

## Spatial variation in the temporal change of male and female melanic ornamentation in the barn owl

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### Keywords:

intralocus genetic conflict;  
melanin-based colouration;  
sexual dimorphism;  
sexually antagonistic selection.

### Abstract

Because the magnitude of selection can vary between sexes and in space and time, sexually antagonistic selection is difficult to demonstrate. In a Swiss population of barn owls (*Tyto alba*), a heritable eumelanic colour trait (size of black spots on ventral feathers) was positively selected with respect to yearling survival only in females. It remains unclear whether the absence of negative selection in males is typical in this species. To tackle this issue indirectly, we measured the size of black spots in 1733 skin specimens collected by museums from 1816 to 2001 in seven European countries and in the Middle-East. The temporal change in spot size was sex- and country-specific. In males, spots became smaller particularly in three countries (Middle-East, Italy and Switzerland). In females, the size of spots increased significantly in two countries (UK and Spain) and decreased in two others (Germany and Switzerland). Because migration and phenotypic plasticity cannot explain these results, selection is the most likely cause. The weaker temporal change in spot size in females than males may be because of the combined effect of strong genetic correlation between the sexes and stronger negative selection in males than positive selection in females. We thus suggest that in the barn owl, spot size (or genetically correlated traits) is sexually antagonistically selected and that its pattern of selection may account for the maintenance of its variation and sexual dimorphism.

### Introduction

The differing reproductive roles of males and females promote sex-specific adaptations. Each sex can evolve specific strategies to maximize fitness often at the expense of the other sex. Because variation in female and male breeding success is primarily because of variation in access to resources in females and to mates in males (Bateman, 1948), males can be selected to force copulations or to be overly attractive, whereas females are selected to resist male eagerness to mate (Holland & Rice, 1998). The two sexes are therefore caught in a coevolutionary arms race where males evolve new ways to obtain mates in response to newly evolved female resistance strategies. Such so-called sexually antagonistic

selection acts either on different sex-specific traits or on the same trait differentially expressed in the two sexes. The latter situation is particularly interesting because the trait is positively selected when expressed in one sex and negatively selected when expressed in the other sex, a case of intralocus genetic conflict (Bonduriansky & Chenoweth, 2009). This conflict imposes a net cost because alleles harmful to one sex accumulate within populations owing to their beneficial effects in the other sex.

The ecological and social factors that determine the costs and benefits of expressing a sexually antagonistically selected trait may not be the same in the two sexes (Connallon *et al.*, 2010). The extent of the positive and negative selective effects of displaying a trait to values typical of the other sex or of their own sex is likely to vary spatially and temporarily (Mank *et al.*, 2010). Because the magnitude of selection exerted on males and females is not necessarily correlated through time and space, detecting positive selection in one sex and

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negative selection in the other sex (i.e. sexually antagonistic selection) may not be easy. This could be explained by spatial (Yoccoz & Mesnager, 1998), temporal (Schulte-Hostedde *et al.*, 2002) and ontogenetic shifts (Schluter *et al.*, 1991) in sex-specific selection (Jormalainen & Merilaita, 1995). For instance, a long-term study in threespine sticklebacks (*Gasterosteus aculeatus*), where females display more spines than males, showed that selection on the number of spines varied between the sexes both among life-history stages and among years. As a consequence, no net selection for increased or decreased sexual dimorphism could be detected over the 15-year study period (Reimchen & Nosil, 2004).

In a Swiss barn owl (*Tyto alba*) population, we showed that over the course of a 12-year study, the size of black spots on the ventral body side was positively selected in females and selectively neutral in males; this pattern of selection led to a microevolutionary increase in spot size not only in females but also in males because spot size is strongly genetically correlated between the sexes (Roulin *et al.*, 2010). Because this pattern of selection might be specific to this study, it is worth considering other populations during longer periods of time to investigate spatio-temporal variation in the sign and magnitude of selection exerted on plumage spottiness in the two sexes. Although members of the two sexes exhibit the trait in the same range of possible values, males have on average smaller spots than females (Roulin, 2004). Given that the maintenance of genetic variation and sexual dimorphism in spot size may be because of sexually antagonistic selection, we would expect spot size to be usually negatively selected in males and positively selected in females.

Examining this prediction is difficult because it necessitates the long-term monitoring of several populations to measure selection exerted on the size of black spots in the two sexes. Thus, an alternative approach is needed. To this end, we measured this eumelanin-based trait in barn owls sampled in seven European countries and in the Middle-East. These animals were collected between 1816 and 2001 and preserved in museum collections. Our specific goal here is to examine the sex-specific change in spot size through time in geographically distinct regions where the magnitude of sex-specific selection is likely to differ due to contrasting ecological conditions. Because the expression of spot size is strongly heritable and weakly sensitive to environmental factors, a change in spot size is most likely due to a change in its breeding value (Roulin *et al.*, 2010). Even if a temporal change in mean population phenotypic values can be because of migration rather than selection, it is worth testing whether the change in spot size over time in eight regions is consistent with the hypothesis of sexually antagonistic selection. In other words, we predict that with time spot size became smaller in males and larger in females, an outcome that can vary from one region to another.

## Materials and methods

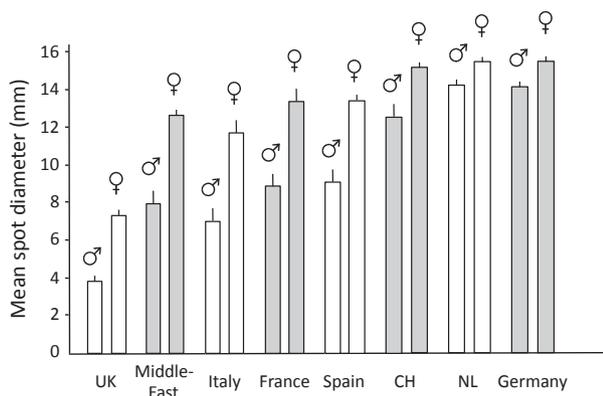
### Data collection

From 1999 to 2009, A. Roulin visited 43 museums (see Acknowledgments) where he had access to skin specimens. Information on site of collection, date and sex was written on the labels, and we determined latitude using a geographical atlas. Because in the present paper, our aim is to examine the sex-specific change in spot size in geographically distinct regions where ecological conditions are different, we decided to pool individuals by countries. Even if this method is not perfect (but defining discrete regions where individuals are pooled is in any case arbitrary), it could be biologically relevant because the barn owl is an open-field bird and agricultural practices are country-specific (Stoate *et al.*, 2009). Moreover, as shown by patterns of isolation by distance at neutral genetic markers (Antoniazza *et al.*, 2010), barn owls from one region are genetically more similar than barn owls from farther away. We thus considered only countries with a sufficiently large number of specimens. This was the case for Germany where *Tyto alba alba/guttata* individuals were collected between 1816 and 1997 (191 males and 253 females), the UK (i.e. England, Scotland and Wales) between 1867 and 1999 (131 males and 141 females), the Netherlands between 1839 and 1985 (125 males and 146 females), Switzerland between 1910 and 1997 (114 males and 129 females), Spain between 1844 and 1997 (72 males and 106 females), Italy between 1891 and 1962 (65 males and 39 females) and France between 1858 and 2001 (46 males and 52 females). Individuals of the subspecies *Tyto alba erlangeri* from the Middle-East (Arabia, Egypt, Iraq, Israel, Palestine and Yemen) were collected between 1835 and 1995 (53 males and 70 females). Pooling birds by countries is further justified by the fact that the mean size of black spots differed significantly between countries (ANOVA, country:  $F_{7,1748} = 164.68$ ,  $P < 0.0001$ ; sex:  $F_{1,1748} = 201.63$ ,  $P < 0.0001$ ; Fig. 1). We did not consider one bird collected in the Czech Republic, six in Hungary, three in Luxemburg, one in Poland, four in Portugal, one in Slovenia and 32 in Sweden.

On each specimen, black spots located within a  $60 \times 40$  mm frame placed on the breast and belly were measured to the nearest millimeter. A mean spot diameter value was calculated and used in the statistical analyses. The method of measuring spot diameter is reliable (Roulin, 2004). Bill length was also measured to the nearest 0.1 mm.

### Cross-validation of museum data against field data

Museum collections have been successfully used to analyse historical phenotypic changes across small to large spatial scales (Shaffer *et al.*, 1998; Suarez & Tsutsui, 2004; Wandeler *et al.*, 2007). To demonstrate that measurements of barn owl skin specimens provide



**Fig. 1** Mean ( $\pm$ SE) size of black eumelanin spots in male and female barn owls collected by museums. UK refers to the United Kingdom, CH to Switzerland and NL to the Netherlands.

biologically relevant data, we investigated whether we obtain similar results using field data and museum collections. In 2003, it was shown that on the European continent, clinal variation in plumage spottiness is more pronounced in male than female barn owls (Roulin, 2003). To test whether we obtain similar results with field data, we asked amateur ornithologists to collect breast feathers on free-living barn owls located throughout the European continent in 2007. We obtained feathers from the Czech Republic (20 birds), Denmark (16), France (74), Germany (148), Hungary (16), the Netherlands (29), Portugal (21), Spain (11) and Switzerland (27). We had on average 6.1 (range is 1 and 8) feathers per individual, and S. Antoniazza counted spots on each feather and measured the length and width of each spot. For each individual, we calculated mean values over all feathers. We then measured the distance between collection site and a southern locality in Portugal (Évora) as done in Roulin (2003). We then performed one ANCOVA with each plumage trait being the dependent variable in separate analyses, sex a factor and distance to Évora a covariate. A significant interaction between sex and distance would indicate that the relationship between plumage traits and distance differs between the sexes. This was the case for number of spots (sex:  $F_{1,358} = 18.54$ ,  $P < 0.0001$ ; distance:  $F_{1,358} = 10.96$ ,  $P = 0.001$ ; interaction:  $F_{1,358} = 7.01$ ,  $P = 0.0085$ ), but not with respect to spot length (sex:  $F_{1,358} = 22.91$ ,  $P < 0.0001$ ; distance:  $F_{1,358} = 22.12$ ,  $P < 0.0001$ ; interaction:  $F_{1,358} = 0.58$ ,  $P = 0.45$ ) and width (sex:  $F_{1,358} = 36.89$ ,  $P < 0.0001$ ; distance:  $F_{1,358} = 0.01$ ,  $P = 0.92$ ; interaction:  $F_{1,358} = 1.74$ ,  $P = 0.19$ ). The significant interaction is explained by the positive relationship between mean number of spots and distance to Évora in males (Pearson's correlation,  $r = 0.30$ ,  $n = 184$ ,  $P < 0.0001$ ), but not in females ( $r = 0.04$ ,  $n = 178$ ,  $P = 0.64$ ). This demonstrates that we obtain similar results using field data as with data collected on skin specimens.

## Statistical procedure

To investigate whether mean spot size changed over the years in a country- and sex-specific way, we performed an ANCOVA with spot size as the dependent variable, sex and country as factors and year of collection as a continuous covariate. The three-way interaction between sex, country and year being significant, we performed two other ANCOVAs for each sex with country as a factor and year as a covariate to test whether the magnitude of the temporal change in spot diameter within each sex varied from one country to another. Furthermore, we carried out an analysis per country to test whether the temporal change in spot diameter was significantly different between males and females. Statistical analyses were carried out with the software JMP 7.0 (SAS Institute Inc., Cary, NC, USA). Tests are two tailed and significance level set to 0.05.

## Results

Spot diameter changed differently during the years in male and female barn owls, a difference that was country-specific (interaction Country  $\times$  Year  $\times$  Sex in Table 1a). An analysis restricted to males revealed a clear tendency for individuals to display smaller black spots during the years (term Year in Table 1b), an effect for which the magnitude differed between countries (term Country  $\times$  Year in Table 1b), being particularly pronounced in the Middle-East, Italy and Switzerland (Table 2, Fig. 2a). Across countries, the size of black spots did not change in any consistent way in females through time (term Year in Table 1c). However, the relationship between spot size and year differed between countries (term Country  $\times$  Year in Table 1b), with females displaying significantly larger spots now than before in the UK and Spain, but smaller spots in Switzerland and Germany (Table 2, Fig. 2b). The temporal change in spot size was significantly different between the sexes in the UK, the Middle-East and Italy (Table 1d–k).

## Testing for potential confounding variables

Introducing bill length as an index of body size (Roulin *et al.*, 2001a) as an extra covariate did not modify the conclusions (Country:  $F_{7,1568} = 135.64$ ,  $P < 0.0001$ ; Year:  $F_{1,1568} = 12.56$ ,  $P = 0.0004$ ; Sex:  $F_{1,1568} = 165.43$ ,  $P < 0.0001$ ; Bill length:  $F_{1,1568} = 23.57$ ,  $P < 0.0001$  (birds with longer bill display larger black spots); Country  $\times$  Year:  $F_{7,1568} = 3.03$ ,  $P = 0.0036$ ; Country  $\times$  Sex:  $F_{7,1568} = 8.09$ ,  $P < 0.0001$ ; Year  $\times$  Sex:  $F_{1,1568} = 16.31$ ,  $P < 0.0001$ ; Country  $\times$  Year  $\times$  Sex:  $F_{7,1568} = 4.24$ ,  $P = 0.0001$ ).

Because spot diameter varies clinally on the European continent, with birds displaying larger black spots in the North than South (Roulin, 2003), we introduced latitude in a preliminary model. Inclusion of this variable did not modify the conclusions, because in the model where we

**Table 1** Sex- and country-specific change in the size of eumelanic spots along the years in barn owls located in different European countries and in the Middle-East. A first analysis was carried out after pooling individuals of the two sexes; then, two separate analyses were performed for each sex, and finally one analysis per country to test whether the change in spot diameter was significantly different between males and females. Interactions are indicated by the symbol ×.

	<i>F</i>	d.f.	<i>P</i>
(a) Both sexes pooled			
Country	145.97	7,1701	< 0.0001
Year	13.69	1,1701	0.0002
Sex	215.04	1,1701	< 0.0001
Country × Year	3.85	7,1701	0.0004
Country × Sex	9.16	7,1701	< 0.0001
Year × Sex	20.05	1,1701	< 0.0001
Country × Year × Sex	4.54	7,1701	< 0.0001
(b) Males			
Country	86.6	7,781	< 0.0001
Year	30.40	1,781	< 0.0001
Country × Year	4.81	7,781	< 0.0001
(c) Females			
Country	60.28	7,920	< 0.0001
Year	0.33	1,920	0.56
Country × Year	3.62	7,920	0.0007
(d) UK			
Year	0.26	1,268	0.61
Sex	45.71	1,268	< 0.0001
Year × Sex	4.93	1,268	0.027
(e) Middle-East			
Year	6.89	1,119	0.0098
Sex	44.56	1,119	< 0.0001
Year × Sex	15.27	1,119	0.0002
(f) France			
Year	0.58	1,94	0.45
Sex	20.29	1,94	< 0.0001
Year × Sex	0.38	1,94	0.54
(g) Germany			
Year	1.81	1,440	0.18
Sex	20.31	1,440	< 0.0001
Year × Sex	2.19	1,440	0.14
(h) the Netherlands			
Year	2.86	1,267	0.09
Sex	11.87	1,267	0.0007
Year × Sex	0.18	1,267	0.67
(i) Italy			
Year	0.45	1,100	0.50
Sex	35.19	1,100	< 0.0001
Year × Sex	9.10	1,100	0.0032
(j) Spain			
Year	0.55	1,174	0.46

**Table 1** (Continued).

	<i>F</i>	d.f.	<i>P</i>
Sex	41.19	1,174	< 0.0001
Year × Sex	1.99	1,174	0.16
(k) Switzerland			
Year	15.11	1,239	0.0001
Sex	24.47	1,239	< 0.0001
Year × Sex	1.77	1,239	0.18

Populations refer to the UK, Middle-East, France, Germany, the Netherlands, Italy, Spain and Switzerland. Skin barn owl specimens were collected during 1816–2001.

pooled the two sexes, the three-way interaction ‘Country × Year × Sex’ was again significant ( $F_{7,1667} = 3.45$ ,  $P = 0.0011$ ), and in the separate models for each sex, the interaction ‘Country × Year’ was still significant in males ( $F_{7,768} = 2.96$ ,  $P = 0.0045$ ) and in females ( $F_{7,899} = 3.01$ ,  $P = 0.0039$ ). There is still the possibility that the temporal trend in the change in spot size is spatially autocorrelated with nearby countries showing similar trends. If this would be the case, the assumption that countries are statistically independent units would be violated. Using the correlation coefficients reported in Table 2, for each country, we calculated the pair-wise difference in the coefficients and geographic distances (using mean coordinates of the location where owls were collected) with all other countries for which we measured the temporal trend in spot size. Permutation Mantel tests for male and female barn owls were not significant indicating that our results are not inflated by spatial autocorrelation ( $z = -965.63$ ,  $P = 0.96$  vs.  $z = -2136.31$ ,  $P = 0.41$ ; each time 1000 permutations). Finally, we calculated the index Moran I for females ( $-0.003$ ,  $P = 0.30$ ) and males ( $0.276$ ,  $P = 0.055$ ). The tendency in males is explained by the fact that the strongest temporal change in spot size was found in the Middle-East which is far from the other considered countries.

Because different museums could have alternative sampling methods, we performed a preliminary analysis including the identity of the museum where each skin specimen was measured as a random variable in a mixed model analysis. Including this random variable did not modify our conclusions (interaction ‘Country × Year × Sex’:  $F_{7,1687} = 4.73$ ,  $P < 0.0001$ ).

Because for all countries, we could measure specimens collected from 1910 onwards, we carried out statistical analyses on this subset of individuals (i.e. 1461 individuals instead of 1733). We obtained qualitatively similar results as the ones presented in Table 1a (Country:  $F_{7,1429} = 110.44$ ,  $P < 0.0001$ ; Year:  $F_{1,1429} = 10.11$ ,  $P = 0.0015$ ; Sex:  $F_{1,1429} = 162.52$ ,  $P < 0.0001$ ; Country × Year:  $F_{7,1429} = 2.26$ ,  $P = 0.027$ ; Country × Sex:  $F_{7,1429} = 8.01$ ,  $P < 0.0001$ ; Year × Sex:  $F_{1,1429} = 17.96$ ,  $P < 0.0001$ ; Country × Year × Sex:  $F_{7,1429} = 2.74$ ,  $P = 0.0079$ ).

**Table 2** Pearson's correlations between the diameter of black plumage spots and year in barn owls from different European countries and in the Middle-East. Significant relationships are written in bold.

	Male	Female
UK	$r = -0.10, n = 131, P = 0.24$	<b><math>r = 0.17, n = 141, P = 0.05</math></b>
Middle-East	<b><math>r = -0.47, n = 53, P = 0.0004</math></b>	$r = 0.13, n = 70, P = 0.27$
France	$r = -0.01, n = 46, P = 0.93$	$r = -0.17, n = 52, P = 0.23$
Germany	$r = 0.006, n = 191, P = 0.93$	<b><math>r = -0.14, n = 253, P = 0.03</math></b>
the Netherlands	$r = -0.12, n = 125, P = 0.17$	$r = -0.08, n = 146, P = 0.34$
Italy	<b><math>r = -0.34, n = 65, P = 0.0057</math></b>	$r = 0.27, n = 39, P = 0.09$
Spain	$r = -0.04, n = 72, P = 0.73$	<b><math>r = 0.20, n = 106, P = 0.037</math></b>
Switzerland	<b><math>r = -0.31, n = 114, P = 0.0009</math></b>	<b><math>r = -0.17, n = 129, P = 0.05</math></b>

Finally, sampling effort varied between countries and to control for this potential problem, for each country, we calculated mean spot diameter of males collected in the same decade (and similarly for females). We then ran a similar model as in Table 1a. The three-way interaction between country, sex and year was again significant ( $F_{7,174} = 3.09, P = 0.0043$ ). Furthermore, our results could potentially be an artefact of the significant differences between countries in mean and variance in year of collection (ANOVA:  $F_{7,1740} = 39.97, P < 0.0001$  and Welch test:  $F_{7,1740} = 73.59, P < 0.0001$ ). To tackle this issue, we again calculated mean spot diameter per country and decade, and then we selected the decades 1890 (corresponding to the first decade when owls were collected in Italy) to 1990. By doing so, mean and variance in year of collection were no more significantly different between countries ( $F_{7,149} = 1.08, P = 0.37$  vs.  $F_{7,63.40} = 1.43, P = 0.21$ ). Using this sample, we again found a significant interaction between country, sex and year (Country:  $F_{7,125} = 42.44, P < 0.0001$ ; Year:  $F_{1,125} = 1.58, P = 0.21$ ; Sex:  $F_{1,125} = 99.76, P < 0.0001$ ; Country  $\times$  Year:  $F_{7,125} = 1.38, P = 0.22$ ; Country  $\times$  Sex:  $F_{7,125} = 3.65, P = 0.0013$ ; Year  $\times$  Sex:  $F_{1,125} = 10.86, P = 0.0013$ ; Country  $\times$  Year  $\times$  Sex:  $F_{7,125} = 2.30, P = 0.03$ ).

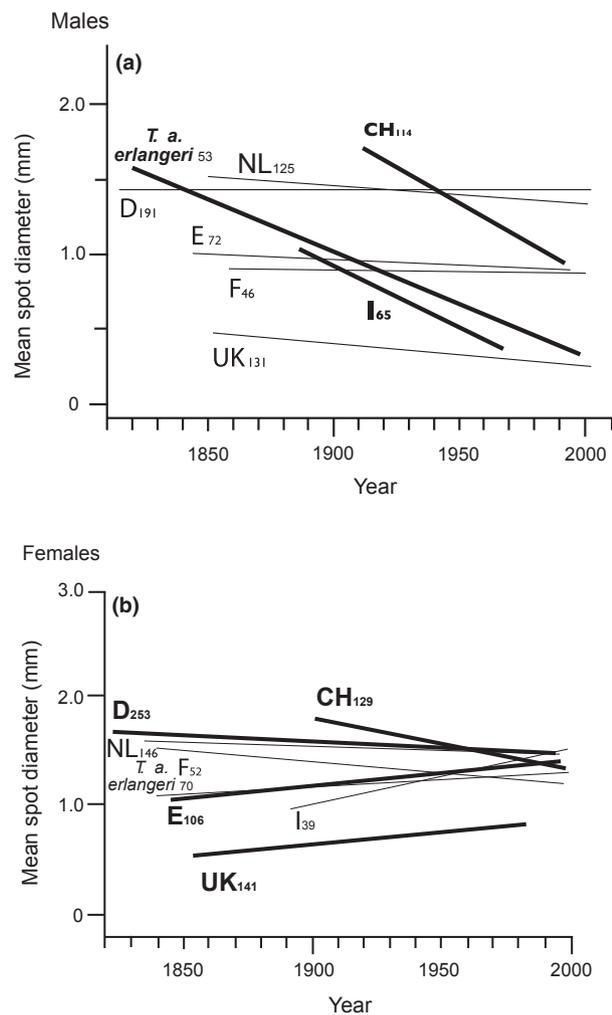
## Discussion

Across Europe and the Middle-East, male barn owls became significantly smaller spotted during the last two centuries in some countries, whereas in females, the pattern was less clear with individuals becoming significantly larger spotted with time in two countries and smaller spotted in two other countries. As can be seen in Fig. 2, the change in spot size during the years was more pronounced in males than in females. Given that genetic drift is unlikely to cause such country-specific temporal change in spot diameter, these changes may be because of selection. Because the size of black spots is genetically correlated between the sexes (Roulin & Dijkstra, 2003), the effect of negative selection on males may overwhelm the positive effect of selection on females on the evolution of female spot size (e.g. Poissant *et al.*, 2010). This may explain why in females the absolute change in spot size was relatively weak compared with males.

## Spatio-temporal variation in sexually antagonistic selection

Although no measurement of selection exerted on spot diameter or any genetically correlated trait has been carried out in the considered countries, the present study is consistent with the hypothesis of spatio-temporal variation in sexually antagonistic selection. Even if we could not analyse yearly variation in mean spot diameter given that insufficient numbers of individuals were collected each year, we could examine whether the size of black spots decreased or increased with time; thus, an absence of change in spot size with time does not imply an absence of sexually antagonistic selection. Such changes in spot size could have been driven by a change in climate or agricultural practices that did not affect all countries in a similar way implying that their impact on terrestrial ecosystems and biodiversity differs between regions (Lemoine *et al.*, 2007; Feehan *et al.*, 2009; Stoate *et al.*, 2009). For instance, one of most important prey species of the European barn owl, the common vole *Microtus arvalis*, shows geographic variation in population dynamics (Tkadlec & Stenseth, 2001), which could have changed in time because of changes in agricultural practices (Jacob, 2003). This may explain why the temporal change in the size of black spots differed between countries, an effect that apparently affected males to a larger extent than females.

Although the genetic correlation between the sexes for spot diameter is strong in all investigated barn owl species and subspecies (Roulin *et al.*, 2001b), the results reported in the present study suggest that the size of black spots is commonly negatively selected in males and positively selected in females, a pattern that can vary in time and space. This finding is important because a previous study in a Swiss population showed that this melanin-based trait is negatively selected in females while being neutral in males (Roulin *et al.*, 2010); we are aware that the micro-evolutionary change in spot size might be because of direct selection on spot size or indirect selection on genetically correlated traits such as immunocompetence (Roulin & Ducrest, 2011). The results reported here suggest that negative selection in males may in fact be more frequent and may often be stronger than previous studies performed in a 190-km<sup>2</sup> study area in Switzerland suggested. Furthermore, the magnitude of negative



**Fig. 2** Relationships between year and the size of black plumage spots in male (panel a) and female (panel b) barn owls from different countries. Regression lines are presented. Significant relationships are drawn in bold. Numbers indicate sample size. *Tyto alba erlangeri* refers to the Middle-East population, CH to Switzerland, D to Germany, E to Spain, F to France, I to Italy, NL to the Netherlands and UK to the United Kingdom.

selection exerted on males may be often stronger than positive selection on females, in contrast to results found in the Swiss population (Roulin *et al.*, 2010). The fact that the pattern of the temporal change in spot diameter in Switzerland in the present study is different from the micro-evolutionary change observed over 12 years in the 190-km<sup>2</sup> study area also in Switzerland further exemplifies the possibility of spatio-temporal variation in the magnitude of sex-specific selection exerted on spot diameter or genetically correlated traits. This is not surprising because in females, spot size is strongly positively associated with survival in the first year of life (Roulin *et al.*, 2010), a fitness component that strongly varies between years (Altwegg *et al.*, 2006). We speculate

that selection against spot size in males was particularly strong in Switzerland during the last century, whereas positive selection in females was pronounced in a small part of this country between 1996 and 2007. This demonstrates the complementarity of observational and experimental data collected at a local scale (in Switzerland) with data collected on skin specimens at the scale of a continent over a longer period of time.

### Alternative hypotheses

The sex-specific spatio-temporal variation in the change in the size of black spots could be explained by four other hypotheses than sexually antagonistic selection. First, the data from museum collections are biased and hence provide biologically nonrelevant information. Obviously, we cannot discard this possibility, but as explained earlier, the European cline variation in spottiness was significantly stronger in males than females both using data from museum collections and data collected by field ornithologists. This indicates that museum collections have a biological value that needs to be exploited. The second hypothesis states that the expression of black spots is sensitive to environmental factors particularly in males; these factors would have changed during the last 200 years modifying the physiology of spot production leading males to produce smaller spots now than in the past. Although we cannot discard this scenario, it is unlikely for the following reasons. In Switzerland, the expression of spot diameter is under strong genetic control ( $h^2 = 0.823$ ), the nest of rearing does not explain any part of the inter-individual variation in spot diameter and the year of hatching explained twice as much of the phenotypic variance in females (0.103%) as in males (0.059%) (Roulin *et al.*, 2010). Thus, if the temporal trend was because of a change in environmental conditions, it should have been stronger in females than males, and the opposite pattern was found. Third, spots become slightly larger in females between the first and second year of age, whereas within males, spot size does not change with age in any particular direction (Dreiss & Roulin, 2010). This indicates that results reported in the present study cannot be explained by spatio-temporal variation in the age of the collected males.

Finally, migration may be responsible for the temporal change in spot size. Because birds are less spotted in southern than northern Europe, particularly in males (Roulin, 2003; Fig. 1), this scenario proposes that birds became less spotted in Switzerland and Germany because of gene flow from Southern Europe. Even if we cannot reject this hypothesis for these two countries, it cannot explain why birds became less spotted in Italy and more spotted in the UK. Indeed, the Alps act as a barrier to immigration in Italy (Maumary *et al.*, 2007) where birds are anyway much smaller spotted than elsewhere on the European continent. Similarly, immigration of birds from the continent to the UK is rare (Martin, 2008). We thus

conclude that selection is a more likely explanation for the spatio-temporal change in spot diameter.

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