
30
40
50
Latitude
Box-cox coefficient of variationof spot diameter
0.50
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
-4.00
0
10
20
30
40
50
Absolute latitude

Figure 1.

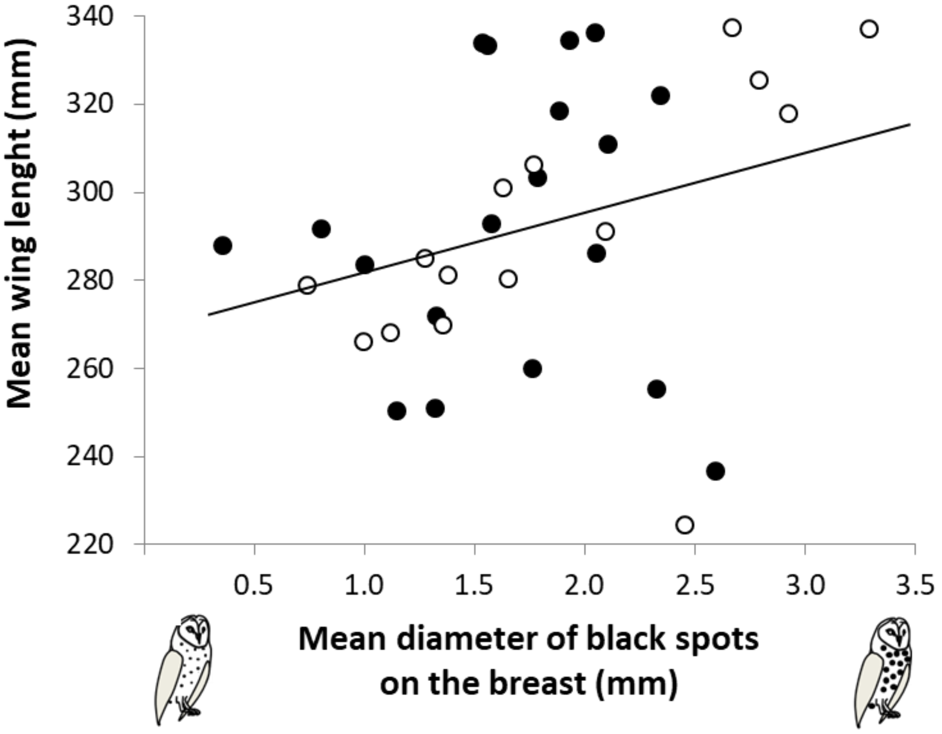
340
320
300
280
260
240
220
Mean wing lenght (mm)
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Mean diameter of black spots on the breast (mm)

Figure 2.

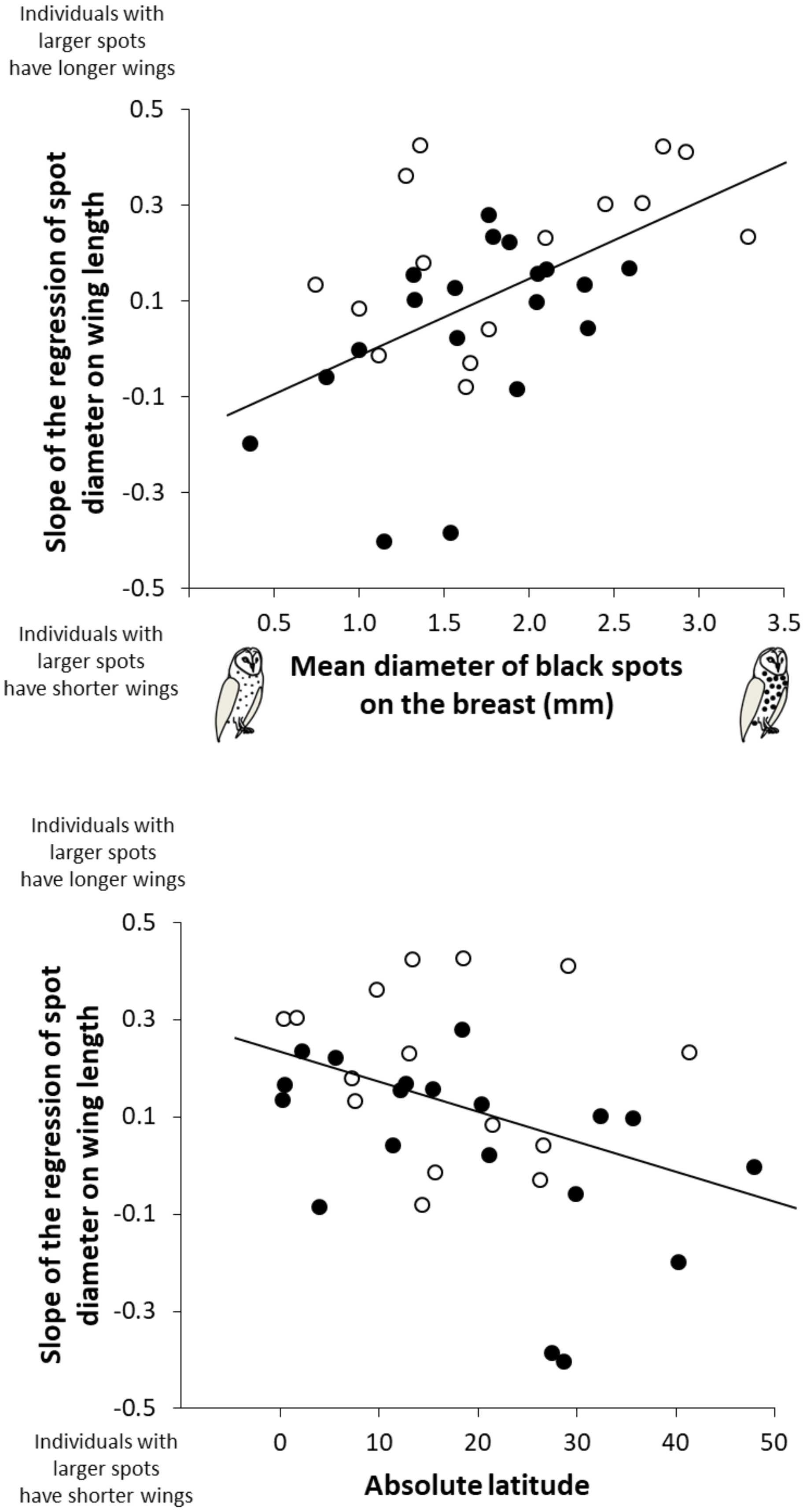
Individuals with larger spots have longer wings
0.5
0.3
0.1
-0.1
-0.3
-0.5
Slope of the regression of spot diameter on wing length
Individuals with larger spots have shorter wings
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Mean diameter of black spots on the breast (mm)
Individuals with larger spots have longer wings
0.5
0.3
0.1
-0.1
-0.3
-0.5
Slope of the regression of spot diameter on wing length
Individuals with larger spots have shorter wings
0
10
20
30
40
50
Absolute latitude

Figure 3.

Appendix 1. Number of *Tytonidae* skins measured in each of 136 natural history museums. Total number of skins used in the present study is reported.

Country (State)	City	Museum	Number *Tytonidae* skins
Australia	Adelaide	South Australian Museum	112
Australia	Brisbane	Queensland Museum	59
Australia	Canberra	CSIRO Ecoystem Sciences	116
Australia	Darwin	Museum and Art Gallery of the Northern	4
Australia	Melbourne	Museum Victoria	168
Australia	Perth	Western Australian Museum	69
Australia	Sydney	Australian Museum	154
Austria	Vienna	Naturhistorisches Museum Wien	50
Belgium	Bruxelles	Royal Belgian Institute of Natural Sciences	96
Belgium	Tervuren	Royal Museum for Central Africa	111
Canada (Ontario)	Ottawa	Musée canadien de la Nature	10
Canada (Ontario)	Toronto	Royal Ontario Museum, Department of	65
Crete	Heraklion	Natural History Museum of Crete	61
Czech Republic	Praha	Narodni Muzeum	10
Denmark	Copenhagen	Natural History Museum of Denmark	50
Finland	Helsinki	Finnish Museum of Natural History	13
France	Lyon	Musée des Confluences	1
France	Nantes	Musée d'histoire naturelle de Nantes	17
France	Paris	Musée national d'Histoire naturelle	111
Germany	Berlin	Museum für Naturkunde	67
Germany	Bonn	Alexander Koenig Research Museum	155
Germany	Braunschweig	Staatliches Naturhistorisches Museum	19
Germany	Bremen	Ubersee-Museum	32
Germany	Dresden	Senckenberg Natural History Museum	73
Germany	Frankfurt	Senckenberg Naturmuseum Frankfurt	58
Germany	Görlitz	Senckenberg Museum of Natural History	14
Germany	Halberstadt	Museum Heineanum	79
Germany	Halle	Natural History Museum	10
Germany	Hamburg	Biozentrum Grindel und Zoologisches	33
Germany	Kassel	Museum of Natural History in the	33
Germany	München	Zoologisches Staatssammlung München	41

Germany	Wilhelmshaven	Institut für Vogelforschung	18
Great Britain	Bristol	Bristol Museum and Art Gallery	15
Great Britain	Cambridge	University Museum of Zoology	19
Great Britain	Cardiff	National Museum Cardiff	15
Great Britain	Edinburgh	National Museums of Scotland, Royal	72
Great Britain	Glasgow	Natural History Museum	1
Great Britain	Inverness	Inverness Museum and Art Gallery	1
Great Britain	Liverpool	World Museum Liverpool	41
Great Britain	Manchester	Manchester Museum	9
Great Britain	Newcastle	Great North Museum	28
Great Britain	Perth	Perth Museum and Art Gallery	3
Great Britain	Sevenoaks	Harrisson Institute	53
Great Britain	Tring	Natural History Museum at Tring	473
Ireland	Dublin	National Museum of Ireland	31
Israel	Tel Aviv	National Museum of Natural History at Tel-	51
Italy	Bergamo	Museo di Scienze Naturali Enrico Caffi	3
Italy	Bologna	Italian National Ringing Centre	98
Italy	Brescia	Museo di Storia Naturale	8
Italy	Carmagnola	Museo di Storia Naturale	14
Italy	Faenza	Museo di Storia Naturale	6
Italy	Firenze	Museo di Storia Naturale di Firenze	41
Italy	Forli	Museo Ornitologico Ferrante Foschi	9
Italy	Genova	Museo di Storia Naturale	43
Italy	Milano	Museo Civico di Storia Naturale de Milan	76
Italy	Napoli	Zoological Museum of Naples	2
Italy	Roma	Museo Civico di Zoologia di Roma	51
Italy	Terrasini	Terrasini museum	25
Japan	Abiko City	Yamashina Institute for Ornithology	12
Japan	Hyogo	National Museum of Nature and Science	6
Japan	Tuskuba	Geological Museum	3
Netherlands	Amsterdam	Zoological Museum	184
Netherlands	Leiden	National Museum of Natural History	200
Northern Ireland	Belfast	National Museums Northern Ireland	16
Norway	Oslo	Naturhistorisk museum	2
Portugal	Lisbon	Museu Nacional de Historia Natural	12
South Africa	Bloemfontein	National Museum	8

South Africa	Cape Town	Iziko South African Museum	9
South Africa	Durban	Durban Natural Science Museum	45
South Africa	East London	East London Museum	36
South Africa	Kimberly	McGregor Museum	4
South Africa	Pretoria	Transvaal museum	70
Spain	Barcelona	Museu Geologic del Seminari de Barcelona	8
Spain	Madrid	Museo Nacional de Ciencias Naturales	44
Spain	Sevilla	Estacion Biologica de Donana	102
Sweden	Stockholm	Naturhistoriska riksmuseet	98
Sweden	Uppsala	Evolutions Museet Uppsala Universitet	4
Switzerland	Basel	Naturhistorisches Museum Basel	93
Switzerland	Chaux-de-Fonds	Musée d'histoire naturelle	3
Switzerland	Fribourg	Musée d'histoire naturelle	7
Switzerland	Genève	Musée d'histoire naturelle	14
Switzerland	Lausanne	Musée cantonal de Zoologie	24
Switzerland	Neuchâtel	Musée d'histoire naturelle	22
Switzerland	Zurich	Zoological Museum of the University of	12
Tasmania	Hobart	Tasmanian Museum and Art Gallery	49
Tasmania	Lauceston	Queen Victoria Museum and Art Gallery	48
USA (Arizona)	Flagstaff	Museum of Northern Arizona	5
USA (Arizona)	Tucson	University of Arizona Museum of Natural	15
USA (California)	Berkely	The Museum of Vertebrate Zoology	114
USA (California)	Camarillo	Western Foundation of Vertebrate Zoology	118
USA (California)	Los Angeles	Natural History Museum of Los Angeles	71
USA (California)	Los Angeles	The Moore Laboratory of Zoology	14
USA (California)	Los Angeles	UCLA-Dickey Bird and Mammal Collections	23
USA (California)	Redlands	San Bernardino County Museum	12
USA (California)	San Diego	San Diego Natural History Museum	40
USA (California)	San Francisco	The California Academy of Sciences	88
USA (California)	Santa Barbara	Santa Barbara Museum of Natural History	48
USA (Colorado)	Denver	Denver Museum of Nature & Science	27
USA (Connecticut)	New Haven	Peabody Museum of Natural History at	63
USA (Delaware)	Wilmington	Delaware Museum of Natural History	37
USA (District Columbia)	Washington DC	Smithsonian Institution, Natural Museum	262
USA (Florida)	Gainesville	Florida Museum of Natural History,	43
USA (Hawaii)	Honolulu	Bishop Museum	20

USA (Illinois)	Chicago	The Chicago Academy of Sciences and its	15
USA (Illinois)	Chicago	The Field Museum of Natural History	230
USA (Kansas)	Lawrence	Natural History Museum & Biodiversity	39
USA (Louisiana)	Bâton Rouge	Louisiana Museum of Natural Science	79
USA (Massachusetts)	Cambridge	Harward Museum of Natural History	157
USA (Michigan)	Ann Arbor	University of Michigan, Museum of	80
USA (Michigan)	East Lansing	Michigan State University Museum	12
USA (Minnesota)	Minneapolis	The University of Minnesota, Bell Museum	11
USA (Montana)	Missoula	Philip L. Wright Zoological Museum	2
USA (Nebraska)	Lincoln	University of Nebraska State Museum	12
USA (New Mexico)	Albuquerque	Museum of Southwestern Biology	47
USA (New Mexico)	Las Cruces	The Vertebrate Museum, New Mexico	7
USA (New York)	Buffalo	Buffalo Museum of Science	7
USA (New York)	Cornell	Cornell University	29
USA (New York)	New York	American Museum of Natural History	640
USA (Ohio)	Cleveland	Cleveland Museum of Natural History	36
USA (Ohio)	Columbus	The Ohio State University	25
USA (Oklahoma)	Norman	Sam Noble Oklahoma Museum of Natural	12
USA (Pennsylvania)	Philadelphia	Academy of Natural Sciences	73
USA (Pennsylvania)	Pittsburgh	Carnegie Museum of Natural History	69
USA (Texas)	Alpine	Sul Ross State University	2
USA (Texas)	College Station	Texas A&M University College Station	41
USA (Texas)	Dallas	Museum of Nature and Science	7
USA (Texas)	El Paso	Centennial Museum, University of Texas	7
USA (Texas)	Lubbock	Texas Technology University	12
USA (Texas)	San Angelo	Angelo State University, Department of	7
USA (Utah)	Provo	Young University	16
USA (Utah)	Salt Lake City	Utah Museum of Natural History	3
USA (Washington)	Pullman	Washington State University, Charles R.	54
USA (Washington)	Seattle	Burke Museum	62
USA (Washington)	Tacoma	Slater Museum of Natural History	50
USA (Wisconsin)	Madison	University of Wisconsin, Zoological	10
Zimbabwe	Bulawayo	Natural History Museum of Zimbabwe	139
Total		136 museums	7’224

Fig. S1. Molecular phylogeny of the *Tyto* taxa included in the analyses. The analysis was performed in BEAST using five mitochondrial and two nuclear markers for a subset of 28 taxa, using one individual per taxon (for a full phylogeny and methods see Uva *et al*. 2018).