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Age and sex differences in niche use at molt and its effect on plumage coloration characteristics in a bird

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Abstract

Bird plumage is often very colorful and can communicate the quality of the bearer to conspecifics. These plumage-based signals of quality are composed of multiple pigments (e.g., melanin and carotenoids). Therefore, sex and age classes, which often show marked differences in plumage coloration, may have different dietary needs for the different plumage components and this might promote preferences for different dietary niches at different molting stages. However, no study has addressed the role that changes in niche use play in the expression of multiple component plumage signals in birds. We used stable isotope analysis to test the hypothesis that niche use is related to age and sex and to differently cultured plumage patches, yellow carotenoid-based and black melanin-based, in great tits *Parus major*. We recorded high niche overlap between plumage patches, although δ^{15}N was higher in black than yellow plumage. Niche overlap was relatively low for age classes and relatively high for sex classes, and age classes showed a contrasting pattern of niche overlap between carotenoid- and melanin-based plumages. Moreover, δ^{13}C, but not δ^{15}N, had a significant negative relationship with carotenoid-based plumage, which was only apparent in juveniles. Taken together, our results demonstrate that niche use had a moderate influence on plumage coloration characteristics of great tit individuals, mostly associated with δ^{13}C rather than with δ^{15}N and with age rather than with sex. Therefore, our study is significant because it confirms the relevance of niche use during ornament production in free-living birds.

Key words: carotenoids, great tits, melanin, multiple ornaments, niche overlap, stable isotopes

Ornaments are often composed of multiple components that may convey information about different aspects of individual quality (Møller and Pomiankowski 1993). The different colors of ornaments are derived from different pigments (McGraw 2006a, 2006b). The two most common pigments used by birds for coloration are melanin pigments (browns and blacks) and carotenoids (yellows, oranges, and reds). Both pigments are reliant on access to required nutrients during the molting phase, although to a different degree. Although melanin pigments are endogenously synthetized, carotenoids can only be acquired from the diet (McGraw 2006a, 2006b). Therefore, the expression of these pigments may have different environmental constraints to one another.

Melanin-based coloration is highly heritable and its expression is regulated by hormones (Bókony et al. 2008; Gasparini et al. 2009; Saino et al. 2013; but see also Roulin 2016). Yet, environmental (Talloen et al. 2004; Fargallo et al. 2007; Giraudeau et al. 2015) and nutritional constraints (e.g., availability of calcium; McGraw 2007) also have a role in determining colors. Some arthropods (e.g.,

spiders) contain high levels of calcium on which melanin metabolism depends and so are a commonly used food source for insectivorous birds (Graveland and Van Gijzen 1994; Bureš and Weidinger 2003). Conversely, carotenoid-based coloration has a strong environmental component often associated with habitat use and diet and its expression mainly depends on the allocation trade-off of carotenoid pigments between competing physiological and signaling functions (Slagsvold and Lifjeld 1985; Alonso-Alvarez et al. 2008; Pagani-Núñez et al. 2014; see also Hill & McGraw 2006). Although the birds derive their carotenoids exclusively from their diets, its expression in bird plumage coloration is moderately heritable (Evans and Sheldon 2012). Carotenoid-based ornaments are considered honest signals of quality because plumage coloration can be related to an individual's ability to find these carotenoids, which are present in fruits and caterpillars in high concentrations (Hill 1991; Hill and McGraw 2006). Yet, a minority of authors suggest that carotenoids are not limiting and other physiological factors are more important for maintaining signal honesty (Isaksson et al. 2008; Simons et al. 2014).

The expression of ornaments may also vary according to sex and age differences, which may determine differential niche use and access to food containing the required nutrients. Sex- and age-related color dimorphism can be gradual or discrete and niche use may vary accordingly. Several studies have reported age-related changes in coloration (e.g., Val et al. 2010; Pagani-Núñez and Senar 2012) and niche use (Polis 1984; see also Vitz and Rodewald 2012; Kernaléguen et al. 2016) although no study has integrated these two factors in a single study. In addition, the sexes often show differences in niche use, which have been associated with sexual dimorphism and sexually divergent life-history strategies in reptiles (Hochkirch et al. 2007), amphibians (Bell and Zamudio 2012), and birds (Bell 1982; Pasinelli 2000). Therefore, it is surprising that almost no study has focused on the role of sexual niche partitioning as a driver of sexual dichromatism (but see Bortolotti et al. 1996). Different individuals may also have different abilities to acquire nutrients and accordingly may influence ornament expression (García-Navas et al. 2012, Pagani-Núñez and Senar 2014). Finally, previous research has reported differences in the timing of molt based on age (Bojarinova et al. 1999; Kiat and Sapir 2017), sex class (Siikamäki et al. 1994; Hemborg 1999), and between different plumage patches (i.e., contour and flight feathers; O'Hara et al. 2002), in many bird species. Therefore, ascertaining bird's niche use and diet during the molting period of different plumage patches is critical to understanding the expression of colored ornaments.

Great tits *Parus major* are an ideal species to study the relationships between niche use and multiple component signaling (Hegyi et al. 2007) because they have different colored plumage patches that have different dietary needs. They have a melanin-based black tie, which is much larger in males than in females and is a signal of social dominance (Järvi and Bakken 1984; Pöysä 1988; see also Senar 2006). They also have yellow carotenoid-based feathers covering the rest of the breast, that darkens with age (Val et al. 2010) and so, can function as an indicator of individual's quality as parents (Hill 1991). Members of the family Paridae have age and sex structured social hierarchies (e.g., Carrascal et al. 1998; Ratcliffe et al. 2007) which are reinforced behaviorally and expressed through individual's plumage ornaments. However, no study has investigated if there are differences in the timing of molt of differently colored plumage patches and whether free-living birds change their habitat or foraging niches at different molt stages to account for different dietary needs.

A powerful tool for examining differences in niche use during feather production is stable isotopes analysis (SIA) (Quinn et al. 2016). Carbon and nitrogen isotope ratios are commonly used to investigate niche use between individuals, populations, or species. Carbon isotope ratios are linked to the consumption of C^3 or C^4 plants, and more generally with habitat structure, whereas nitrogen isotopes ratios are positively correlated to the trophic level in which individuals are feeding (Boecklen et al. 2011). Quantifying those isotope ratios enable researchers to provide direct estimates of habitat and diet overlap among species, communities, or social groups (Swanson et al. 2015).

In this study, we tested the hypothesis that bird's niche use is related to sex and age (which correlate to dominance), and that also determines access to nutrients at different stages of the molt and, thus, differences in coloration among individuals. This allowed us to make four predictions. First, we predicted that birds would show asynchrony in the timing of molt for differently colored plumage patches. This asynchrony of molt would provide the necessary temporal variation for niche shift during molt. Second, we predicted individuals would exploit different niches (measured using stable isotopes) at different molt stages in order to obtain the specific nutrients required while molting different color patches. Third, we predicted that sex and age differences in coloration would be associated with differences in niche use. Finally, we predicted that differences in niche use would affect ornament production regardless of age and sex, and therefore act as a signal of an individual's foraging ability. Together, these predictions will enable us to examine whether niche use is an important factor determining the expression of multi-component visual signals.

Materials and Methods

Study area and sampling procedures

This study was conducted in a Mediterranean mixed forest located at the Can Catà field station (45°27'N, 2°8'E, Catalonia, northeast Spain). Here, the main tree species are pines *Pinus halepensis*, oaks *Quercus cerrioides*, and holm oaks *Q. ilex*. This forest shows a marked pattern of variation. Oaks dominate valley bottom, whereas the proportion of pines increases with elevation (Pagani-Núñez et al. 2014). This pattern is associated with $\delta^{13}C$ variation, with less negative values associated with pine-dominated patches and vice versa (Pagani-Núñez et al. 2016). During the study, we captured 59 individual great tits using funnel traps (Senar et al. 1997). We sexed and aged individuals according to plumage coloration characteristics (Svensson 1992).

We had two sampling periods. The first was during August 2012, when we collected data on the temporal molting patterns of yellow and black contour feathers of great tits within the study area. We captured 18 individuals (7 males, 6 females, and 5 individuals of unknown sex; 9 juvenile and 9 adult individuals). Molting birds are generally less active and more difficult to capture than non-molting birds, which explain our low number of captures. We used these individuals to quantify the intensity of molt of contour plumage of different plumage patches. Therefore, we counted the numbers of contour feathers in active molt at the yellow breast patches and the ventral black tie on the breast. Our second sampling period was during January and February 2013 when we collected feathers, which were used for stable isotope analyses. We captured 25 females (18 adult and 7 juvenile individuals) and 34 males (30 adult and 4 juvenile individuals). Our sample was biased toward adults because sampling took place during late winter, when the ratio

juvenile/adult had declined due to post-fledgling mortality of juveniles.

Plumage coloration characteristics

We measured coloration on the yellow plumage patch at both the upper and the lower part of the belly (and computed the mean of both measurements). We used a portable colorimeter to measure the color of feathers (Minolta CR200, Minolta Corporation 1994). We computed chroma values using the 1976 CIELAB Uniform Color Space standard algorithms implemented in this colorimeter (Minolta Corporation 1994; Fairchild 2005). Chroma is the color purity or color saturation and is measured on a continuous scale from 0 to 100. It quantifies how different from gray, at constant lightness (i.e., relative luminance), is an object's color and it is related to carotenoid content in feathers (Isaksson et al. 2008). We repeated each measurement two times for each individual and used the mean value in analyses. Repeatability of color measurements obtained with this procedure was high (i.e., > 0.9; Figuerola et al. 1999). We obtained samples over a short time-period, so plumage abrasion should not have affected these coloration measurements (Figuerola and Senar 2005). We computed chroma, but excluded other relevant traits for coloration (e.g., achromatic components), because previously researchers have focused on chroma of yellow plumage and black tie size (see e.g., Norris et al. 1990; Dauwe and Eens 2008). We also measured the width of bird's black ties at the level of the clavicle with digital calipers (0.01 mm) (Järvi and Bakken 1984), which we used as a proxy of overall tie size (Figuerola and Senar 2000).

Stable isotopes analyses

In order to conduct niche use analyses, we collected feathers from yellow and black plumage patches to extract stable isotope signatures. We focused on $\delta^{13}C$ (as a proxy of differential exploitation of C3 and C4 plants) and $\delta^{15}N$ values (as a proxy of trophic level in the food web), which are commonly employed to quantify niche use (Boecklen et al. 2011). Previous research conducted at our study site on great tits has shown that low values of $\delta^{13}C$ were associated with dense low altitude Oak-dominated areas, whereas high values were associated with higher altitude open Pine-dominated areas (Pagani-Núñez et al. 2016). Conversely, values of $\delta^{15}N$ were related to trophic level. High $\delta^{15}N$ values were associated with a high intake of spiders (high $\delta^{15}N$ values due to their role as predators) and low $\delta^{15}N$ values were associated with high intake of caterpillars, which eat leaves and fruits (Pagani-Núñez et al. 2016). We collected at least 15–20 contour feathers from each individual at each plumage patch. We collected feathers from both the upper and lower parts of the yellow breast patch (which mirrored our coloration measurement) and the black ventral tie (i.e., 2 samples of yellow and 1 sample of black feathers [45–60 feathers per individual]). We kept feathers from each patch in sealed plastic bags and stored in dry and dark conditions in the laboratory until their later analysis. We pooled multiple feathers from each patch for analysis.

We conducted the stable isotope analyses at the Scientific Technical Services Department at the University of Barcelona. We cleaned the feathers in a NaOH solution (0.25 M) and oven-dried them at 40°C for 24 h. We then powdered the feathers and loaded subsamples (0.35 mg) into tin packets, which were crimped for combustion. We powdered together upper and lower breast yellow feathers, so that we had one measurement per plumage patch and individual. We conducted SIA using an elemental analysis-isotope ratio mass spectrometer (EA-IRMS) with a Flash 1112 (for C and N) elemental analyzer. These were coupled to a Delta C IRMS via a CONFLOIII interface (Thermo Fisher Scientific, Bremen, Germany). We expressed stable isotope ratios as parts per thousand (‰), according to the equation $\delta X = [(Rsample/Rstandard)-1]$, where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. We referenced our samples against international standards Pee Dee Belemnite (VPDB) for ^{13}C and atmospheric nitrogen (AIR) for ^{15}N. As internal standards, we used IAEACH7, USGS40 and IAEACH6 for ^{13}C, and USGS40, IAEAN1, IAEANO3 and IAEAN2 for ^{15}N. We paired internal standards against international standards and analyzed them each for 16 sample measurements. Measurement precision was 0.15‰ for $\delta^{13}C$ and 0.25‰ for $\delta^{15}N$.

Statistical analyses

We made a number of different analyses in order to test our predictions. First, we assessed time asynchrony at molt of different plumage patches by computing a generalized linear regression (Poisson distribution with a log link-function). We used the number of yellow feathers in active molt as dependent variable and date (measured as the number of days since 1 July) as independent variable. We repeated this procedure with the number of black feathers showing active molt.

Second, we determined whether there birds were using different niches when molting yellow and black plumage patches. We ran a linear mixed-effect model, fitted by restricted maximum likelihood, using $\delta^{13}C$ values as dependent variable. As fixed-factors we include plumage patch (black melanin-based or yellow carotenoid-based), age (juvenile or adult), and sex classes (male or female), and computed the interactions between plumage patch and sex and between plumage patch and age. We included individual identity as random factor. Therefore, we compared plumage patches after accounting for sex and age differences in niche use. For this procedure, we used nlme v3.1-131 (Pinheiro et al. 2017). We repeated this procedure with $\delta^{15}N$ values. We report mean $\pm$ SD of isotope values throughout.

We examined niche overlap between the sexes and age classes, and for the different plumage patches, using nicheRover v1.0 (Swanson et al. 2015). This allowed us to combine information from the two isotope signatures into a single measure of niche overlap in order to quantify differences in niche use between age and sex classes. This software uses a Bayesian approach to compute niche regions (i.e., elliptical projections of niche space) using any continuous variable of niche use (Swanson et al. 2015). There is a probability of an individual from group A to be found in the modeled niche region of group B and vice versa (i.e., overlap probability). The difference between these two values facilitates the assessment of asymmetric niche overlap between groups. We modeled 1000 samples and ran 1000 iterations to compute niche regions with a probability level of alpha = 0.95 (i.e., 95% probability) and 95% confidence intervals, and used 10 Monte Carlo draws to plot the elliptical projection (i.e., default priors).

Chroma of yellow feathers and black tie width fitted a normal distribution, which we assessed using qq-plots. We computed these qq-plots using car v2.1-5 (Fox et al. 2017) and MASS v7.3-47 (Ripley et al. 2013) packages. Hence, we computed a linear model (Gaussian model and identity link-function) with chroma of yellow plumage as the dependent variable; sex and age as categorical factor; and isotope values ($\delta^{13}C$ and $\delta^{15}N$) of yellow plumage as independent variables, also including significant interactions from previous models. We assessed the relative explanatory power of these variables through model selection procedures based on second-order Akaike Information Criteria (AICc, corrected for small sample sizes)

using MuMIn v1.15.6 (Bartoń 2015). We considered models with ΔAICc < 2 as optimal, which is the difference in AICc between the best and a given model (Burnham and Anderson 2002). We only report models with ΔAICc < 10, which are the models presumed to be biologically meaningful (Symonds and Moussalli 2011). We repeated this analysis using black tie width as dependent variable. All analyses were conducted using R software v3.4 (R Core Team 2017) and used ggplot2 v2.2.1 (Wickham 2009) to create the figures.

Results

Temporal asynchrony at molt of different plumage patches

We recorded certain asynchrony in the molting patterns of yellow and black feathers. Individuals showed a tendency to start molting yellow feathers first with black feathers molting later (N yellow feathers *vs.* date: Estimate $\pm$ SD $= -0.08 \pm 0.01$, $t = -7.12$, $P < 0.01$; whilst N black feathers *vs.* date: Estimate $\pm$ SD $= 0.04 \pm 0.01$, $t = 4.92$, $P < 0.01$) (Figure 1). This provided the necessary temporal variation in molt times between color patches that is required for niche switching during the molting phase. Age and sex effects were not significant when included in these models (all $P > 0.07$) whilst date-related effects remained significant (all $P < 0.01$) (data not shown).

Plumage patch, sex, and age differences in isotopic signatures

We did not find significant differences in $\delta^{13}C$ values between plumage patches (black: -22.31 ± 1.20 *vs.* yellow $= -22.70 \pm 1.15$) or between the sexes (females: -22.76 ± 1.31 *vs.* males: -22.66 ± 1.12), but we did find a significant difference in $\delta^{13}C$ values between adults and juveniles (juveniles: -21.21 ± 1.19 *vs.* adults: -22.56 ± 1.07) (Table 1). The interaction between plumage

patch and sex was not significant, whilst the interaction between plumage patch and age was significant (Table 1). The difference in $\delta^{13}C$ values between adults and juveniles was stronger for black than for yellow feathers (Figure 2A).

When we conducted the analysis for nitrogen isotopes, we found a different pattern from the pattern found for the carbon isotopes. We recorded significant differences in $\delta^{15}N$ values between plumage patches (black: 1.51 ± 1.41 *vs.* yellow: 1.45 ± 1.26), whilst there were no differences in $\delta^{15}N$ values between age (juveniles: 3.18 ± 1.45 *vs.* adults: 1.11 ± 0.93) or sex classes (females: 1.61 ± 1.44 *vs.* males: 1.40 ± 1.21) (Table 2). Moreover, we found significant interactions between plumage patch and age class and between plumage patch and sex class (Table 2). Age differences in $\delta^{15}N$ were more marked for yellow than for black plumage (Figure 2B), whilst sexual differences were more marked for black than for yellow plumage (Figure 2C).

Niche overlap at molt of different plumage patches

Overall, we recorded higher overlap between the sexes than between age classes (Figure 3). When birds were molting yellow plumage, they had relatively high niche overlap between the sexes with females showing an overlap percentage of 81.36% (95% CI = 64%–95%) with males' niche regions, and males showed an overlap percentage of 94.39% (83%–100%) with the niche regions of females. However, the niche overlap percentage was relatively low between age classes, with juvenile individuals showing a niche overlap percentage of 40.3% (19%–65%) with adults and adults showing a niche overlap percentage of 75.39% (35%–99%) with juveniles.

When we examined the niche overlap of the later black plumage, we recorded a similar fashion of overlap although age classes showed the opposite pattern. Females again showed a high niche overlap percentage of 82.13% (65%–95%) with males, whilst male niche overlap percentage was 94.56% (84%–100%) of the female
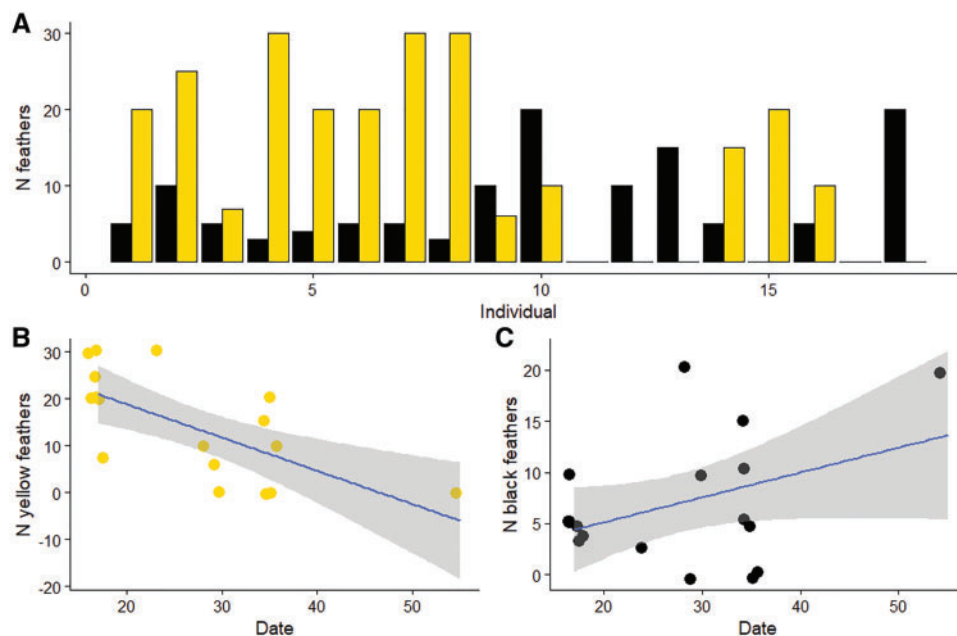

Figure 1. Temporal variation at molt of black and yellow contour feathers: **(A)** linear relationships between the number of black and yellow contour feathers being molted and date, and **(B)** number of black and **(C)** yellow feathers being molted per individual (ordered by date). In **(B)** and **(C)**, regression lines and 95% confidence intervals are provided.
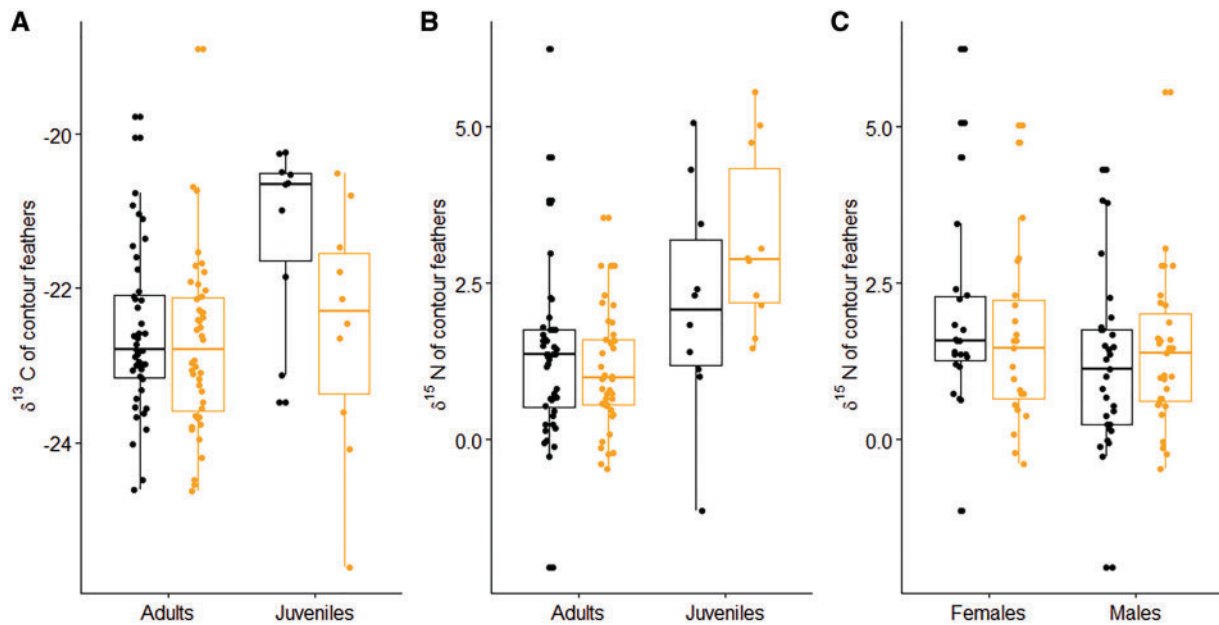
Figure 2. (A) Age differences in δ^{13}C of black (black color) and yellow (dark yellow color) contour feathers. (B) Age differences in δ^{15}N of black (black color) and yellow (dark yellow color) contour feathers. (C) Sex differences in δ^{15}N of black (black color) and yellow (dark yellow color) contour feathers. Horizontal lines represent median and first and third quartiles.

Table 1. Results from non-linear mixed-effect model analyzing the effect of plumage patch (yellow carotenoid-based or black melanin-based), sex (female or male), and age (juvenile or adult) on δ^{13}C values

	Estimate	SE	t	P
(Intercept)	−22.72	0.27	−83.35	< 0.001
Plumage patch	−0.13	0.24	−0.54	0.594
Sex	0.25	0.33	0.76	0.451
Age	**1.42**	**0.42**	**3.38**	**0.001**
Plumage patch* Sex	−0.08	0.29	−0.29	0.775
Plumage patch* Age	**−1.13**	**0.37**	**−3.03**	**0.004**

Significant effects are marked in bold.

Table 2. Results from non-linear mixed-effect model analyzing the effect of plumage patch (yellow carotenoid-based or black melanin-based), sex (female or male), and age (juvenile or adult) on δ^{15}N values

	Estimate	SE	t	P
(Intercept)	1.76	0.30	5.92	< 0.001
Plumage patch	**−0.79**	**0.24**	**−3.33**	**0.002**
Sex	−0.64	0.36	−1.78	0.081
Age	0.61	0.46	1.34	0.185
Plumage patch × Sex	**0.87**	**0.29**	**3.02**	**0.004**
Plumage patch × Age	**1.53**	**0.37**	**4.17**	**< 0.001**

Significant effects are marked in bold.

niche region. Conversely, juveniles showed a niche overlap percentage of 60.36% (31%–89%) with the adults' niche regions, whilst adults showed a niche overlap percentage of 47.34% (20%–84%) with juveniles.

The effect of niche use on plumage coloration characteristics

Model selection procedures showed that only 1 out of 22 models had ΔAICc < 2 using yellow chroma as dependent variable (Table 3). This model included sex and age effects, and significant negative relationships with δ^{13}C values of yellow plumage and with the interaction between δ^{13}C values and age (Table 3; Figures 4 and 5). Similarly, in the case of black tie width only 1 out of 17 models had ΔAICc < 2. This model only included sex effects (Table 4).

Discussion

In this study, we recorded several age and sex dependent differences in δ^{13}C and δ^{15}N isotopic signatures and also moderate to high niche overlap at molt of differently colored plumage patches and between age and sex classes. Therefore, we found mixed support for our hypothesis that niche use during molt are the basis of color differences in plumage based sexually selected ornaments. First, we found that δ^{13}C rather than δ^{15}N correlated with plumage coloration characteristics of great tits. In our study area, δ^{13}C variation is associated with the spatial niche, while δ^{15}N variation is associated with the trophic niche (Pagani-Núñez et al. 2016). In this study, only δ^{15}N values were found different between carotenoid- and melanin-based plumages. Second, we found that although niche overlap was generally high between classes, there was relatively low niche overlap between adult and first year birds, whereas the sexes showed relatively high niche overlap. Juveniles had broader niches than adults at molt of black melanin-based plumage and showed the opposite pattern at molt of yellow carotenoid-based plumage. Overall, we recorded a limited influence of niche use, mostly associated with δ^{13}C rather than with δ^{15}N and with age rather than sex classes, on plumage coloration characteristics of great tit individuals.
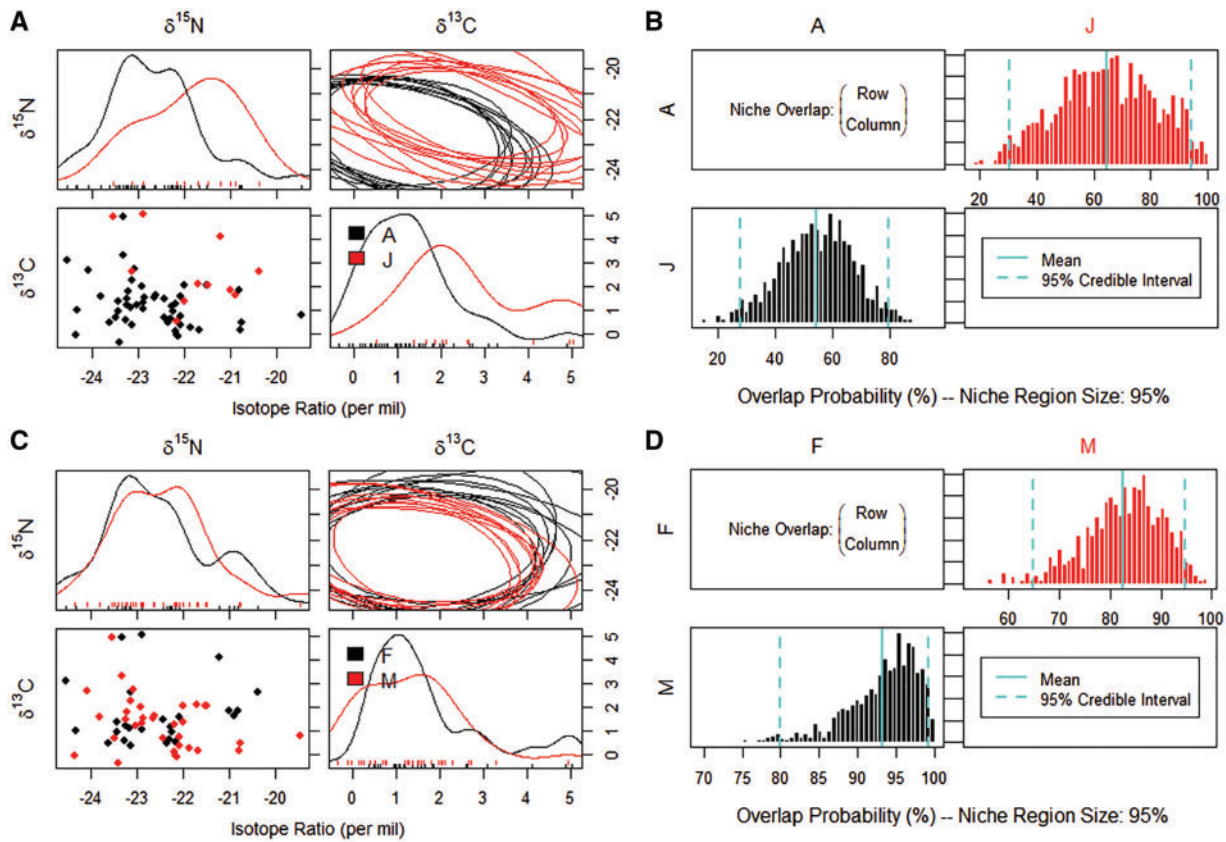
Figure 3. Graphical representation of niche overlap and asymmetries between **(A)** age classes (adults and juveniles) and **(C)** sexes (males and females). Left-up and right-down: density plots in function of age and sex class for δ^{15}N and δ^{13}C, respectively. Left-down: scatterplots of δ^{13}C vs. δ^{15}N values. Right-up: elliptical projections of niche regions for the different sexes and age classes. Niche overlap was much higher for the sexes than for age classes. A graphical representation of the overlap probability (and 95% confidence intervals) in niche regions between **(B)** age classes (adults and juveniles) and **(D)** sexes (males and females) is also provided.

Table 3. Results from model selection procedures based on second-order AICc

Yellow chroma

Age	Sex	Y δ^{13}C	Y δ^{15}N	Y δ^{13}C × Age	Y δ^{15}N × Age	Y δ^{15}N × Sex	df	logLik	AICc	ΔAICc	Weight	Intercept
+	**+**	**+**		**+**			**6**	**−144.76**	**303.40**	**0.00**	**0.365**	**18.29**
+	+	+					5	−147.17	305.60	2.26	0.118	−0.60
+	+	+	+	+			7	−144.73	306.00	2.60	0.099	18.63
+	+	+	+	+	+		8	−143.68	306.60	3.28	0.071	19.90
+	+						4	−149.02	306.90	3.52	0.063	21.85
+	+	+	+	+		+	8	−144.19	307.60	4.29	0.043	15.28
+		+		+			5	−148.27	307.80	4.46	0.039	22.37
+	+	+	+				6	−147.13	308.10	4.74	0.034	−0.19
+	+	+	+	+	+	+	9	−142.97	308.10	4.77	0.034	16.24
+		+					5	−148.85	309.00	5.62	0.022	21.54
+	+	+	+			+	7	−146.55	309.60	6.23	0.016	−3.44
+	+	+	+		+		7	−146.69	309.90	6.52	0.014	−0.92
+		+	+	+			6	−148.13	310.10	6.73	0.013	23.03
+		+					4	−150.75	310.30	6.98	0.011	2.05
+		+	+	+	+		7	−147.09	310.70	7.31	0.009	24.30
+	+		+		+		6	−148.48	310.80	7.44	0.009	21.19
+							3	−152.28	311.00	7.69	0.008	23.76
+	+		+			+	6	−148.63	311.10	7.73	0.008	21.09
+	+	+	+		+	+	8	−145.99	311.30	7.90	0.007	−4.52
+		+	+				5	−150.60	312.50	9.12	0.004	2.89
+			+				4	−151.93	312.70	9.35	0.003	23.23
+	+		+		+	+	7	−148.21	312.90	9.56	0.003	20.67

The dependent variable was yellow chroma of breast feathers. The independent variables were sex (male or female), age (young or adult), and δ^{13}C and δ^{15}N of yellow plumage. We included the interactions δ^{13}C × age, δ^{15}N × age and δ^{15}N × sex, which were significant in previous models (Tables 1 and 2). We considered optimal those models with ΔAICc <2 but here are reported models with ΔAICc <10. Models were also weighted (column "Weight") according to their relative likelihood. A "+" indicates that the variable is included in the model.
Models with ΔAICc < 2 are marked with bold letters.

Age- and sex-related effects of niche use on plumage coloration

Visual color displays of birds are key signals and because they are molted annually, they can act as a signal of the individual's foraging ability, condition, and social-status at the time of molt (Hawkins et al. 2012; Dale et al. 2015). However, no study has addressed the implications of niche use during ornament production in natural conditions. We found that juveniles showed high overlap in $\delta^{13}C$ and low overlap in $\delta^{15}N$ with adults whilst molting yellow plumage. Conversely, juveniles showed low overlap in $\delta^{13}C$ and high overlap in $\delta^{15}N$ with adults for the black plumage patches. Moreover, juveniles' niches were smaller and were recorded within adults' niches at molt of yellow plumage, whereas we found the opposite pattern at molt of black plumage. This suggests that niche partitioning may play a role in driving color differences in carotenoid-based ornaments recorded between age classes in birds (see e.g. Val et al. 2010; Pagani-Núñez and Senar 2012). Niche constraints driven by competitive exclusion could also influence age-based color differences in species displaying gradual color variation and might therefore, be non-adaptive (Hawkins et al. 2012; see also Ebenman 1987).
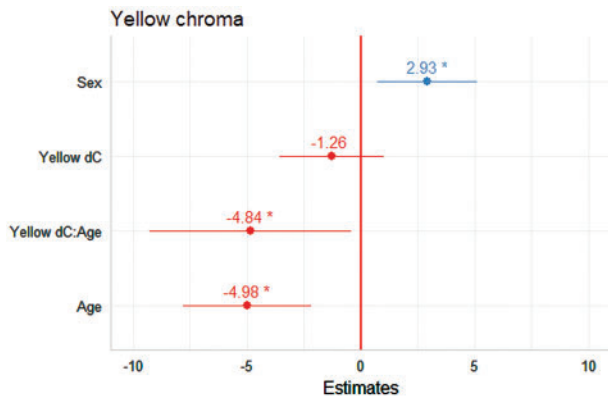

Figure 4. Plot of point estimates and SE of the correlations between factors sex, age, $\delta^{13}C$ of yellow feathers, and the interaction between $\delta^{13}C$ and age, with chroma of yellow feathers, which was the best model product of Akaike Information Criterion procedures.
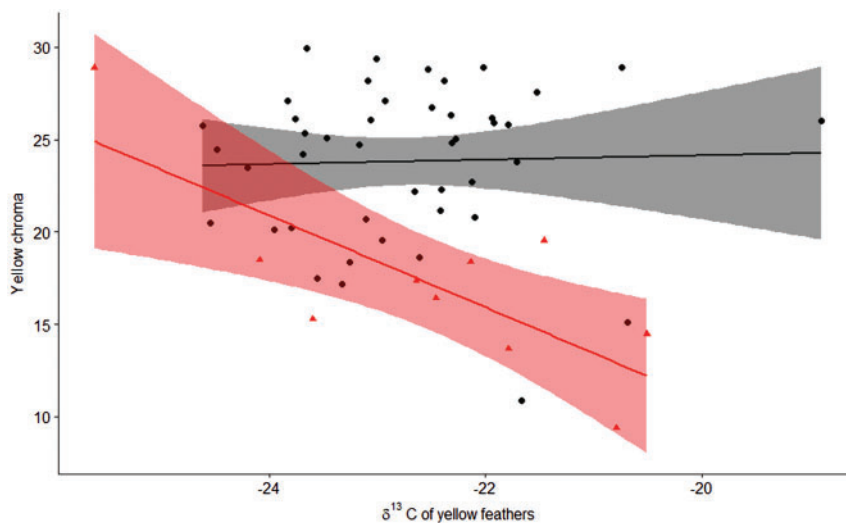
Nonetheless, age-related changes in hematocrit levels (Fair et al. 2007), oxidative stress (Bize et al. 2008), digestion efficiency (Madonia et al. 2017), or the level of parasite infection (Hõrak et al. 2001) might also have influenced age-related differences in carotenoid-based coloration (see also Simons et al. 2012). There was also high niche overlap between males and females, which indicates they used similar niches during ornament production. This suggests that factors other than niche use (for instance, genetic and life-history differences), may play a role in determining sexual differences in plumage coloration among individuals (Dale et al. 2015).

Ornaments as indicators of foraging ability of individuals

Carotenoid-based yellow coloration is thought to be an honest signal of foraging ability (Mateos-Gonzalez et al. 2011; García-Navas et al. 2012; Pagani-Núñez and Senar 2014). Our results showed that $\delta^{13}C$ variation effects on coloration were only relevant for juveniles. Chromaticity of carotenoid-based plumage patches increased when molted in oak dominated areas of the forest (Pagani-Núñez et al. 2014; 2016). Although previous research has shown multiple ways in which diet quality may affect plumage coloration and quality (see e.g. McGlothlin et al. 2007; Isaksson et al. 2008), we found no support for this idea. Indeed, the spatial niche (i.e., $\delta^{13}C$) was more important than trophic niche (i.e., $\delta^{15}N$) on plumage chromaticity of great tits. This pattern was more important for carotenoid-based plumage patches rather than melanin-based plumage patches and was only apparent in juveniles. Juvenile individuals molting in Oak-dominated areas characteristic of valley bottoms were more chromatic with regards to carotenoid-based yellow coloration. Therefore, our results support previous studies that have reported higher environmental sensitivity of carotenoid based-plumage compared with melanin-based plumage (Senar and Quesada 2006; Evans and Sheldon 2012; Roulin 2016). Other factors such as age and the spatial niche were more relevant than the individual's access to particular prey types or trophic niches. In this study, we were unable to establish a direct link between individual's trophic niche use and their plumage coloration during feather molt. Therefore, we found no support for the idea that chroma of carotenoid-based plumage coloration is indicative of the foraging ability. This was

Figure 5. Relationships between $\delta^{13}C$ of and chroma of carotenoid-based yellow feathers depending on age (adults: $r = 0.03$, $P = 0.84$; juveniles: $r = -0.77$, $P < 0.01$). Black color represents adults and red color represents juveniles. Regression lines and 95% confidence intervals are provided.

Table 4 Results from model selection procedures based on second-order AICc

Black tie width (mm)

Age	Sex	B δ^{13}C	B δ^{15}N	B δ^{13}C × Age	B δ^{15}N × Age	B δ^{15}N × Sex	df	logLik	AICc	ΔAICc	Weight	Intercept
	+						**3**	**−99.13**	**204.70**	**0.00**	**0.383**	**5.59**
	+	+					4	−99.02	206.90	2.13	0.132	7.53
+	+						4	−99.06	207.00	2.22	0.126	5.53
	+		+				4	−99.09	207.00	2.27	0.123	5.67
	+		+			+	5	−98.93	209.10	4.39	0.043	5.50
+	+	+					5	−99.01	209.30	4.55	0.039	7.17
	+	+	+				5	−99.01	209.30	4.55	0.039	7.39
+	+		+				5	−99.01	209.30	4.56	0.039	5.62
+	+		+			+	6	−98.84	211.50	6.76	0.013	5.44
	+	+	+			+	6	−98.86	211.50	6.80	0.013	7.11
+	+		+		+		6	−98.99	211.80	7.06	0.011	5.58
+	+	+	+				6	−98.99	211.80	7.06	0.011	6.76
+	+	+		+			6	−99.00	211.80	7.09	0.011	7.01
+	+		+		+	+	7	−98.75	214.00	9.25	0.004	5.34
+	+	+	+			+	7	−98.82	214.10	9.39	0.003	6.31
+	+	+	+		+		7	−98.97	214.40	9.68	0.003	6.59
+	+	+	+	+			7	−98.99	214.50	9.72	0.003	6.73

The dependent variable was black tie width (mm). The independent variables were sex (male or female), age (young or adult), and δ^{13}C and δ^{15}N of black plumage. We included the interactions δ^{13}C × age, δ^{15}N × age and δ^{15}N × sex, which were significant in previous models (Tables 1 and 2). We considered optimal those models with ΔAICc <2 but here are reported models with ΔAICc <10. Models were also weighted (column "Weight") according to their relative likelihood. A "+" indicates that the variable is included in the model.
Models with ΔAICc < 2 are marked with bold letters.

particularly evident for male adults, for which carotenoid- and melanin-based ornaments are thought to play a key role during mate selection (Hill and McGraw 2006). Nonetheless, our results emphasize the importance of considering multiple plumage components and their complex relationships when studying ornament production under natural conditions (Freeman-Gallant et al. 2010; Guindre-Parker et al. 2013; Chaine and Lyon 2015).

We conducted the first study to examine the possibility that different levels of coloration could be related to differences in molting times of differently colored plumage patches and found mixed support to this idea. Stable isotope analysis is clearly an important tool for understanding the role of diets and in determining sources of phenotypic variation. Examining access to resources and how this may affect the expression of other traits in the field is important because most research addressing the effect of dietary access to carotenoids on ornament production has been laboratory-based (Hill 1991; Saks et al. 2003; McGlothlin et al. 2007; Alonso-Alvarez and Galván 2011). Therefore, we encourage further field-based research to examine the multiple factors that affect the variation in niche use on different types of ornaments.

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