



Male plumage signal expression is related to feather corticosterone concentration in the Pied Flycatcher *Ficedula hypoleuca*

Juan Moreno¹ · Jimena López-Arrabé²

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Abstract

Male signals may express the capacity to sustain environmental challenges. In some migratory birds like the Pied Flycatcher *Ficedula hypoleuca*, plumage ornaments are molted in the winter quarters shortly before spring migration while most feathers are replaced shortly after the breeding season in the breeding areas. The concentration of corticosterone in feathers (CORTf) may relate to baseline CORT levels at the time of molt which could be expressed through plumage signals. Male Pied Flycatchers present white patches on forehead feathers and tertials which are molted before spring migration and on secondaries and primaries replaced after breeding. They also express a variable degree of melanisation of head and back feathers molted in the wintering areas. All these plumage traits have been previously shown to function in social contests and/or mate attraction. Here we have collected tertials on the two wings and two tail feathers, molted in wintering and breeding areas respectively, of males in a Spanish montane population and analysed CORTf in the laboratory with standard enzyme immunoassays. There is no correlation within individuals between CORTf in the two types of feathers, although levels are similar. The size of the forehead patch is negatively associated with CORTf in tail feathers, mainly in small males, while the blackness of head and back is negatively related to CORTf in tertials, mainly in large males. The size of the wing patch composed of patches on feathers molted both in wintering (tertials) and breeding areas (primaries and secondaries) is not related to CORTf in any type of feather. Different male plumage traits thus may reflect circulating CORT levels during molt processes occurring in the wintering respectively breeding range as expressed by CORTf in different types of feathers.

Keywords Body size · Melanic plumage · Physiological capacity · White plumage patches

Zusammenfassung

Die Ausprägung männlicher Gefiedersignale bei Trauerschnäppern *Ficedula hypoleuca* hängt mit der Kortikosteronkonzentration in den Federn zusammen.

Männliche Signale könnten die Fähigkeit, schwierige Umweltbedingungen auszuhalten, ausdrücken. Bei einigen Zugvögeln, wie dem Trauerschnäpper *Ficedula hypoleuca*, werden Gefiederornamente im Winterquartier kurz vor dem Frühjahrszug gemausert, während die meisten Federn kurz nach der Brutsaison im Brutgebiet ersetzt werden. Die Konzentration von Kortikosteron in den Federn (CORTf) dürfte mit dem Ausgangs-CORT-Spiegel während der Mauser, der sich in Gefiedersignalen manifestieren könnte, zusammenhängen. Männliche Trauerschnäpper haben weiße Flecken auf den Stirn- und Schirmfedern, die vor dem Frühjahrszug gemausert werden, und auf den Arm- und Handschwingen, die nach der Brut ersetzt werden. Zudem variiert das Ausmaß der Einlagerung von Melanin in die Federn auf Kopf und Rücken, die im

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✉ Juan Moreno
jmoreno@mncn.csic.es

¹ Museo Nacional de Ciencias Naturales-CSIC, J. Gutiérrez
Abascal 2, 28006 Madrid, Spain

² Departamento de Biodiversidad, Ecología y Evolución,
Facultad de Ciencias Biológicas, Universidad Complutense
de Madrid, José Antonio Nováis, 28040 Madrid, Spain

Überwinterungsgebiet gemausert werden. Für all diese Gefiedermerkmale ist gezeigt worden, dass sie im sozialen Wettstreit und/oder bei der Anziehung von Partnern eine Rolle spielen. Hier haben wir Schirmfedern von beiden Flügeln und zwei Schwanzfedern, die im Überwinterungs- bzw. Brutgebiet gemausert wurden, von Männchen einer Trauerschnäpperpopulation in einer spanischen Gebirgsregion gesammelt und CORTf im Labor mittels Standard-Enzym-Immunoassays analysiert. Bei Individuen gab es keine Korrelation zwischen CORTf in den beiden Federtypen, obwohl die Spiegel ähnlich waren. Die Größe des Stirnflecks war negativ mit CORTf in den Schwanzfedern assoziiert (hauptsächlich bei kleinen Männchen), während die Intensität der Schwarzfärbung von Kopf und Rücken negativ mit CORTf in den Schirmfedern zusammenhing (hauptsächlich bei großen Männchen). Die Größe des Flügelbereichs, der aus Flecken auf Federn besteht, die im Überwinterungsgebiet (Schirmfedern) bzw. im Brutgebiet (Hand- und Armschwingen) gemausert wurden, hing nicht mit CORTf in anderen Federtypen zusammen. Unterschiedliche Gefiedermerkmale der Männchen könnten daher den CORT-Spiegel im Blut während im Überwinterungs- bzw. Brutgebiet ablaufenden Mauserprozessen anzeigen, der sich als CORTf-Spiegel in verschiedenen Federtypen manifestiert.

Introduction

Sexually selected signals are assumed to express the capacity to sustain environmental challenges and compete successfully for resources during the breeding season (Anderson 1994). Glucocorticoid hormones mediate associations between organisms and their environment, and their activity may be associated with signal expression. The main glucocorticoid in birds, corticosterone (CORT), has been shown to respond to situations of increased metabolic demand (Wingfield 2013; Sapolsky et al. 2000). Thus, potentially adverse external conditions favoring reassignment of resources to immediate survival may stimulate an activation of the hypothalamic – pituitary axis resulting in elevated circulating CORT (Wingfield 2013; Henschen et al. 2018; but see Dickens and Romero 2013; MacDougall-Shackleton et al. 2019). CORT has been frequently measured in plasma of wild birds immediately or briefly after capture (Sapolsky 1982; Wingfield et al. 1982; Wingfield and Romero 2001), although concentration in faeces and other biological materials including feathers has also been recorded (e.g. Möstl et al. 2005; Bortolotti et al. 2008). Signals of individual quality may express the ability to cope with reassignments in organismic metabolic demands (Lobato et al. 2010; Douglas et al. 2018; Henschen et al. 2018). Thus, if plumage traits are indicators of metabolic coping capacity, their expression may be negatively linked to baseline CORT levels during feather growth (Lobato et al. 2010; Schull et al. 2018; Hasegawa et al. 2019). In accordance with this possibility, postnatal exposure to CORT has been shown to have a negative impact on the expression of plumage signals (Dupont et al. 2019).

Due to its potentially noninvasive character, CORT concentration in feathers (CORTf) is increasingly being used to study metabolic responses to environmental conditions during molt (Bortolotti et al. 2008; Romero and Fairhurst 2016), despite its potential methodological problems (e.g. Harris et al. 2016). CORTf has been experimentally shown to constitute the best feather steroid marker for detecting

nutritional problems in developing (Will et al. 2019) and adult birds (Grunst et al. 2015). Furthermore, CORTf has shown negative associations with certain fitness-related traits (Harms et al. 2015; Lodjak et al. 2015; Mougeot et al. 2016). It is assumed that a poor condition during molt may imply impaired ability to cope with environmental demands at that time, so CORTf should be negatively associated with individual condition at the time of production of analyzed feathers (Jenkins et al. 2013; Romero and Fairhurst 2016). For plumage signals to be informative about individual condition or quality in the mating season, information about coping capacity at the time of molt as expressed by baseline CORT levels could be of value (Moreno and López-Arrabé 2021). Information about the association of CORTf with melanin-based plumage signals of adult birds is still scarce (Moreno and López-Arrabé 2021). The area of white plumage patches has been shown to be condition dependent (McGlothlin et al. 2007; D’Alba et al. 2011) and predict reproductive success (Doucet et al. 2005). It has also been proposed that differential eumelanisation of body plumage may express antioxidant capacity (Moreno and Møller 2006; McGraw 2008) and immune defence (Moreno and Møller 2006). Thus, both darker body plumage and larger contrasting white patches within it may express coping capacity during molt and thereby be associated with CORTf.

Most migratory birds experience a post-breeding complete molt, while some of these species replace their nuptial plumage at the winter quarters shortly before migrating to the breeding range (Ginn and Melville 1983). Measuring CORTf in feathers molted at different times of the year may give an indication of metabolic demands experienced at different phases of the annual cycle as expressed by baseline CORT levels while molting. In some species like the European Pied Flycatcher *Ficedula hypoleuca*, males renew in Africa part of their plumage, including the white forehead, tertials, and the dark mantle body feathers, while all feathers are replaced annually immediately after breeding for birds older than 1 year (Lundberg and Alatalo 1992). The extent of male achromatic plumage patches is phenotypically

plastic (Moreno et al. 2019) and has been positively related to age (Galván and Moreno 2009), mate attraction (Sirkiä and Laaksonen 2009; Sirkiä et al. 2015), social dominance (Osorno et al. 2006; Järvisjö et al. 2013), stress (Lobato et al. 2010) and oxidative status (Moreno et al. 2011; López-Arrabé et al. 2014). A higher melanisation of dorsal body feathers has also shown individual variation related to age (Potti and Montalvo 1991) and positive associations with breeding success (Saetre et al. 1995, 1997; Grinkov and Kerimov 1998; Galván and Moreno 2009; Sirkiä et al. 2010), mate choice (Potti and Montalvo 1991; Saetre et al. 1994; Galván and Moreno 2009; Sirkiä and Laaksonen 2009), social dominance (Järvi et al. 1987; Huhta and Alatalo 1993), survival (Belskii and Lvakhov 2004), condition (Slagsvold and Lifjeld 1992) and immune response (Kerimov et al. 2013). Dorsal melanisation and extent of the white forehead patch are not correlated in our population (Galván and Moreno 2009).

In the present study, we have measured concentration of CORTf in tail feathers and tertials of males captured during the breeding season while provisioning young at the nest in a Spanish montane population of Pied Flycatchers. We have also measured in these males the size of the white forehead and wing patches and estimated the proportion of black feathers on head, back and wing coverts. Thus, CORTf is estimated in some of the feathers molted after the previous breeding season in Spain as well as in Africa prior to spring migration. We wanted to establish if values of CORTf in tail feathers and tertials are correlated within individuals and differ in magnitude, and to determine which are more predictive of male signaling status. Moreover, we wanted to test if any or both estimates of CORTf are related negatively to the size of forehead and wing patches as previously shown in the same population for female forehead patches (Moreno and López-Arrabé 2021).

Material and methods

General field methods

The study was carried out in 2021 at the Lozoya field site (central Spain, 40° 58' N, 3° 48' W, 1400–1750 m a.s.l.), where a long-term study of Pied Flycatchers is being conducted since 2001 (Moreno 2015, 2020). The habitat is a montane deciduous forest of Pyrenean oak *Quercus pyrenaica* below roughly 1550 m a.s.l. and coniferous forest of pine *Pinus sylvestris* above this altitude. In the deciduous habitat there are 100 nest boxes covering an area of roughly 85 ha (for size and other properties on nest boxes see Lambrechts et al. 2010). Another 80 nest boxes, installed in 2019, cover a roughly similar area in the coniferous habitat at 1600–1750 m a.s.l. Nest-box occupation has been checked

every year since 2001, being markedly higher in the deciduous habitat. The breeding period of the species in the area lasts from the middle of April, when the first males arrive from migration, to the beginning of July, when most chicks have fledged. We clean all nest boxes every year after breeding is over. Daily checking of all nest boxes from April 15 to the beginning of June is done to detect the presence and settlement of every flycatcher breeding pair as well as their reproductive activities (daily checks cease after occupation of nest boxes by other species). The number of Pied Flycatcher nests was 30 in the study year, 23 in the deciduous habitat and 7 in the coniferous habitat.

Capture and sampling

Most males were captured in their nest boxes while feeding 7–8 day-old nestlings (fledging takes place 16–19 days after hatching, Moreno 2020) during daytime, by using conventional nest-box traps set at the entrance (Moreno 2020). The trap was removed once both adults were trapped or after a maximum of 90 min (in every season some trap-shy males are not captured). All captured males ($n = 21$) were measured as to tarsus length with a digital caliper to the nearest 0.01 mm following Svensson (1984). Digital photographs of the forehead patch and of the right white wing patch were taken from above at distances of 10 cm from the bird with a ruler aligned beside the head or wing for reference. The wing was completely extended when taking the photograph. All photographs were taken during the morning hours with the same digital camera and following the method described in previous studies (Sirkiä et al. 2015; Moreno et al. 2019). Surfaces of the forehead and wing patches were estimated with Adobe Photoshop CS5 v.11.0. following Sirkiä et al. (2015) (some photos were of insufficient quality which reduced sample size below 21 in some cases). The proportion of black feathers on head and mantle were estimated visually in the field on a scale of 10–100% with intervals of 10% between levels (0–10% = 5, 10–20% = 15, etc.) (Galván and Moreno 2009).

After measurements and before release, the three tertials on each wing were removed with scissors at the base as well as the two extreme tail feathers. Many males overlap molt of wing feathers with nestling-provisioning (Moreno et al. 2001; Sanz et al. 2004), so the manipulation can be considered mild. Moreover, tertials and tail feathers are less important for flight than primaries and secondaries.

Corticosterone extraction

A methanol-based extraction technique was used to extract CORTf, since steroid hormones are generally soluble in polar alcohols such as methanol (Pötsch and Moeller 1996). All glassware used for CORT extraction was silanized

using 1% v/v dichloromethylsilane ($(\text{CH}_3)_2\text{SiCl}_2$) in hexane (C_6H_{14}), rinsed twice with methanol, and air dried. Silanization prevents CORT from adhering to glassware and was shown to significantly reduce losses of CORT during the extraction process (Bicudo et al. 2020; Kouwenberg et al. 2015).

To measure CORTf levels in tertials we followed the protocol of Bortolotti et al. (2008), but all the feathers from the same individual were pooled before extraction as the sum of lengths of these short, wide feathers could be estimated less reliably than the pooled mass (Koren et al. 2012; Lendvai et al. 2013; Sepp et al. 2018). For comparative reasons the same procedure was used for tail feathers. Concentrations were normalized by mass of feathers allowing for the representation of CORT levels per unit mass (pg/mg) (Freeman and Newman 2018), and controlling that we obtained a small range of pooled feather masses per individual (tertials: 12.7 ± 2.8 (Mean \pm SD) mg; tail feathers: 10.2 ± 2.0 (Mean \pm SD) mg) in order to avoid potential confounding effects on CORTf (Romero and Fairhurst 2016). The feather vanes and the rachis were cut into small pieces (< 5 mm) with scissors. All feathers per individual were pooled and weighed in tared silanized glass tubes. To these were added 6 ml of methanol, after which the tubes were capped with a rubber plug crossed with two syringe needles and parafilm to avoid evaporation, and incubated for 30 min in an ultrasound water bath at room temperature. The tubes were left overnight (around 19 h: from 15:00 to 10:00) in a shaking (60 rpm) water bath at 50 °C. They were then decanted into a syringe. Tubes were then washed with 2 additional ml of methanol, vortexed and to them added the previous extract, after which they were filtered using a nylon syringe filter (0.45 μ). They were placed in a heated block at 50 °C under a stream of nitrogen until evaporation which took approx. 50 min. The dried extracts were then resuspended in 150 μ l of steroid-free serum (DRG, Germany) and vigorously vortexed for 10 min. The reconstituted samples were frozen at -20° in microtubes.

Corticosterone quantification

To measure CORTf concentrations we used an enzyme immunoassay (EIA-4164 DRG, Germany) following kit protocol. Inter-assay variability was assessed using the coefficient of variation (CV) of three known controls across four assays, while samples, controls and standards were run in duplicate in each assay to assess intra-assay variability. Repeatability was high (intra-assay %CV = 8.280, $N = 96$; inter-assay %CV = 14.463, $N = 3$). All samples were above detection limits (1.1 pg/ml). Serial dilutions of an extraction pool were included in the assay to test for the linearity of the association between expected and observed concentration of CORT in the dilutions by using the set of ratios performed

($y = 1.382x - 0.709$, $r^2 = 0.969$). A Synergy HT Multi-Mode Microplate Reader (Biotek, USA) at 450 nm was used.

Assay plates can differ in certain factors, such as temperature, which in turn can affect the amount of hormone found in each feather (Bosholt et al. 2020). There were no significant associations of CORTf (see below) with plate for either tertials (Kruskal – Wallis $H_{3,21} = 1.45$, $p = 0.6931$) or tail feathers ($H_{3,20} = 1.45$, $p = 0.6928$). There was no significant association of tertial CORTf (CORTf t) with the mass of the tertials analysed ($r_{s20} = -0.13$, $p > 0.50$), nor of tail feather CORTf (CORTf r) with the mass of tail feathers ($r_{s20} = -0.42$, $p > 0.05$).

Statistical analyses

We hypothesized that plumage ornamentation should be related to CORTf in tertials and/or to CORTf in tail feathers when controlling for size (tarsus length) of the male. Tarsus length is not correlated with wing length in the sample and is independent of feather wear. Two-way interactions were also included. As dependent variables we included the size (cm^2) of forehead and wing patches and the percentage of black feathers on mantle (BLACKNESS henceforth). The areas of both patches were normally distributed (Kolmogorov – Smirnov, $p > 0.20$). BLACKNESS was subjected to the arcsine transformation to attain normality (Kolmogorov – Smirnov, $p > 0.10$). Correlations between the independent variables were lower than 0.30 and nonsignificant (all $p > 0.05$). An extreme value of CORTf r (more than 3 SD away from the mean) was removed as statistical outlier from analyses in order to avoid errors of inference (Osborne and Overbay 2004).

Model selection in the GLIM module of STATISTICA was conducted independently for the three plumage traits (normal distribution, identity link function), using AICc to estimate the relative strength of the associations with individual male traits including CORTf. Only models showing differences in AICc lower than two with the best model (that with the lowest AICc value) will be presented in Tables. Linear regressions of the main effects are presented after checking that quadratic terms were not significant.

Results

The mean concentration of CORT in tertials was $47.45 \pm \text{SD}30.78$ pg/mg ($n = 21$). The mean concentration of CORT in tail feathers was $47.86 \pm \text{SD}34.89$ pg/mg ($n = 20$). CORTf in tertials and CORTf in tail feathers were not correlated ($r_s = 0.32$, $p > 0.10$). CORTf per mg did not differ between tertials and tail feathers (Wilcoxon matched pairs test: $Z = 0.33$, $p = 0.74$).

CORTf r was present in all the 12 models explaining variation in forehead patch size with an AICc difference less than 2 with the best, while CORTf t and tarsus length turned up in 5 models each (Table 1). Moreover, CORTf r was implicated in interactions with CORTf t in 6 best models and in interactions with tarsus length in 9 best models.

Table 1 Selection of Generalized Linear Models with normal distribution and identity link function of male forehead patch size using AICc to estimate the relative strength of associations with CORTf for tail feathers (CORTf r), CORTf for tertials (CORTf t), tarsus length and their two-way interactions (1 = CORTf t, 2 = CORTf r, 3 = Tarsus length)

| | df | AICc | χ^2 | p |
|------------------------------------|----|---------|----------|-------|
| CORTf r CORTf t Tarsus 1*2 2*3 | 5 | -29.277 | 14.457 | 0.013 |
| CORTf r Tarsus 1*2 2*3 1*3 | 5 | -29.252 | 14.433 | 0.013 |
| CORTf r | 1 | -29.005 | 6.186 | 0.013 |
| CORTf r 2*3 | 2 | -28.579 | 7.760 | 0.021 |
| 2*3 | 1 | -28.543 | 5.724 | 0.017 |
| CORTf r Tarsus 2*3 | 3 | -28.199 | 9.380 | 0.025 |
| CORTf r 1*2 2*3 1*3 | 4 | -27.992 | 11.173 | 0.025 |
| CORTf r CORTf t 1*2 2*3 1*3 | 5 | -27.474 | 12.655 | 0.027 |
| CORTf r Tarsus | 2 | -27.440 | 6.621 | 0.036 |
| CORTf r CORTf t | 2 | -27.325 | 6.507 | 0.039 |
| CORTf r CORTf t 1*2 2*3 | 4 | -27.300 | 10.481 | 0.033 |
| CORTf r CORTf t Tarsus 1*2 2*3 1*3 | 5 | -27.282 | 14.463 | 0.025 |

Log-likelihood tests against null models are presented. Only models with AICc differences less than 2 from the best are presented

These two-way interactions with CORTf r were present in the best single model (Table 1). CORTf r was negatively associated with forehead patch size and explained nearly 20% of its variation (Fig. 1). However, this was mainly due to a nearly significant association for males with tarsi shorter than 17 mm ($r_9 = -0.66, p = 0.053$), while the correlation for males with longer tarsi was far weaker ($r_{11} = -0.25, p = 0.459$). The association of forehead patch size with CORTf r was also stronger for males with high CORTf t ($r_8 = -0.64, p = 0.086$) than for those with low CORTf t values ($r_{12} = -0.40, p = 0.19$).

Model selection for wing patch size did not lead to any model significantly different from a null model (Table 2). Tarsus length turned up singly in the best model, although it did not show a significant association with wing patch size ($r_{19} = 0.33, p = 0.15$).

CORTf t was present singly in the second best model explaining variation in transformed BLACKNESS and in 6 of 13 models with AICc differences less than 2 with the best (Table 3). CORTf r turned up in only three of these models and in no case singly, while tarsus length appeared only in two models (Table 3). The best model included only the interaction between CORTf t and tarsus length. CORTf t was negatively associated with transformed BLACKNESS and explained nearly 15% of its variation (Fig. 2). This association was due to a very strong correlation for males with tarsi longer than 17 mm ($r_{11} = -0.78, p = 0.005$), while the correlation for smaller males was far weaker ($r_9 = -0.28, p = 0.43$).

Fig. 1 Association between male forehead patch size and CORT concentration in tail feathers ($y = 0.497 - 0.002x$, Adjusted $r^2 = 0.200, F_{1,18} = 5.74, p = 0.0277$)

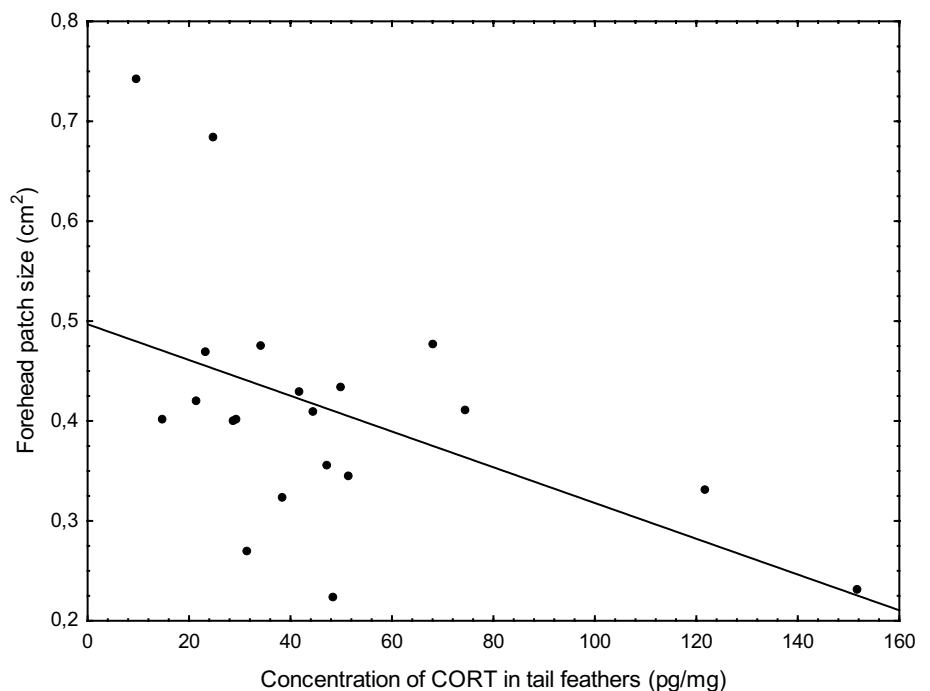


Fig. 2 Association between transformed percentage black feathers on mantle and black feathers on tail and CORT concentration in tertials ($y = 1.276 - 0.003x$, Adjusted $r^2 = -0.146$, $p = 0.0491$)

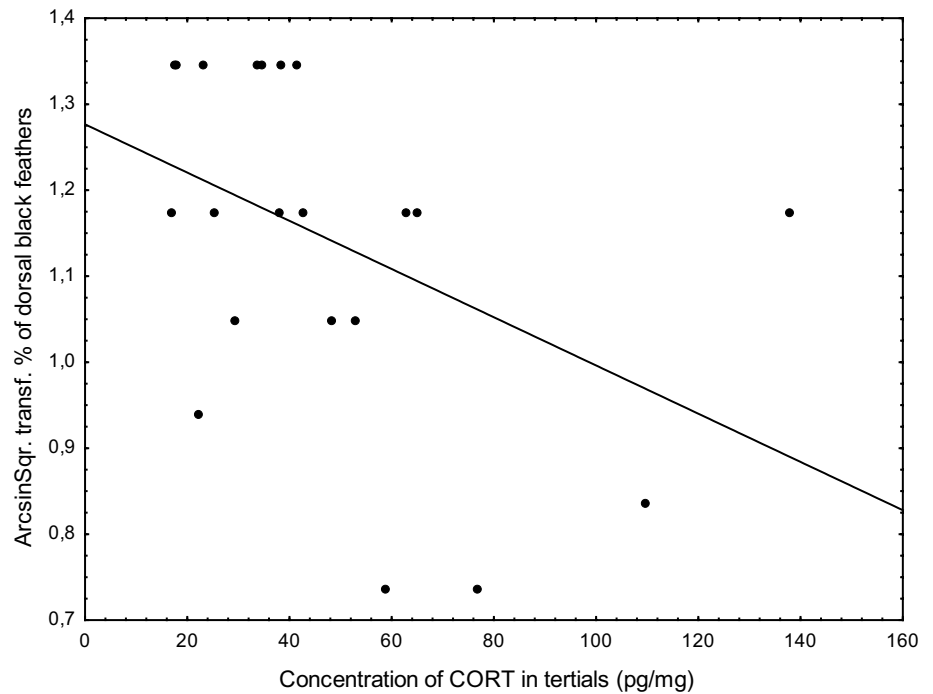


Table 2 Selection of Generalized Linear Models with normal distribution and identity link function of male wing patch size using AICc to estimate the relative strength of associations with CORTf for tail feathers (CORTf r), CORTf for tertials (CORTf t), tarsus length and their two-way interactions (1=CORTf t, 2=CORTf r, 3=Tarsus length)

| | df | AICc | χ^2 | p |
|----------------|----|--------|----------|-------|
| Tarsus | 1 | 80.920 | 2.038 | 0.153 |
| CORTf r | 1 | 82.351 | 0.607 | 0.436 |
| 1*2 | 1 | 82.428 | 0.530 | 0.467 |
| CORTf r Tarsus | 2 | 82.458 | 2.500 | 0.286 |
| 2*3 | 1 | 82.460 | 0.498 | 0.480 |
| Tarsus 2*3 | 2 | 82.463 | 2.495 | 0.287 |
| CORTf t | 1 | 82.756 | 0.202 | 0.653 |
| Tarsus 1*2 | 2 | 82.774 | 2.185 | 0.335 |
| 1*3 | 1 | 82.826 | 0.132 | 0.716 |
| CORTf r 2*3 | 2 | 82.827 | 2.132 | 0.344 |
| CORTf t Tarsus | 2 | 82.910 | 2.045 | 0.359 |
| Tarsus 1*3 | 2 | 82.910 | 2.048 | 0.359 |

Log-likelihood tests against null models are presented. Only models with AICc differences less than 2 from the best are presented

Discussion

Our results indicate that the size of the white forehead patch of males is negatively related to levels of CORT in tail feathers replaced on the breeding grounds. This association is only detected in smaller males. On the other

Table 3 Selection of Generalized Linear Models with normal distribution and identity link function of male transformed BLACKNESS (Arcsin-Sqrt transformed percentage of black feathers on head and mantle) using AICc to estimate the relative strength of associations with CORTf for tail feathers (CORTf r), CORTf for tertials (CORTf t), age and their two-way interactions (1=CORTf t, 2=CORTf r, 3=Tarsus length)

| | df | AICc | χ^2 | p |
|--------------------|----|--------|----------|-------|
| 1 * 3 | 1 | -8.898 | 4.988 | 0.026 |
| CORTf t | 1 | -8.603 | 4.693 | 0.030 |
| CORTf r 1*3 | 2 | -8.419 | 6.509 | 0.039 |
| 1*3 2*3 | 2 | -8.381 | 6.472 | 0.039 |
| CORTf t CORTf r | 2 | -8.136 | 6.226 | 0.044 |
| 1*2 1*3 | 2 | -8.093 | 6.184 | 0.045 |
| CORTf t 2*3 | 2 | -8.074 | 6.164 | 0.046 |
| CORTf t 1*2 | 2 | -7.800 | 5.891 | 0.053 |
| CORTf t Tarsus 1*3 | 3 | -7.689 | 7.779 | 0.050 |
| CORTf r 1*2 | 2 | -7.629 | 5.719 | 0.057 |
| CORTf t 1*3 | 2 | -7.405 | 5.495 | 0.064 |
| 1*2 2*3 | 2 | -7.125 | 5.215 | 0.074 |
| Tarsus 1*3 | 2 | -6.927 | 5.017 | 0.081 |

Log-likelihood tests against null models are presented. Only models with AICc differences less than 2 from the best are presented

hand, male BLACKNESS is negatively associated with concentration of CORT in tertials molted in the wintering range. This correlation is only apparent in large males. Finally, wing patch size based on feathers molted both

in the breeding area (primaries, secondaries) and in winter quarters (tertials), shows no association with CORTf. Although CORTf concentration in both feather types do not differ as to levels, these are not correlated within individuals, indicating that they are two independent measures of CORT concentration in blood during molt.

Several studies have associated the expression of plumage signals to levels of CORT in adult birds (Kennedy et al. 2013; Henderson et al. 2013; Saino et al. 2013; Henschen et al. 2018; Angelier et al. 2018; Berg et al. 2019; Windsor et al. 2019), or after experimental post-natal exposure (Dupont et al. 2019; Fairhurst et al. 2015), although structural colors have in some cases shown less association with CORT (Sarpong et al. 2019). Some of these studies include measurements of CORTf and show that CORTf can be negatively or positively related to signal intensity for carotenoid-based signals (Kennedy et al. 2013; Lendvai et al. 2013; Fairhurst et al. 2014; Grunst et al. 2015). Black, grey, and white plumage (termed achromatic plumage) has been shown to have a role in the context of status signaling (Møller and Erritzoe 1992; Senar et al. 2000; González et al. 2002). The expression of achromatic plumage signals has been related to social dominance and mating preferences in both male and female birds (Mennill et al. 2003; Woodcock et al. 2005; Santos et al. 2011; Crowhurst et al. 2012). Due to the highly conspicuous contrast between melanistic and white body regions, white plumage may be an efficient mode of visual communication (Galván 2008). Because individuals of both sexes risk injury during agonistic encounters, these signals could also be socially costly to maintain and therefore possibly favored by social selection in both sexes (Lyon and Montgomerie 2012). Sexual and social achromatic plumage signals may thus express the capacity to accommodate environmental challenges during molt as expressed by baseline CORTf.

In male Pied Flycatchers the size of achromatic patches and dorsal melanisation is determined during the yearly partial molt in the wintering areas when body feathers and tertials are molted prenuptially (Lundberg and Alatalo 1992; Moreno et al. 2019). We have shown previously that the extents of white feather patches in males are phenotypically plastic and respond to environmental conditions at the breeding grounds (Moreno et al. 2019). One physiological mechanism explaining these responses would be through circulating CORT levels during molt processes immediately after breeding. This would be expressed through CORTf levels in tail feathers. Accordingly, the sign of the association with CORTf in tail feathers in males was negative, as in females when analyzing CORTf in tertials (Moreno and López-Arrabé 2021). As shown for females (Moreno and López-Arrabé 2021), we could also expect that poor condition at the time of molt in Africa before migration could affect levels of plasma CORT as revealed by CORTf

in tertials. By analyzing CORTf in tertials we could thus gauge the environmental challenges experienced by birds at the time of prenuptial molt on the wintering grounds. However, in males CORTf in tertials showed no association with the size of the forehead patch. Unfortunately, Moreno and López-Arrabé (2021) did not analyze CORTf in tail feathers which precludes directly comparing both studies to ascertain if there are sex differences in these associations. The relationship found for forehead patch size confirms with feathers the same negative association found in another study on Iberian Pied Flycatcher males based on fecal CORT metabolites (Lobato et al. 2010). On the other hand, CORTf in any feather type did not contribute to explaining the size of the wing patch. A similar result was obtained for tertials in females (Moreno and López-Arrabé 2021). Interestingly, melanisation of body feathers on head and back was better explained by CORTf in tertials than in tail feathers. The black breeding plumage of males is developed before spring migration and may better express physiological status shortly before the impending breeding season than after the previous reproductive effort.

Small males may experience greater challenges while breeding due to social costs imposed by larger rivals for nest sites and/or mates (Andersson 1994; Ligon 1999). Interestingly, the negative association of CORT in feathers molted soon after breeding with forehead patch size was only marked for smaller males. This implies that for large males the conditions experienced during and immediately after the previous breeding season are not relevant when acquiring the subsequent breeding body plumage, while the more socially challenged smaller males express during molt in winter quarters their capacity to cope while breeding by adjusting the expression of the forehead patch during the subsequent molt. This result is related to the negative association of forehead patch size with experimentally altered breeding effort in the previous season found in the closely related Collared flycatcher *Ficedula albicollis* (Gustafsson et al. 1995). It seems that environmental challenges experienced during the breeding season have long-term consequences for signal expression presumably mediated by long-term changes in baseline CORT levels. On the other hand, large males have to cover a larger body surface with new body feathers at winter quarters (Walsberg and King 1978). They would therefore experience a greater challenge during prenuptial molt of body plumage as expressed by the negative association of baseline CORT levels in tertials molted at that stage with BLACKNESS. Thus, darker large males are those presenting low baseline glucocorticoid circulation before migrating to the breeding grounds.

Although the results from a single-year observational study based on a small sample of individuals need to be interpreted with caution, our results suggest that larger

white forehead patches are developed in the winter quarters in males with low CORT baseline concentration in plasma at the end of breeding, especially if they are small. On the other hand, a darker body plumage is produced by males that present low baseline CORT circulation before spring migration, especially if they are large. Males in nuptial plumage could thus be expressing through the extent of their forehead patches or dorsal plumage melanisation their physiological state after the previous breeding season or before migrating to the breeding grounds. The capacity to cope physiologically when recovering from breeding exertion or when preparing for the presumably costly prenuptial migration may be crucial variables to evaluate in social and sexual interactions.

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References

- Andersson M (1994) Sexual selection. Princeton Univ Press, Princeton
- Angelier F, Parenteau C, Trouve C, Angelier N (2018) The behavioural and physiological stress responses are linked to plumage coloration in the rock pigeon (*Columbia livia*). *Physiol Behav* 184:261–267. <https://doi.org/10.1016/j.physbeh.2017.12.012>
- Belskii EA, Lvakhov AG (2004) Breeding plumage coloration in *Ficedula hypoleuca* (Passeriformes, Muscicapidae) males from the Middle Urals. *Zool Zhurn* 83:1468–1475
- Berg ML, Knott B, Ribot RFH, Buchanan KL, Bennett ATD (2019) Do glucocorticoids or carotenoids mediate plumage coloration in parrots? An experiment in *Platyercus Elegans*. *General Compar Endocrinol* 280:82–90. <https://doi.org/10.1016/j.ygcen.2019.04.014>
- Bicudo T, Anciães M, Arregui L, Gil D (2020) Effects of forest fragmentation on feather corticosterone levels in an Amazonian avian community. *Ardeola* 67:229–245
- Bortolotti GA, Marchant TA, Blas J, German T (2008) Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Func Ecol* 22:494–500. <https://doi.org/10.1111/j.1365-2435.2008.01387.x>
- Bosholn M, Anciães M, Gil D, Weckstein JD, Dispoto JH, Fecchio A (2020) Individual variation in feather corticosterone levels and its influence on haemosporidian infection in a Neotropical bird. *Ibis* 162(1):215–226
- Crowhurst CJ, Zanollo V, Griggio M, Robertson J, Kleindorfer S (2012) White flank spots signal feeding dominance in female Diamond Firetails, *Stagonopleura guttata*. *Ethology* 118:63–75
- D’Alba L, Van Hemert C, Handel CM, Shawkey MD (2011) A natural experiment on the condition-dependence of achromatic plumage reflectance in Black-Capped Chickadees. *PLoS One* 6:8
- Dickens MJ, Romero LM (2013) A consensus endocrine profile for chronically stressed wild animals does not exist. *Gen Comp Endocrin* 191:177–189
- Doucet SM, Mennill DJ, Montgomerie R, Boag PT, Ratcliffe LM (2005) Achromatic plumage reflectance predicts reproductive success in male black-capped chickadees. *Behav Ecol* 16:218–222
- Douglas HD, Hector D, Kitaysky AS, Kitaiskaia EV (2018) Odor is linked to adrenocortical function and male ornament size in a colonial seabird. *Behav Ecol* 29:736–744. <https://doi.org/10.1093/beheco/ary032>
- Dupont SM, Grace JK, Brischox F, Angelier F (2019) Post-natal corticosterone exposure affects ornaments in adult male house sparrows (*Passer domesticus*). *Gen Comp Endocrinol* 276:45–51. <https://doi.org/10.1016/j.ygcen.2019.02.021>
- Fairhurst GD, Dawson RD, van Oort H, Bortolotti GR (2014) Synchronizing feather-based measures of corticosterone and carotenoid-dependent signals: what relationships do we expect? *Oecologia* 174:689–698. <https://doi.org/10.1007/s00442-013-2830-5>
- Fairhurst GD, Damore N, Butler MW (2015) Feather corticosterone levels independent of developmental immune challenges predict carotenoid-based, but not melanin-based, traits at adulthood. *Auk* 132:863–877. <https://doi.org/10.1642/AUK-15-34.1>
- Freeman NE, Newman AEM (2018) Quantifying corticosterone in feathers: validations for an emerging technique. *Conserv Physiol* 6:1–9
- Galván I, Moreno J (2009) Variation in effects of male plumage ornaments: the case of Iberian pied flycatchers. *Ibis* 151:541–546
- Ginn HB, Melville DS (1983) Molt in birds. *BTO Guide* 19:1–112
- González G, Sorci G, Smith LC, de Lope F (2002) Social control and physiological cost of cheating in status signaling male house sparrows (*Passer domesticus*). *Ethology* 108:289–302
- Grinkov VG, Kerimov AB (1998) The reproduction strategies and color variability of male breeding plumage in the pied flycatcher (*Ficedula hypoleuca*). Possible ways of maintenance of a phenotype population structure. *Zool Zhurnal* 77:825–837
- Grunst ML, Grunst AS, Parker CE, Romero LM, Rotenberry JT (2015) Pigment-specific relationships between feather corticosterone concentrations and sexual coloration. *Behav Ecol* 26:706–715. <https://doi.org/10.1093/beheco/aru210>
- Gustafsson L, Qvarnström A, Sheldon BC (1995) Trade-offs between life-history traits and a secondary sexual character in male colored flycatchers. *Nature* 375:311–313
- Harms NJ, Legagneux P, Gilchrist HG, Betty J, Love OP, Forbes MR, Bortolotti GR, Soos C (2015) Feather corticosterone reveals effect of molting conditions in the autumn on subsequent reproductive output and survival in an Arctic migratory bird. *Proc R Soc B* 282:2014–2085. <https://doi.org/10.1098/rspb.2014.2085>
- Harris CM, Madliger CL, Love OP (2016) Temporal overlap and repeatability of feather corticosterone levels: practical

- considerations for use as a biomarker. *Conserv Physiol* 4:cow051. <https://doi.org/10.1093/conphys/cow051>
- Hasegawa M, Arai E, Ito S, Wakamutsu K (2019) Evolution of short tails and breakdown of honest signaling system during a severe winter in the Pacific swallow *Hirundo tahitica*. *Evol Ecol* 33:403–416. <https://doi.org/10.1007/s10682-019-09978-z>
- Henderson LJ, Heidinger BJ, Evans NP, Arnold KE (2013) Ultraviolet crown coloration in female blue tits predicts reproductive success and baseline corticosterone. *Behav Ecol* 24:1299–1305. <https://doi.org/10.1093/beheco/art066>
- Henschen AE, Whittingham LA, Dunn PO (2018) Male stress response is related to ornamentation but not resistance to oxidative stress in a warbler. *Func Ecol* 32:1810–1818. <https://doi.org/10.1111/1365-2435.13104>
- Huhta E, Alatalo RV (1993) Plumage color and male-male interactions in the Pied Flycatcher. *Anim Behav* 45:511–518
- Järvi T, Röskaft E, Bakken M, Zumsteg B (1987) Evolution of variation in male secondary sexual characteristics—a test of eight hypotheses applied to pied flycatchers. *Behav Ecol Sociobiol* 20:161–169
- Järvistö PE, Laaksonen T, Calhim S (2013) Forehead patch size predicts the outcome of male-male competition in the pied flycatcher. *Ethology* 119:662–670
- Jenkins BR, Vitousek MN, Safran RJ (2013) Signaling stress? An analysis of pheomelanin-based plumage color and individual corticosterone levels at two temporal scales in North American barn swallows, *Hirundo rustica erythrogaster*. *Horm Behav* 64:665–672. <https://doi.org/10.1016/j.yhbeh.2013.08.006>
- Kennedy EA, Lattin CR, Romero LM, Dearborn DC (2013) Feather coloration in museum specimens is related to feather corticosterone. *Behav Ecol Sociobiol* 67:341–348. <https://doi.org/10.1007/s00265-012-1454-9>
- Kerimov AB, Rogovin KA, Ivankina EV, Bushuev AV, Sokolova OV, Ilyna TA (2013) Specific immunity and polymorphism of breeding plumage in pied flycatcher (*Ficedula hypoleuca*) males (Aves: Passeriformes). *Biol Bull Rev* 3:232–240
- Koren L, Nakagawa S, Burke T, Soma KK, Wynne-Edwards KE, Gefen E (2012) Non-breeding feather concentrations of testosterone, corticosterone and cortisol are associated with subsequent survival in wild house sparrows. *Proc R Soc B* 279:1560–1566. <https://doi.org/10.1098/rspb.2011.2062>
- Kouwenberg AL, McKay DW, Fitzsimmons MG, Storey AE (2015) Measuring corticosterone in feathers using an acetonitrile/hexane extraction and enzyme immunoassay: feather corticosterone levels of food-supplemented Atlantic Puffin chicks. *J Field Orn* 86:73–83. <https://doi.org/10.1111/jofo.12090>
- Lambrechts MM, Adriaensen F, Ardia DR, Artemyev AV, Atiénzar F, Bánbura J, Barba E, Bouvier J-C, Camprodon J, Cooper CB, Dawson RD, Eens M, Eeva T, Faivre B, Garamszegi LZ, Goodenough AE, Gosler AG, Grégoire A, Griffith SC, Gustafsson L, Johnson LS, Kania W, Keiš O, Llambias PE, Mainwaring MC, Mänd R, Massa B, Mazgajski TD, Møller AP, Moreno J, Naef-Daenzer B, Nilsson J-A, Norte AC, Orell M, Otter KA, Park CR, Perrins CM, Pinowski J, Porkert J, Potti J, Remeš V, Richner H, Rytönen S, Shiao M-T, Silverin B, Slagsvold T, Smith HG, Sorace A, Stenning MJ, Stewart I, Thompson CF, Török J, Tryjanowski P, van Noordwijk AJ, Winkler DW, Ziane N (2010) The design of artificial nestboxes for the study of secondary hole-nesting birds: a review of methodological inconsistencies and potential biases. *Acta Ornithol* 45:1–26
- Lendvai AZ, Giraudeau M, Németh J, Bakó V, McGraw KJK (2013) Carotenoid-based plumage coloration reflects feather corticosterone levels in male house finches (*Haemorrhous mexicanus*). *Behav Ecol Sociobiol* 2013(67):1817–1824
- Ligon JD (1999) The evolution of avian breeding systems. Oxford University Press, Oxford
- Lobato E, Moreno J, Merino S, Morales J, Tomás G, Martínez J, Vázquez R, Kuchar A, Möstl E, Osorno JL (2010) Arrival date and territorial behavior are associated with corticosterone metabolite levels in a migratory bird. *J Ornithol* 151:587–597. <https://doi.org/10.1007/s10336-009-0488-x>
- Lodjak J, Mägi M, Roonit U, Tilgar V (2015) Context-dependent effects of feather corticosterone on growth rate and fledging success of wild passerine nestlings in heterogeneous habitat. *Oecologia* 179:937–946. <https://doi.org/10.1007/s0042-015-3357-8>
- López-Arrabé J, Cantarero A, Pérez-Rodríguez L, Palma A, Moreno J (2014) Plumage ornaments and reproductive investment in relation to oxidative status in the Pied Flycatcher *Ficedula hypoleuca iberiae*. *Can J Zool* 92:1019–1027
- Lundberg A, Alatalo RV (1992) The pied flycatcher. Poyser, London
- Lyon BE, Montgomerie R (2012) Sexual selection is a form of social selection. *Phil Trans Roy Soc B-Biol Sci* 367:2266–2273
- MacDougall-Shackleton SA, Bonier F, Romero LM, Moore IT (2019) Glucocorticoids and “stress” are not synonymous. *Integr Organism Biol*. <https://doi.org/10.1093/iob/obz017>
- McGraw KJ (2008) An update on the honesty of melanin-based color signals in birds. *Pigm Cell Melanoma Res* 21:133–138
- Mennill DJ, Doucet SM, Montgomerie R, Ratcliffe LM (2003) Achromatic color variation in black-capped chickadees, *Poecile atricapilla*: black and white signals of sex and rank. *Behav Ecol Sociobiol* 53:350–357
- Møller AP, Erritzoe J (1992) Acquisition of breeding coloration depends on badge size in male house sparrows *Passer domesticus*. *Behav Ecol Sociobiol* 31:271–277
- Moreno J (2015) The incidence of clutch replacements in the Pied Flycatcher *Ficedula hypoleuca* is related to nest-box availability: Evidence of female-female competition? *Ardeola* 62:67–80
- Moreno J (2020) Effects of food availability and parental risk taking on duration of the nestling period: a field experiment. *Ardeola* 67:29–38
- Moreno J, López-Arrabé J (2021) The extent of white plumage patches in female Pied flycatchers *Ficedula hypoleuca* is negatively associated with corticosterone concentration in partly unpigmented feathers. *J Ornithol* 162:511–520. <https://doi.org/10.1007/s10336-020-01851-z>
- Moreno J, Møller AP (2006) Are melanin ornaments signals of antioxidant and immune capacity in birds? *Acta Zool Sin* 52:202–208
- Moreno J, Sanz JJ, Merino S, Arriero E (2001) Daily energy expenditure and cell-mediated immunity in pied flycatchers while feeding nestlings: interaction with molt. *Oecologia* 129:492–497. <https://doi.org/10.1007/s004420100767>
- Moreno J, Velando A, Ruiz-de-Castañeda R, Cantarero A, González-Braojos S, Redondo A (2011) Plasma antioxidant capacity and oxidative damage in relation to male plumage ornamental traits in a montane Iberian Pied Flycatcher *Ficedula hypoleuca* population. *Acta Ornithol* 46:65–70. <https://doi.org/10.3161/000164511X589929>
- Moreno J, Cantarero A, Plaza M, López-Arrabé J (2019) Phenotypic plasticity in breeding plumage signals in both sexes of a migratory bird: responses to breeding conditions. *J Avian Biol*. <https://doi.org/10.1111/jav01855>
- Möstl E, Rettenbacher S, Palme R (2005) Measurement of corticosterone metabolites in birds’ droppings: an analytical approach. *Ann NY Acad Sci* 1046:17–34
- Mougeot F, Lendvai AZ, Martínez-Padilla J, Pérez-Rodríguez L, Giraudeau M, Casas F, Moore IT, Redpath S (2016) Parasites, mate attractiveness and female feather corticosterone levels in a socially monogamous bird. *Behav Ecol Sociobiol* 70:277–283. <https://doi.org/10.1007/s00265-015-2048-0>
- Osborne JW, Overbay A (2004) The power of outliers (and WHY researchers should always check for them). *Pract Assess Res Evaluat*. <https://doi.org/10.7275/qf69-7k43>

- Osorno JL, Morales J, Moreno J, Merino S, Tomás G, Vázquez RA (2006) Evidence for differential maternal allocation to eggs in relation to manipulated male attractiveness in the Pied Flycatcher (*Ficedula hypoleuca*). *J Ornithol* 147:605–611
- Pötsch L, Moeller MR (1996) On pathways for small molecules into and out of human hair fibers. *J Forensic Sci* 41:121–125
- Potti J, Montalvo S (1991) Male arrival and female mate choice in pied flycatchers *Ficedula hypoleuca* in central Spain. *J Avian Biol* 22:45–54
- Romero LM, Fairhurst GD (2016) Measuring corticosterone in feathers: strengths, limitations, and suggestions for the future. *Comp Biochem Physiol A* 202:112–122
- Saetre GP, Dale S, Slagsvold T (1994) Female pied flycatchers prefer brightly colored males. *Anim Behav* 48:1407–1416
- Saetre GP, Fossnes T, Slagsvold T (1995) Food provisioning in the Pied Flycatcher—do females gain direct benefits from choosing bright-colored males. *J Anim Ecol* 64:21–30
- Saetre GP, Slagsvold T, Kruszewicz A, Viljugrein H (1997) Paternal care in Pied flycatchers *Ficedula hypoleuca*: energy expenditure in relation to plumage color and mating status. *Ardea* 85:233–242
- Saino N, Canova L, Costanzo A, Rubolini D, Roulin A, Møller AP (2013) Immune and stress response covary with melanin-based coloration in the Barn Swallow. *Evol Biol* 40:521–531. <https://doi.org/10.1007/s11692-013-9228-5>
- Santos ESA, Scheck D, Nakagawa S (2011) Dominance and plumage traits: meta-analysis and metaregression analysis. *Anim Behav* 82:3–19
- Sanz JJ, Moreno J, Merino S, Tomás G (2004) A trade-off between two resource-demanding functions: post-nuptial molt and immunity during reproduction in male pied flycatchers. *J Anim Ecol* 73:441–447. <https://doi.org/10.1111/j.0021-8790.2004.00815.x>
- Sapolsky RM (1982) The endocrine-stress response and social status in the wild baboon. *Horm Behav* 16:279–287
- Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrin Rev* 21:55–89
- Sarpong K, Madliger CL, Harris CM, Love OP, Doucet SM, Bitton PP (2019) Baseline corticosterone does not reflect iridescent plumage traits in female tree swallows. *Gen Comp Endocrinol* 270:123–130. <https://doi.org/10.1016/j.ygcen.2018.10.015>
- Schull Q, Robin JP, Dobson FS, Saadaoui H, Viblanc VA, Bize P (2018) Experimental stress during molt suggests the evolution of condition-dependent and condition-independent ornaments in the king penguin. *Ecol Evol* 8:104–1095. <https://doi.org/10.1002/ece3.3677>
- Senar JC, Polo V, Uribe F, Camerino M (2000) Status signalling, metabolic rate and body mass in the siskin: the cost of being a subordinate. *Anim Behav* 59:103–110
- Sepp T, Desai S, Lendvai AZ, Németh Jm McGraw KJ, Giraudeau M (2018) Feather corticosterone levels are not correlated with health or plumage coloration in juvenile house finches. *Biol J Linn Soc* 20:1–8
- Sirkiä PM, Laaksonen T (2009) Distinguishing between male and territory quality: females choose multiple traits in the pied flycatcher. *Anim Behav* 78:1051–1060
- Sirkiä PM, Virolainen M, Laaksonen T (2010) Melanin coloration has temperature-dependent effects on breeding performance that may maintain phenotypic variation in a passerine bird. *J Evol Biol* 23:2385–2396
- Sirkiä PM, Adamík P, Artemyev AV, Belskii E, Both C, Bureš S, Burgess M, Bushuev AV, Forsman JT, Grinkov V, Hoffmann D, Järvinen A, Král M, Krams I, Lampe HM, Moreno J, Mägi M, Nord A, Potti J, Ravussin PA, Sokolov L, Laaksonen T (2015) Fecundity selection does not vary along a large geographical cline of trait means in a passerine bird. *Biol J Linn Soc* 114:808–827
- Slagsvold T, Lifjeld JT (1992) Plumage color is a condition-dependent sexual trait in male pied flycatchers. *Evolution* 46:825–828
- Svensson L (1984) Identification guide to European passerines. Stockholm
- Walsberg GE, King JR (1978) The relationship of the external surface area of birds to skin surface area and body mass. *J Exp Biol* 76:185–189
- Will A, Wynne-Edwards K, Zhou RK, Kitaysky A (2019) Of 11 candidate steroids, corticosterone concentration standardized for mass is the most reliable steroid biomarker of nutritional stress across different feather types. *Ecol Evol* 9:11930–11943. <https://doi.org/10.1002/ece3.5701>
- Windsor RL, Fox GA, Bowman R (2019) Consistency of structural color across molts: The effects of environmental conditions and stress on feather ultraviolet reflectance. *Auk* 136:1–17
- Wingfield JC (2013) Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. *Funct Ecol* 27:37–44
- Wingfield JC, Smith JP, Farner DS (1982) Endocrine responses of white-crowned sparrows to environmental stress. *Condor* 84:399–409
- Wingfield JC, Romero LM (2001) Adrenocortical responses to stress and their modulation in free-living vertebrates. In: McEwen BS, Goodman HM (eds) *Handbook of Physiology*, Section 7, vol IV, pp 211–234
- Woodcock EA, Rathburn MK, Ratcliffe LM (2005) Achromatic plumage reflectance, social dominance and female mate preference in black-capped chickadees (*Poecile atricapillus*). *Ethology* 111:891–900

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