

Variation in effects of male plumage ornaments: the case of Iberian Pied Flycatchers

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It has been proposed that mate preferences by female Pied Flycatchers *Ficedula hypoleuca* differ between southern (Iberian) and northern (Scandinavian) European populations. Whereas the size of the white forehead patch, but not plumage colour, has been reported to be a sexually selected trait in the former, only plumage darkness apparently acts as an ornament in the latter. In addition, northern male Pied Flycatchers become darker with age, a trend not detected until the present study in southern birds. Here we show that in an Iberian population of Pied Flycatchers breeding only a few tens of kilometres from previously studied populations, plumage darkness is associated with mating success and increases with age, whereas the size of the white forehead patch is not related to mating success and is only weakly correlated with age, trends similar to those reported for Scandinavian rather than other Iberian Pied Flycatcher populations. This represents a case of variation in sexually selected traits between geographically close populations of Pied Flycatchers that cannot be explained by sympatry with closely related species. It is proposed that differences in the identity and abundance of environmental stressors may be the cause of this regional variation in sexually selected traits.

Keywords: coloration, *Ficedula hypoleuca*, melanin, sexual selection.

Condition-dependent sexually selected traits capture a large proportion of genetic variance in the condition of individuals that bear them, which makes these traits more variable than most ordinary traits not sexually selected (Cuervo & Møller 2001). Therefore, environmental variables affecting condition can cause variation in sexual selection between geographic areas (e.g. Hegyi *et al.* 2002). Finding regional differences in the expression of sexually selected traits is thus important to understand sexual selection. There are several hypotheses that attempt to explain the causes of regional variation in sexual selection. In particular, it has been suggested that geographical differences in the prevalence of parasites or in the availability of pigments such as carotenoids needed to generate the sexual traits, are responsible for the regional variation in sexually selected traits (Hegyi *et al.* 2002, Dunn *et al.* 2008). However, there are only a few

cases in which this variation has been reported (Dunn *et al.* 2008), making it difficult either to determine how general this phenomenon may be or to gain an understanding of the mechanisms underlying observed variation in sexually selected traits.

The Pied Flycatcher *Ficedula hypoleuca* is a small passerine bird in which sexual selection has been intensively studied, especially the signalling roles of their conspicuous black and white plumage coloration and the white forehead patch of males (Dale *et al.* 1999). In Scandinavian populations, females apparently prefer darker males as mates, and the white forehead patch of males does not act as an ornament (Dale *et al.* 1999, 2002). In contrast, the sexually selected trait in a population from central Iberia (La Hiruela; 41°04'N, 3°27'W), located close to the southernmost limit of the species' breeding range, is the white forehead patch, with females preferring males with larger patches as mates, whereas the black plumage coloration does not act as an ornament (Potti & Montalvo

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1991a, 1991b). There are two other key differences between Scandinavian and central Iberian populations. First, the white forehead patch of Scandinavian males is small, whereas that of Iberian birds is large, as expected from the general exaggeration experienced by sexually selected traits (Potti & Montalvo 1991b, Dale *et al.* 1999). Secondly, there are differences in the ontogeny of plumage colour, so while Scandinavian birds become darker with age (Dale *et al.* 1999), the opposite trend has been reported for Iberian birds (Potti & Montalvo 1991a).

We obtained data on male plumage to test whether sexually selected traits were the same in nearby populations. Our aim here is to test for a possible role in mate choice of the plumage coloration and white forehead patch of male Pied Flycatchers from a different Spanish population from those studied by Potti and Montalvo (1991a, 1991b), Sanz (2001) and Osorno *et al.* (2006), which are the only Iberian populations from which results on the signalling function of these traits have been reported previously. Our population is located approximately 30 km from those reported in previous studies. Given the regional variation in the expression of sexually selected traits in Pied Flycatchers (see above), we predicted that our results would be more similar to those found in other Iberian populations (i.e. white forehead patch being sexually selected) than to those found in Scandinavia (i.e. plumage blackness being sexually selected). We also predicted that the coefficient of variation of the sexually selected trait in our population would be higher than that for other traits (Cuervo & Møller 2001). Therefore, the coefficient of variation of the white forehead patch should be higher than that of plumage blackness if the former, but not the latter, is sexually selected in our population. Further, we predicted that the expression of sexually selected traits would increase during ontogeny (Galván & Møller 2009). Finally, we expected to find a positive relationship between body condition and the expression of those plumage traits that are sexually selected, as predicted by the signal theory for traits whose honesty is maintained by costs (i.e. handicaps; Hasson 1997).

METHODS

The study was conducted between 2001 and 2005 and during 2007 in a population of Pied Flycatch-

ers breeding in nest-boxes close to the village of Lozoya, central Spain (40°58'N, 3°48'W). The study area is a montane Pyrenean Oak *Quercus pyrenaica* forest on an east-facing slope at 1500 m asl. Nest-boxes were checked daily to detect the days of clutch initiation and hatching of the majority of the clutch. When nestlings were 12 days old (hatching day = day 0), males were captured with nest-box traps while visiting the nest and classified as first-year birds or older from the colour of primaries (brown in first-year birds). They were ringed if necessary, identified, weighed to the nearest 0.25 g with a PESOLA spring balance and tarsus length was measured with a digital calliper to the nearest 0.01 mm. Body condition was calculated as the residuals of tarsus length regressed against body mass. The maximum height and width of the white forehead patch was measured to the nearest 0.1 mm with a calliper. Width and height are positively correlated ($r = 0.41$, $F_{1,116} = 23.93$, $P < 0.0001$), so we have used their product as an estimate of patch size. The percentage of black feathers in the head and mantle was scored as 'blackness', which is the exact inverse of the brownness score used by Potti and Montalvo (1991a). This score is highly correlated (Potti & Montalvo 1991b) with Drost's (1936) scale from I (darkest) to VII (lightest) used by other researchers (e.g. Dale *et al.* 1999) for the Scandinavian population. Blackness scores were converted to 10-point intervals, with values ranging from 0 (0–10%) to 9 (90–100%), and measurements being collected by a single observer (J.M.). No experimental manipulations were performed on the birds used in this study.

We used laying date as a measure of mating and nesting success, as in Pied Flycatchers pairing date and laying date are strongly correlated (Potti & Montalvo 1991b). Moreover, the earliest males obtain the best territories, territory quality being an important determinant of reproductive success in this species (Alatalo *et al.* 1986, Lundberg & Alatalo 1992). In our study population, laying date is negatively correlated with the number of fledged young (ANOVA with laying date as a covariate and year as a fixed factor in a dataset obtained during the period 2001–2005: laying date: $F_{1,136} = 5.74$, $P = 0.018$; year: $F_{4,136} = 3.35$, $P = 0.012$) and with clutch size ($F_{1,136} = 74.46$, $P < 0.0001$; year: $F_{4,136} = 4.01$, $P = 0.004$). We used clutch size as another measure of reproductive success. In the Scandinavian population, mating success was

calculated as the time elapsed until females were attracted to males because it was an experimental study in which the number of nest-boxes was limited (Dale *et al.* 1999), and so the measures used in their study (Dale *et al.* 1999) were not strictly comparable to those used in ours.

A dataset on 119 male Pied Flycatchers for which we had information on plumage colour, forehead patch size and laying date from at least 1 of the 6 years of study was used, though not all variables could be determined for some birds. Of these, 22 birds were captured in two breeding seasons and one bird in four breeding seasons. Therefore, for these individuals we could analyse variation in plumage colour and forehead patch size in relation to age. However, as we did not know the exact age of the birds at the time of first capture, except for those captured when aged 1 year or less, we could only analyse the time elapsed between the first and subsequent recaptures as an estimation of age. Although only an approximation, this measure might be correlated with the real age of birds because the Pied Flycatcher is a short-lived bird, with a lifespan of 4–5 years (Lundberg & Alatalo 1992), which makes our age estimates highly probable because there are few real yearly age classes. Furthermore, the variance in the exact age of the birds during the first capture might be higher than that for recaptured birds, as there were probably both young and old birds at first capture but we know that captures during subsequent years correspond only to older birds. Moreover, due to the few real yearly age classes the probability that our age estimates would fall into the wrong age class is low. Accordingly, our tests should be viewed as being conservative.

We compared the variance in blackness score and forehead patch size by testing for significant differences in the corresponding coefficients of variation. This was done by using the statistic developed by Forkman (2005), which is approximately F-distributed. For those individuals for whom we had more than one observation corresponding to different years, we selected one data point at random to avoid pseudoreplication. General linear models (GLM) were used to investigate the factors that determined the laying date and clutch size of Pied Flycatchers, introducing plumage colour score and white forehead patch size as covariates. Tarsus length was also introduced as a covariate to control for the effects of body size.

Year was introduced as a fixed factor, as well as age (first year or older). The interactions of age with plumage blackness and forehead patch size were included in the models, as the information content of sexually selected traits can differ between age classes (e.g. Hegyi *et al.* 2006). We used a backward stepwise procedure to remove non-significant terms, setting a probability of 0.1 to drop them from the model.

To analyse changes in blackness and forehead patch size with age, we performed two separate general linear mixed models (GLMMs) with plumage blackness and forehead patch size as dependent variables. Individual identity was introduced as a random factor to consider its interaction with time interval between recaptures (as an estimate of age), which was a covariate (assigning a value of 0 to first captures, 1 to recaptures after 1 year, 2 to recaptures after 2 years, etc.). Zero values were included because this category corresponds to the lowest possible age estimate that we could assign to birds, and thus it is a reference lowest value (i.e. age class) with respect to longer age intervals obtained during subsequent recaptures. Body condition was also included as a covariate. We corroborated the results of our models by testing whether the increment in years from the first capture to the second was correlated with the increment in either blackness score or forehead patch size during that time.

RESULTS

The mean (\pm se) size of the white forehead patch of male Pied Flycatchers in our population was $58.4 \pm 1.43 \text{ mm}^2$, with values that ranged from 19.4 to 93.2 mm^2 . The mean blackness score was 7.2 ± 0.1 (range: 3–9). The white forehead size in our Iberian population is therefore markedly larger than in Scandinavian populations (25 mm^2 ; Dale *et al.* 1999), but more similar to the mean values found in other Iberian populations studied by Potti and Montalvo (1991b; *c.* 47 mm^2) and Osorno *et al.* (2006; 49.7 mm^2). The mean blackness score is similar to that reported by Potti and Montalvo (1991a; 30.8% brown), but much higher than in Scandinavian Pied Flycatchers, which tend to be brown instead of black (Sætre *et al.* 1997).

The coefficient of variation of blackness score was significantly lower (18.72%) than that of the white forehead patch size (26.58%; $F_{117,119} = 1.81$, $P = 0.001$). Body condition on day 12 of the

nestlings was correlated neither with plumage blackness (Pearson correlation: $r = 0.01$, $n = 118$, $P = 0.898$) nor with forehead patch size (Pearson correlation: $r = 0.00$, $n = 116$, $P = 0.983$).

The factors affecting the laying date of male Pied Flycatchers explained 26% of the variance in that variable ($F_{7,106} = 5.03$, $P < 0.0001$) and showed a negative correlation with blackness score ($\beta = -0.20$, $F_{1,106} = 4.74$, $P = 0.032$, Fig. 1a) after controlling for the effects of body size ($F_{1,106} = 0.10$, $P = 0.755$) and year ($F_{5,106} = 4.95$, $P < 0.001$). Age ($F_{1,105} = 0.14$, $P = 0.71$), as well as its interaction with blackness ($F_{1,104} = 0.85$, $P = 0.357$), was not significantly correlated with laying date. In contrast, neither the size of the white forehead patch ($F_{1,101} = 0.39$, $P = 0.536$,

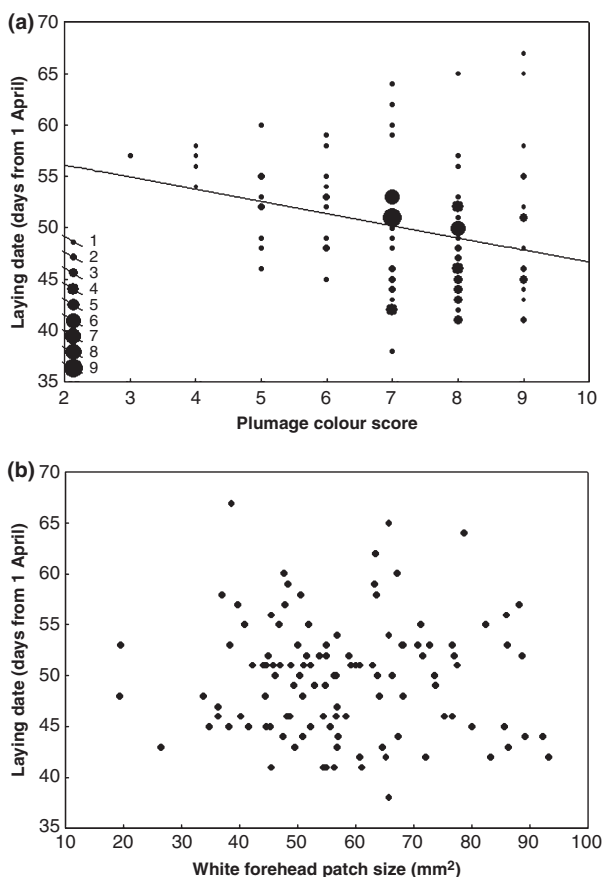


Figure 1. Relationship between laying date and: (a) plumage colour (Pearson correlation test: $r = -0.27$, $n = 115$, $P = 0.003$) and (b) white forehead patch size (Pearson correlation test: $r = -0.02$, $n = 113$, $P = 0.817$) in male Iberian Pied Flycatchers. The size of points shown in (a) represents the extent of overlapping data (legend shown in the bottom left of the graph). The line is the regression line: $y = 58.48 - 1.18 \times x$.

Fig. 1b) nor its interaction with age ($F_{1,100} = 0.18$, $P = 0.670$) were retained in this model.

A different model explained 34% of the variance in clutch size ($F_{12,99} = 4.22$, $P < 0.0001$; body size: $F_{1,99} = 0.15$, $P = 0.698$; year: $F_{5,99} = 1.57$, $P = 0.175$; laying date: $F_{1,99} = 16.84$, $P < 0.0001$). The effect of age was not significant ($F_{1,99} = 0.00$, $P = 0.971$). Clutch size was not affected by male plumage blackness *per se* ($F_{1,99} = 2.58$, $P = 0.111$), but the interaction between blackness and age was significant ($F_{1,99} = 4.70$, $P = 0.032$), as the correlation between clutch size and blackness was positive and significant for first-year birds ($\beta = 0.72$, $P = 0.034$) but was not significant for older birds ($\beta = -0.11$, $P = 0.667$). Interestingly, clutch size was negatively correlated with forehead patch size ($\beta = -0.38$, $F_{1,99} = 9.53$, $P = 0.003$), and the interaction between this variable and age was also significant ($F_{1,99} = 5.95$, $P = 0.016$). This suggests that although there was an overall effect, the slope of the regression between clutch size and forehead patch size was only significant for first-year birds ($\beta = -0.86$, $P = 0.003$), not for older birds ($\beta = -0.13$, $P = 0.412$).

When we analysed the variation of male plumage blackness with age, the model explained a high proportion of variance in that variable ($R^2 = 0.86$, $F_{23,24} = 6.69$, $P < 0.0001$), and showed that male Pied Flycatchers became darker with age ($\beta = 0.32$, $F_{1,24} = 15.36$, $P = 0.001$, individual identity: $F_{22,24} = 6.24$, $P < 0.0001$, Fig. 2a). The interaction between age and individual identity was not significant ($F_{22,2} = 5.50$, $P = 0.165$), indicating that the age-related colour variation was a general trend. This result was corroborated to a limited extent by the weak relationship between the increment in plumage blackness score between two different years and the time elapsed between those recaptures ($R^2 = 0.16$, $\beta = 0.40$, $F_{1,20} = 3.78$, $P = 0.066$). This increase did not depend on the interannual change in body condition ($F_{1,19} = 0.21$, $P = 0.653$). Similarly, the size of the white forehead patch increased with age ($R^2 = 0.89$, $F_{23,24} = 8.73$, $P < 0.0001$, age: $\beta = 0.22$, $F_{1,24} = 9.16$, $P = 0.006$, individual identity: $F_{22,24} = 7.68$, $P < 0.0001$, age \times individual identity: $F_{22,2} = 0.78$, $P = 0.701$, Fig. 2b). However, the relationship between the increment in the size of the white forehead patch size and the time elapsed between recaptures in 2 years was far from significant ($F_{1,20} = 0.82$, $P = 0.377$).

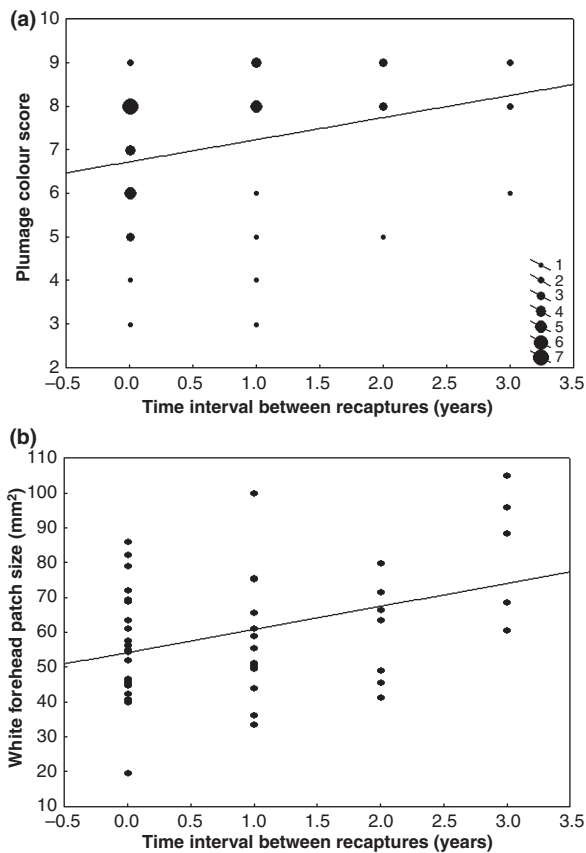


Figure 2. Relationship between time elapsed between different recaptures and: (a) plumage colour and (b) white forehead patch size in male Iberian Pied Flycatchers. The size of points shown in (a) represents the extent of overlapping data (legend shown in the bottom right of the graph). The time elapsed between recaptures is considered an indicator of male age, 0 values represent first captures. The lines are regression lines: (a) $y = 6.72 + 0.51 \cdot x$; (b) $y = 54.20 + 6.61 \cdot x$.

A similar result was obtained for the association with body condition during that period ($F_{1,19} = 0.00$, $P = 0.986$).

DISCUSSION

We found evidence of regional variation in sexually selected traits in Pied Flycatchers but, contrary to predictions, our results were similar to those obtained in Scandinavian populations of Pied Flycatchers (Dale *et al.* 1999, 2002). First, there was evidence of a higher mating success for darker males overall and also of larger clutches for at least darker first-year males. There was no association of white forehead patch size with either mating or nesting success. In first-year males, clutch size

shows a positive correlation with blackness but a negative one with forehead patch size. Secondly, male Pied Flycatchers became darker with age, and although there was also a tendency to increase the size of the forehead patch with age, this last result was not supported when the correlation between increments in that trait between first and second captures and the time elapsed between those captures was tested. Thus, the age-related increment in forehead patch size was weak, whereas both tests showed the existence of a clear tendency towards a darkening in plumage colour in older birds. Therefore, plumage colour, but not forehead patch size, seems to be a sexually selected trait in our population of Pied Flycatchers.

These results contrast with those reported by Potti and Montalvo (1991a, 1991b) for another Iberian population located only about 30 km from our study area. In this population, the size of the white forehead patch, but not body plumage colour, was a sexually selected trait when their studies were conducted (1987–89). In a third Iberian sub-population, also situated several tens of kilometres away from our study population, experimentally reduced forehead patches induced a higher male parental allocation (Sanz 2001) and a reduced female investment in eggs (Osorno *et al.* 2006). In our population, the coefficient of variation of the sexually selected trait (i.e. plumage blackness) was smaller than that of a trait that is apparently not used in mate choice (i.e. forehead patch size). If there was an initial preference by females for large forehead patches as in other Iberian populations (Potti & Montalvo 1991a, 1991b, Osorno *et al.* 2006), it could be that the direction of sexual selection has changed rapidly, now acting on the plumage darkness of males. A recent shift in the direction of sexual selection could explain the high variance that can still be observed in forehead patch size. Although the variance in plumage blackness scores and forehead patch size differed, both values are still high and characteristic of sexually selected traits (Cuervo & Møller 2001). This gives support to the idea that there has been a rapid change in mate preferences.

The divergence in sexually selected traits that we are reporting here may have been favoured by the fact that there are no selective forces acting in favour of brown males in Iberia, as this is not an area of sympatry with the closely related Collared Flycatcher *Ficedula albicollis* (Sætre *et al.* 1997, Dale *et al.* 1999). Given that the synthesis of

melanins, the pigments that create the black colour of Pied Flycatchers (Lundberg & Alatalo 1992), depends on the level of environmental oxidative stress (Galván & Solano 2009), variation in the identity and abundance of environmental stressors may well be the cause of the divergence in sexually selected traits in Iberian Pied Flycatchers. Future studies should attempt to identify the exact causes of divergence in the mating preferences of Iberian Pied Flycatchers.

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