Short communication

Assessing the usefulness of ptilochronology in the study of melanin- and carotenoid-based ornaments in the Great Tit

Parus major

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Ptilochronology is a method for assessing the nutritional condition of birds based on the width of daily growth bars on feathers. Wide growth bars reflect fast feather growth and as feather growth is costly, the width of the bars reflects the condition of a bird during moult (Grubb 1989). It is a very simple and inexpensive method, which makes it ideal for field research (Grubb 2006). In addition, as a sampled feather is soon replaced by a new feather, a process that would take place during natural moult, this method is also harmless to the bird.

Ptilochronology has therefore become a popular method for assessing the nutritional state of birds in the wild (Grubb 2006). However, the efficacy of the method might differ, for example, between sexes (Grubb 1989, Takaki et al. 2001, Bostrom & Ritchison 2006) or age categories (Grubb et al. 1991). Kern and Cowie (2002) failed to find any relationship between the growth of different types of feathers taken from the same individual. Furthermore, other studies have concluded that the general validity of the method is unclear and that it can be used only under strictly controlled conditions (Murphy & King 1991).

Despite these reservations, ptilochronology has been used in several studies of feather ornaments as an indicator of condition (Hill & Montgomery 1994, Eeva et al. 1998, Keyser & Hill 1999, Doucet 2002, Senar et al. 2003, van Oort & Dawson 2005, Hegyi et al. 2007, Siefferman et al. 2008, Kimball 2009). The assumption is that these species moult body and contour feathers at the same time. Thus, if both ornaments and feather growth bars reflect condition (Griffith et al. 2006, Grubb 2006, Hill & McGraw 2006), then these two traits should covary. Carotenoid-based feather ornaments are expected to reflect a bird’s condition and there is evidence supporting this claim (von Schantz et al. 1999, Hill 2002, McGraw 2006a). Although melanin ornaments were thought not to reflect condition (McGraw 2006b), recent evidence suggests that they might be as condition-dependent as carotenoid-based ornaments (Griffith et al. 2006, Galván & Alonso-Alvarez 2008).

No clear-cut relationship between feather ornaments and feather growth has emerged from studies to date (see above). As ptilochronology is a very simple method and has great potential in field ornithology, we examined the relationships between both carotenoid- and melanin-based ornaments and feather growth in a large sample of individuals of a wild passerine. We chose the Great Tit Parus major because expression of its carotenoid-based (Hõrak et al. 2003, Tschierren et al. 2003, but see Fitze & Richner 2002) and melanin-based ornaments (Fitze & Richner 2002, Galván & Alonso-Alvarez 2008) is known to depend on condition during moult and feather growth. Thus, if feather growth also reflects condition during moult, we expected a positive correlation between the width of feather growth bars and the expression of both carotenoid- and melanin-based ornaments.

METHODS

This research was conducted at three adjacent nestbox plots (188 nestboxes in total) in a deciduous forest near the village of Grygov (49°31’N, 17°19’E) in eastern Czech Republic. Nestboxes were placed 1.5 m above the ground and, besides Great Tits, were also inhabited by Blue Tits Cyanistes caeruleus, Collared Flycatchers Ficedula albicollis and Nuthatches Sitta europaea. Fieldwork was carried out between 2005 and 2007 from early April until mid-June.

During feeding of nestlings (median age of young females = 7 days, males = 9 days), we captured parents in the nestbox. We captured females at almost all the nests (n = 165). However, because of time constraints, we captured males only from a subset of nests (n = 109). We measured their tarsus-length with digital callipers (to the nearest 0.01 mm) and weighed them on a spring Pesola balance to the nearest 0.125 g. From each bird we took 10–15 yellow feathers from the upper right part of the breast for spectrophotometric analysis. We photographed the bird’s breast with a digital camera (Panasonic DMC-FZ5). When taking a picture of the breast, we held the bird outstretched by its tarsi and beak and photographed it together with a ruler from a standard distance following the protocol of...
Figuerola and Senar (2000). All measurements and photographs were taken by V.R. We also plucked the second outer rectrix from the right side of the tail for later measurement of growth bars on feathers. We determined the age of the birds based on their plumage as 1 year old or older (Jenni & Winkler 1994). For each bird, we calculated its condition as the residual from the linear regression of body mass on tarsus-length (Brown 1996).

**Analyses of samples**

We quantified reflectance spectra of yellow feathers sampled from the breast using standard procedures (Andersson & Prager 2006). We used 10–15 feathers from each bird, which is sufficient to obtain reliable values from our study species (Quesada & Senar 2006). We used an Avantes AvaSpec-2048 fibre-optic spectrometer together with an AvaLight-XE xenon pulsed light source and WS-2 white reference tile. The probe was used both to provide light and to sample reflected light and was held perpendicular to the feather surface. We took five readings from different parts of each feather. Feathers were arranged on a black, non-reflective surface so that they overlapped extensively.

We obtained reflectance (%) from 320 to 700 nm in 1-nm increments. We calculated carotenoid chroma as \( R_{700} - R_{450} / R_{700} \), where \( R_{700} \) is the reflectance at 700 nm and \( R_{450} \) the reflectance at 450 nm. In statistical analyses we used the average carotenoid chroma calculated from the five readings from each set of feathers. To assess repeatability of our measurements, in a subsample of feathers we rearranged feathers and took another five readings, and again averaged the carotenoid chroma calculated from them. We calculated repeatability of these two average carotenoid chroma estimates as an intraclass correlation coefficient (Lessels & Boag 1987), which was sufficiently high \( r_i = 0.85, P < 0.001, n = 55 \). We use carotenoid chroma here because it reflects the amount of yellow carotenoids (lutein and zeaxanthin) in breast feathers in the Great Tit (Isaksson & Andersson 2008, Isaksson et al. 2008).

We analysed photographs of breast feathers in Adobe Photoshop CS3 Extended. We used the quick selection tool to roughly delimit the black stripe. Then we manually finished the selection so that it was as precise as possible and measured the surface area of the stripe. We used a standard in photographs of each bird to adjust the scale of each photograph and to obtain absolute surface area (cm²). We defined stripe surface as the area of the black feathers between the point of inflexion, where the ventral stripe widens to a throat patch, and the posterior end of the stripe (Figuerola & Senar 2000). All measurements were taken by B.M. To assess repeatability, a different observer measured a subsample of photographs; repeatability was high \( r_i = 0.87, P < 0.001, n = 75 \).

As it is not possible to use the standard technique (see Grubb 1989, 2006) to determine the width of feather growth bars in the Great Tit (Senar et al. 2003), we used the modification suggested by Carrascal et al. (1998). We measured the length of the feather and overall width of the first 10 measurable distal growth bars to the nearest 0.1 mm. Growth bars were not apparent in all the feathers and we excluded these feathers from the analyses. All measurements were taken twice. To obtain the width of one growth bar (mm) we divided the average of the two measurements by 10. Repeatability of the two measurements was high \( r_i = 0.99, P < 0.001, n = 210 \). All measurements were taken by B.M.

**Statistical analyses**

We analysed variation in growth bar width using general linear mixed models (GLMM). As we sampled some individuals repeatedly across years, we included individual identity as a repeated factor in the mixed procedure of SAS. First, we fitted a model with the following factors and covariates: year, sex, age, carotenoid chroma, black stripe, length of tail feather and condition. We subsequently removed non-significant factors (age, black stripe, condition) until we had only statistically significant variables at the level of \( \alpha = 0.05 \) in the model. \( F \) and \( P \) values for non-significant factors given in the Results section are those immediately before the factor was removed from the model. Growth bar width was transformed to the power of four to normalize its originally left-skewed distribution and all the analyses were conducted using this transformation. Residuals from each linear model were checked to conform to the requirements of normal distribution, equal variance and linearity (Grafen & Hails 2002).

**RESULTS**

We obtained tail feathers from 238 birds over 3 years (146 females, 92 males). Average length of tail feathers was 65.9 mm (mean ± 3.15 sd, \( n = 238 \)) and was larger in males than in females \( (F_{1,236} = 22.27, P < 0.001) \). Individual identity as a random repeated factor was significant \( (\text{estimate} = 5.089 ± 0.4685 \text{ se}, \ z = 10.86, P < 0.001) \).

The width of feather growth bars was 2.87 mm (mean ± 0.23 sd, \( n = 210 \)), being larger in females than in males \( (F_{1,202} = 13.14, P < 0.001) \) and differed with year \( (F_{2,202} = 4.82, P = 0.009) \). The growth bar width also correlated negatively with the carotenoid chroma of yellow breast feathers \( (F_{1,202} = 5.82, P = 0.017; \text{Fig. 1a}) \) and positively with the total length of the feather \( (F_{1,202} = 57.91, P < 0.001; \text{whole model } R^2 = 0.56) \). There was no significant relationship of growth bar width to the size of the black breast stripe \( (F_{1,199} < 0.01, P = 0.971; \text{Fig. 1b}) \), to condition...
Individual identity as a random repeated factor was significant in the analysis of feather growth in both the full model (estimate = 332.8 ± 33.88 se, \( z = 9.82 \), \( P < 0.001 \)) and the final model after non-significant fixed-factors were removed (estimate = 322.6 ± 32.10 se, \( z = 10.05 \), \( P < 0.001 \)).

**DISCUSSION**

We found no relationship of growth bar width to the size of the melanin ornament but, unexpectedly, a negative relationship to the chroma of the carotenoid ornament. Thus, the growth rate of tail feathers declined as carotenoid levels in breast feathers increased.

The available evidence suggests that carotenoid-rich feather ornaments are a reflection of good body condition during feather growth (von Schantz et al. 1999, Hill 2002, McGraw 2006a). This is also true for the Great Tit (Hörak et al. 2000, Tschierren et al. 2003, but see Fitz & Richner 2002). Given comparatively well-established condition-dependence of carotenoid-based feather ornaments, we expected positive relationships between their expression and the growth rate of tail feathers. However, contrary to our expectations, there was a significant negative relationship between the carotenoid chroma of yellow breast feathers and growth rate of tail feathers. This runs contrary to previous studies, where the correlation between the intensity of carotenoid-based ornaments and feather growth rate was either positive (Hill & Montgomerie 1994, Senar et al. 2003) or absent (Eeva et al. 1998, van Oort & Dawson 2005, Hegyi et al. 2007). However, the significance of our results should not be overstated, because the relationship between carotenoid content and feather growth was not very strong (\( r = -0.16 \); see also Fig. 1).

Melanin-based ornaments were thought not to be condition-dependent (McGraw 2006b). However, recent evidence suggests that they might be as condition-dependent as carotenoid-based ornaments (Griffith et al. 2006). Potential proximate mechanisms of condition-dependence might include corticosterone-mediated stress (Roulin et al. 2008), oxidative stress (Galván & Alonso-Alvarez 2008), or the allocation of calcium among competing physiological functions (Roulin et al. 2006). However, we found no relationship of growth bar width to the size of the melanin-based black breast stripe, commensurate with the findings of previous studies (Senar et al. 2003, Hegyi et al. 2007, Kimball 2009). Thus, our study adds to a growing body of evidence that feather growth does not correlate with the expression of melanin ornaments, at least in small songbirds.

The usefulness of ptilochronology has been challenged (Murphy & King 1991, Takaki et al. 2001, Kern & Cowie 2002, van Oort & Otter 2005, Bostrom & Ritchison 2006). Here, we did not test methods of conducting ptilochronology but used standard methods to compare the relationship between feather ornaments and feather growth from a large sample of birds. Results of studies conducted to date are highly inconsistent, even when conducted on the same species. For instance, in the Great Tit, feather growth has been shown to correlate positively with hue of yellow breast feathers (Senar et al. 2003), negatively with chroma (this study), or not at all with either brightness (Eeva et al. 1998, Senar et al. 2003, Hegyi et al. 2007) or chroma (Senar et al. 2003, Hegyi et al. 2007). Similar inconsistencies can be found in studies of other bird species (Hill & Montgomerie 1994, van Oort & Dawson 2005). At least two possible conclusions can be drawn from these studies. First, ptilochronology may be an unreliable approach for assessing condition in wild-ranging birds, at least until rigorous methodological studies demonstrate otherwise. Secondly, ptilochronology may be reliable in certain species or for application to certain types of ornaments, but to reveal interspecific patterns would require many more studies to be conducted on a broader spectrum of

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Relationship between the width of growth bars of tail feathers (mm) and (a) carotenoid chroma of yellow breast feathers (for definition see Methods; \( n = 210 \)), and (b) black breast stripe area (cm\(^2\); \( n = 210 \)). For the sake of convenience, untransformed data not adjusted for other covariates are presented. However, note that all analyses were conducted on transformed data.
species. Moreover, differences in results within a species are known to occur due to population differences in the information content of the ornamental traits (Dunn et al. 2008, Galván & Moreno 2009) and different expression of ornaments in different populations and subspecies (Hill 2002). Again, studies conducted on populations differing in resource limitation (e.g. carotenoids, see Hill 2002) or expression and information content of feather ornaments could reveal interesting patterns. The usefulness of ptilochronology as a simple field method to estimate a bird’s condition during moult could still emerge from future studies, especially if these are done in an explicitly inter- or intraspecific comparative framework.

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REFERENCES


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