



Short communication

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

BEATA MATYSIOKOVÁ* & VLADIMÍR REMEŠ
*Department of Zoology and Laboratory of
Ornithology, Palacký University, Tř. Svobody 26,
77146 Olomouc, Czech Republic*

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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 & Montgomerie 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 (Hörak *et al.* 2000, 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

*Corresponding author.
Email: betynec@centrum.cz

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 $(R700-R450)/R700$, where R700 is the reflectance at 700 nm and R450 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 ($r_1 = 0.85$, $P < 0.001$, $n = 55$). 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 ($r_1 = 0.87$, $P < 0.001$, $n = 75$).

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 ($r_1 = 0.99$, $P < 0.001$, $n = 210$). 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 \pm 3.15 sd, $n = 238$) and was larger in males than in females ($F_{1,236} = 226.27$, $P < 0.001$). Individual identity as a random repeated factor was significant (estimate = 5.089 ± 0.4685 se, $z = 10.86$, $P < 0.001$).

The width of feather growth bars was 2.87 mm (mean \pm 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 ($F_{1,202} = 5.82$, $P = 0.017$; Fig. 1a) and positively with the total length of the feather ($F_{1,202} = 57.91$, $P < 0.001$; whole model $R^2 = 0.56$). There was no significant relationship of growth bar width to the size of the black breast stripe ($F_{1,199} < 0.01$, $P = 0.971$; Fig. 1b), to condition

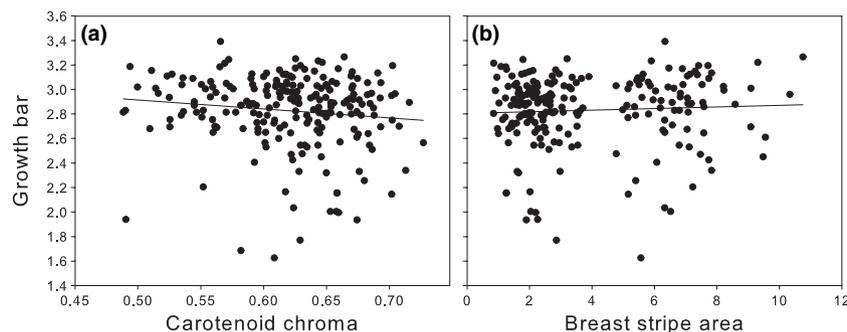


Figure 1. Relationship between the width of growth bars of tail feathers (mm) and (a) carotenoid chroma of yellow breast feathers (for definition see Methods; $n = 210$), and (b) black breast stripe area (cm^2 , $n = 210$). For the sake of convenience, untransformed data not adjusted for other covariates are presented. However, note that all analyses were conducted on transformed data.

($F_{1,200} = 0.83$, $P = 0.362$) or to age ($F_{1,201} = 1.06$, $P = 0.305$).

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 Fitze & 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

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

- Andersson, S. & Prager, M. 2006. Quantifying colors. In Hill, G.E. & McGraw, K.J. (eds) *Bird Coloration, Volume 1. Mechanisms and Measurements*: 41–89. Cambridge, MA: Harvard University Press.
- Bostrom, M.R. & Ritchison, G. 2006. Possible relationships between morphology, territory quality, and skin color of American Kestrels. *J. Field Ornithol.* **77**: 392–398.
- Brown, M.E. 1996. Assessing body condition in birds. *Curr. Ornithol.* **13**: 67–135.
- Carrascal, L.M., Senar, J.C., Mozetich, I., Uribe, F. & Domenech, J. 1998. Interactions about environmental stress, body condition, nutritional status, and dominance in Great Tits. *Auk* **115**: 727–738.
- Doucet, S.M. 2002. Structural plumage coloration, male body size, and condition in the Blue-black Grassquit. *Condor* **104**: 30–38.
- Dunn, P.O., Whittingham, L.A., Freeman-Gallant, C.R. & DeCoste, J. 2008. Geographic variation in the function of ornaments in the Common Yellowthroat *Geothlypis trichas*. *J. Avian Biol.* **39**: 66–72.
- Eeva, T., Lehikoinen, E. & Rönkä, M. 1998. Air pollution fades the plumage of the Great Tit. *Funct. Ecol.* **12**: 607–612.
- Figuerola, J. & Senar, J.C. 2000. Measurement of plumage badges: an evaluation of methods used in the Great Tit *Parus major*. *Ibis* **142**: 482–484.
- Fitze, P.S. & Richner, H. 2002. Differential effects of a parasite on ornamental structures based on melanins and carotenoids. *Behav. Ecol.* **13**: 401–407.
- Galván, I. & Alonso-Alvarez, C. 2008. An intracellular antioxidant determines the expression of a melanin-based signal in a bird. *PLoS ONE* **3**: e3335.
- Galván, I. & Moreno, J. 2009. Variation in effects of male plumage ornaments: the case of Iberian Pied Flycatchers *Ficedula hypoleuca*. *Ibis* **151**: 541–546.
- Grafen, A. & Hails, R. 2002. *Modern Statistics for the Life Sciences*. Oxford: Oxford University Press.
- Griffith, S.C., Parker, T.H. & Olson, V.A. 2006. Melanin versus carotenoid-based sexual signals: is the difference really so black and red? *Anim. Behav.* **71**: 749–763.
- Grubb, T.C. 1989. Ptilochronology: feather growth bars as indicators of nutritional status. *Auk* **106**: 314–320.
- Grubb, T.C. Jr 2006. *Ptilochronology. Feather Time and Biology of Birds*. Oxford: Oxford University Press.
- Grubb, T.C., Waite, T.A. & Wiseman, A.J. 1991. Ptilochronology: induced feather growth in Northern Cardinals varies with age, sex, ambient temperature, and day length. *Wilson Bull.* **103**: 435–445.
- Hegyí, G., Szigeti, B., Török, J. & Eens, M. 2007. Melanin, carotenoid and structural plumage ornaments: information content and role in Great Tits *Parus major*. *J. Avian Biol.* **38**: 698–708.
- Hill, G.E. 2002. *Red Bird in a Brown Bag*. Oxford: Oxford University Press.
- Hill, G.E. & McGraw, K.J. (eds) 2006. *Bird Coloration, Volume 1. Mechanisms and Measurements*. Cambridge, MA: Harvard University Press.
- Hill, G.E. & Montgomerie, R. 1994. Plumage colour signals nutritional condition in the House Finch. *Proc. R. Soc. Lond. B* **258**: 47–52.
- Hórák, P., Vellau, H., Ots, I. & Møller, A.P. 2000. Growth conditions affect carotenoid-based plumage coloration of Great Tit nestlings. *Naturwissenschaften* **87**: 460–464.
- Isaksson, C. & Andersson, S. 2008. Oxidative stress does not influence carotenoid mobilization and plumage pigmentation. *Proc. R. Soc. Lond. B* **275**: 309–314.
- Isaksson, C., Ornborg, J., Prager, M. & Andersson, S. 2008. Sex and age differences in reflectance and biochemistry of carotenoid-based colour variation in the Great Tit *Parus major*. *Biol. J. Linn. Soc.* **95**: 758–765.
- Jenni, L. & Winkler, R. 1994. *Moult and Ageing of European Passerines*. London: Academic Press.
- Kern, M.D. & Cowie, R.J. 2002. Ptilochronology proves unreliable in studies of nestling Pied Flycatcher. *Ibis* **144**: 23–29.
- Keyser, A.J. & Hill, G.H. 1999. Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. *Proc. R. Soc. Lond. B* **266**: 771–777.
- Kimball, S.A. 2009. *Mating system dynamics in passerine birds*. PhD. Thesis, Ohio State University.
- Lessels, C.M. & Boag, P.T. 1987. Unrepeatable repeat abilities: a common mistake. *Auk* **104**: 116–121.
- McGraw, K.J. 2006a. Mechanics of carotenoid-based coloration. In Hill, G.E. & McGraw, K.J. (eds) *Bird Coloration, Volume 1. Mechanisms and Measurements*: 177–242. Cambridge, MA: Harvard University Press.
- McGraw, K.J. 2006b. Mechanics of melanin-based coloration. In Hill, G.E. & McGraw, K.J. (eds) *Bird Coloration, Volume 1. Mechanisms and Measurements*: 243–294. Cambridge, MA: Harvard University Press.
- Murphy, M.E. & King, J.R. 1991. Ptilochronology: a critical evaluation of assumptions and utility. *Auk* **108**: 695–704.
- van Oort, H. & Dawson, R.D. 2005. Carotenoid ornamentation of adult male Common Redpolls predicts probability of dying in a salmonellosis outbreak. *Funct. Ecol.* **19**: 822–827.
- van Oort, H. & Otter, K.A. 2005. Natal nutrition and the habitat distributions of male and female Black-capped Chickadees. *Can. J. Zool.* **83**: 1495–1501.

- Quesada, J. & Senar, J.C.** 2006. Comparing plumage colour measurements obtained directly from live birds and from collected feathers: the case of the Great Tit *Parus major*. *J. Avian Biol.* **37**: 609–616.
- Roulin, A., Dauwe, T., Blust, R., Eens, M. & Beaud, M.** 2006. A link between eumelanism and calcium physiology in the Barn Owl. *Naturwissenschaften* **93**: 426–430.
- Roulin, A., Almasi, B., Rossi-Pedruzzi, A., Ducrest, A.L., Wakamatsu, K., Miksik, I., Blount, J.D., Jenni-Eiermann, S. & Jenni, L.** 2008. Corticosterone mediates the condition-dependent component of melanin-based coloration. *Anim. Behav.* **75**: 1351–1358.
- von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H.** 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond. B* **266**: 1–12.
- Senar, J.C., Figuerola, J. & Domènech, J.** 2003. Plumage coloration and nutritional condition in the Great Tit *Parus major*: the roles of carotenoids and melanins differ. *Naturwissenschaften* **90**: 234–237.
- Siefferman, L., Shawkey, M.D., Bowman, R. & Woolfenden, G.E.** 2008. Juvenile coloration of Florida Scrub-Jays (*Aphelocoma coerulescens*) is sexually dichromatic and correlated with condition. *J. Ornithol.* **149**: 357–363.
- Takaki, Y., Eguchi, K. & Nagata, H.** 2001. The growth bars on tail feathers in the male Styan's Grasshopper Warbler may indicate quality. *J. Avian Biol.* **32**: 319–325.
- Tschieren, B., Fitze, H.S. & Richner, H.** 2003. Proximate mechanisms of variation in the carotenoid-based plumage coloration of nestling Great Tits (*Parus major* L.). *J. Evol. Biol.* **16**: 91–100.

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