

No evidence for survival selection on carotenoid-based nestling coloration in great tits (*Parus major*)

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Abstract

In several vertebrate species evidence supports the hypothesis that carotenoid-based coloration of adults has evolved due to sexual selection. However, in some birds already the nestlings display carotenoid-based coloration. Because the nestling's body plumage is typically moulted before the first reproductive event, sexual selection cannot explain the evolution of these carotenoid-based traits. This suggests that natural selection might be the reason for its evolution. Here we test whether the carotenoid-based nestling coloration of great tits (*Parus major*) predicts survival after fledging. Contrary to our expectation, the carotenoid-based plumage coloration was not related to short- nor to long-term survival in the studied population. Additionally, no pre-fledging selection was detectable in an earlier study. This indicates that the carotenoid-based coloration of nestling great tits is currently not under natural selection and it suggests that past selection pressures or selection acting on correlated traits may have led to its evolution.

Introduction

In many vertebrate species carotenoid-based coloration is considered during female choice and is thus an important trait in sexual selection (e.g. Milinski & Bakker, 1990; Hill, 1991; Faivre *et al.*, 2001). By preferring intensely coloured males (Milinski & Bakker, 1990; Hill, 1991) females select less parasitized (Milinski & Bakker, 1990; Hill & Brawner, 1998; Brawner *et al.*, 2000; Hōrak *et al.*, 2001; Hill & Farmer, 2005), more immunocompetent (Bendich & Shapiro, 1986; Bendich, 1991; Blount *et al.*, 2003; Saks *et al.*, 2003) and better caring fathers (Hill, 1991; Senar *et al.*, 2002). Brighter coloured males are therefore able to reproduce at a higher rate and thus females impose directional selection on the male's plumage coloration (Badyaev *et al.*, 2000).

However, in some bird species already the nestlings show an intense carotenoid-based plumage coloration (Brush, 1978). Because juvenile passerines moult their plumage before their first reproduction (Jenni & Winkler, 1994), sexual selection hardly explains the evolution of

the nestling's carotenoid-based colour traits. This suggests that intensely yellow-coloured nestling plumages may have evolved due to natural selection. Support for this hypothesis is provided by different studies, which indicate that natural selection acts on nestling coloration (e.g. Lyon *et al.*, 1994; Götmark & Olsson, 1997; Hill, 2002). As predators are important determinants of fledgling survival (Naef-Daenzer *et al.*, 2001), plumage colours which provide camouflage will be selected for (e.g. Butcher & Rohwer, 1989; Götmark & Olsson, 1997). Parents may as well select for brightly coloured nestling traits by allocating food to nestlings in relation to their coloration (Lyon *et al.*, 1994; Hill, 2002). Beside these inter- and intraspecific factors, abiotic factors may favour a specific plumage coloration. For example, the colour determines how much light will be reflected and thus advantageous thermoregulatory properties of a given colour may be selected for (e.g. Ellers & Boggs, 2003). The bird's plumage as well protects against mechanical impact, solar radiation and water (see Jenni & Winkler, 1994, for a review). Because the effectiveness of the protective properties depends on the incorporated pigments and on the feather structure, which as well determines the plumage coloration (Bleiweiss, 2004) selection acting on the protective function of the plumage may select for a specific coloration. Therefore

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parents, predators and abiotic factors may impose directional, stabilizing or disruptive selection (Kingsolver & Smith, 1995), and thus lead to the evolution of carotenoid-based nestling plumage coloration.

We recently showed that in great tits the nestling's plumage coloration does neither influence the parental feeding behaviour (Tschirren *et al.*, 2005) nor the nestling's survival before fledging (Tschirren *et al.*, 2003). Thus, selective pressures directly acting on the carotenoid-based coloration during the nestling period may not be present or they may be very weak and thus remained undetected. This suggests that the nestling's plumage coloration may have evolved because of selective advantages after fledging, e.g. camouflage, parental feeding preferences, thermoregulation or the protective functions of the plumage. In this study, we therefore test whether survival selection is acting after fledging on the carotenoid-based plumage coloration of nestling great tits. To test this hypothesis we link nestling survival with plumage coloration (Gregoire *et al.*, 2004). By applying models including short- (first-year) and long-term survival we further examine when selection is acting.

Methods

Species description

The great tit (*Parus major*) is a small hole nesting passerine, which inhabits deciduous forest, hedges, parks and urban areas (Gosler, 1993). Nestling great tits display a bright yellow carotenoid-based plumage coloration, which arises due to the incorporation of the carotenoids lutein and zeaxanthin into the feathers (Partali *et al.*, 1987). Because carotenoids cannot be synthesized by animals the amount of incorporated carotenoids depends on the amounts of the ingested carotenoids as well as on the nestling's condition (Tschirren *et al.*, 2003). Thus the plumage coloration is a reliable signal of early nutritional conditions (Fitze *et al.*, 2003b; Tschirren *et al.*, 2003). The carotenoids incorporated into the feathers have antioxidant functions (e.g. Surai, 2002) and a recent study suggests that lutein and zeaxanthin might be immuno-stimulating (Blount *et al.*, 2003), suggesting that individuals incorporating more carotenoids into their plumage are healthier or of better quality (von Schantz *et al.*, 1999; H orak *et al.*, 2001). The nestling's body plumage is moulted in autumn and it is not correlated with the first-year plumage coloration (Fitze *et al.*, 2003a; Tschirren *et al.*, 2003).

General procedures

The study was performed in a great tit (*P. major*) population in the 'Forst', a forest near Bern, Switzerland (46°54'N, 7°17'E/46°57'N, 7°21'E). The hatching date of the broods was determined by daily visits (hatching day = day 1). Nestlings were individually marked by clipping dorsal tufts on day 2, and were ringed with

individual aluminium rings on day 9. The fledging date was determined by daily visits starting 18 days post-hatching and nests were inspected for dead nestlings on the fledging day. Fifteen days post-hatching the plumage coloration of the nestling's breast was measured under standardized conditions using a digital camera as described in Fitze & Richner (2002). Digital photos were analysed using ADOBE PHOTOSHOP® program (Adobe Systems Incorporated, San Jose, CA, USA) and hue (H), saturation (S) and brightness (B) of the coloration were calculated (for methodological details see Fitze & Richner, 2002). Carotenoid pigments reflect light only in the human visible range of the spectrum (e.g. Hill, 1998, 2002; O'Neil *et al.*, 2001). Therefore quantifying plumage coloration using a digital camera reliably measures differences in the carotenoid content of the feathers (Tschirren *et al.*, 2003).

Selection on nestling plumage coloration

In 1998 the breast plumage coloration of 191 nestlings originating from 30 nests and in 1999 the colour of 164 nestlings originating from 50 nests were measured. The natural range of the plumage coloration was 40.9–47.1° (43.4° ± 0.06 SE) for hue, 37.9–61.3% (50.5% ± 0.002 SE) for saturation, and 64.6–92.3% (80.7% ± 0.002 SE) for brightness. In the years 1999, 2000 and 2001 all great tits breeding in nest boxes within our study area (consisting of approximately 800 nest boxes within 16 km²) were captured to assess the survival of the birds. The survival estimates (Φ) are based on birds recaptured during the breeding period and within the study area. Survival estimates therefore correspond to the probability of recruiting locally. Adult great tits were captured at the nest when their offspring were 14 days old. For the estimation of the probability of recruiting locally and for the estimation of the recapture probability we used the program MARK (White & Burnham, 1999; White, 2000) using Cormack–Jolly–Seber models (e.g. Jolly, 1965; Lebreton *et al.*, 1992). Before analysis we confirmed, by a goodness of fit test, that the starting model adequately fitted the data using a bootstrap approach (White, 2000). As our starting model was over-dispersed (1000 simulations, $P < 0.012$) we adjusted the variance inflation factor ($c\text{-hat}$) with the quotient of the observed inflation factor and the mean simulated inflation factor ($c\text{-hat} = 1.206 \pm 0.069$) from the bootstraps (Lebreton *et al.*, 1992; Burnham & Anderson, 1998; White, 2000). Survival analyses were started with the full model including year and age. Age was modelled as first year or older than 1 year (Clobert *et al.*, 1988). We first modelled the recapture probability (Table 1A) and subsequently the probability of recruiting locally (Table 1B). Model selection was based on the Akaike Information Criterion (AIC) and differences between two models of ≥ 2 AIC were considered as being significantly different (Burnham & Anderson, 1998). The

Table 1. Models of selection on nestling plumage coloration. The effects of age (a) and year (y) are shown for both (A) the recapture probability (p), and (B) the probability of recruiting locally (ϕ). The best model selected in sections (A) and (B) corresponds to the basic model against which the models incorporating the covariates were tested. The basic model is marked in bold. Models are numbered according to decreasing complexity but ordered according to AIC. (C) Long-term survival models incorporating the first (PC1), second (PC2), and third (PC3) principal component of plumage coloration. (D) Models incorporating plumage coloration as an age-dependent covariate acting on first-year survival only (linear and/or quadratic terms were included). (E) Long-term survival models incorporating the covariates body condition, clutch size, and hatching date. (F) Models incorporating body condition, clutch size, and hatching date as age-dependent covariates acting on first-year survival only. (G) Models incorporating both the plumage coloration and the covariates body condition, clutch size and hatching date. Only models with lower AIC than the basic model are given (sections C–G).

Model	QAICc	Δ QAICc	Parameters	Q deviance	QAICc weight (%)	Model explanation	Selection gradient \pm SE (on logit scale)	Models compared, likelihood ratio test test
(A) Recapture probability								
5. $\phi_{a,y}p$	246.1	4.6	6	233.9	1.5	Intercept		4–5, $P = 0.34$
4. $\phi_{a,y}p_a$	247.3	5.8	7	233.0	0.8	Age dependent		3–4, $P = 0.66$
3. $\phi_{a,y}p_y$	250.2	8.7	8	233.8	0.2	Year dependent		2–3, $P = 0.24$
2. $\phi_{a,y}p_{a+y}$	250.9	9.4	9	232.4	0.1	Age + year dependent		1–2, $P = 1$
1. $\phi_{a,y}p_{a,y}$	253.0	11.5	10	232.4	0.1	Age \times year dependent		
(B) Survival estimates								
7. $\phi_{a+y}p$	244.1	2.6	5	233.9	4.2	Age + year dependent		6–7, $P = 1$
6. $\phi_{a,y}p$	246.1	4.6	6	233.9	1.4	Age \times year dependent		
8. $\phi_{a,y}p$	251.0	9.5	4	251.0	0.1	Year dependent		7–8, $P < 0.001$
9. $\phi_{a,y}p$	251.4	9.9	3	245.4	0.1	Age dependent		7–9, $P < 0.01$
(C) Long-term survival models incorporating PC1, PC2, and PC3 of the plumage coloration with lower AIC values than the basic model								
10. $\phi_{a+y}p_{PC1+PC2}$	244.2	2.7	7	229.9	3.8	Stabilizing or disruptive selection on PC1	PC1: 0.228 ± 0.217 ; PC1 ² : -0.365 ± 0.277	7–10, $P = 0.14$
11. $\phi_{a+y}p_{PC1}$	244.4	2.9	6	232.3	3.6	Directional selection on PC1	0.246 ± 0.188	7–11, $P = 0.21$
12. $\phi_{a+y}p_{PC2+PC2^2}$	247.9	6.4	7	233.6	0.6	Stabilizing or disruptive selection on PC2	PC2: 0.126 ± 0.257 ; PC2 ² : 0.028 ± 0.224	7–12, $P = 0.86$
13. $\phi_{a+y}p_{PC2}$	245.8	4.3	6	233.6	1.7	Directional selection on PC2	0.136 ± 0.249	7–13, $P = 0.58$
14. $\phi_{a+y}p_{PC3+PC3^2}$	245.8	4.3	7	231.5	1.8	Stabilizing or disruptive selection on PC3	PC3: 0.264 ± 0.249 ; PC3 ² : -0.331 ± 0.263	7–14, $P = 0.30$
15. $\phi_{a+y}p_{PC3}$	245.7	4.2	6	233.5	1.9	Directional selection on PC3	0.127 ± 0.189	7–15, $P = 0.53$
(D) Models incorporating plumage coloration as age-dependent effect acting on first year survival only, which have lower AIC values than the basic model								
16. $\phi_{a+y}p_{a1}PC1+at1.PC1^2$	245.9	4.4	7	231.7	1.6	Stabilizing or disruptive selection on PC1	PC1: 0.176 ± 0.252 ; PC1 ² : -0.299 ± 0.291	7–16, $P = 0.33$
17. $\phi_{a+y}p_{a1}PC1$	245.1	3.6	6	232.9	2.4	Directional selection on PC1	0.219 ± 0.222	7–17, $P = 0.32$
18. $\phi_{a+y}p_{a1}PC2+at1.PC2^2$	248.0	6.5	7	233.7	0.6	Stabilizing or disruptive selection on PC2	PC2: -0.073 ± 0.297 ; PC2 ² : 0.088 ± 0.237	7–18, $P = 0.91$
19. $\phi_{a+y}p_{a1}PC2$	246.0	4.5	6	233.8	1.6	Directional selection on PC2	-0.059 ± 0.302	7–19, $P = 0.75$
20. $\phi_{a+y}p_{a1}PC3+at1.PC3^2$	246.9	5.4	7	232.6	1.0	Stabilizing or disruptive selection on PC3	PC3: 0.216 ± 0.274 ; PC3 ² : -0.267 ± 0.283	7–20, $P = 0.52$
21. $\phi_{a+y}p_{a1}PC3$	245.8	4.3	6	233.6	2.1	Directional selection on PC3	0.126 ± 0.225	7–21, $P = 0.40$
(E) Long-term survival models incorporating the covariates body condition (BC), clutch size (CS), and hatching date (HD), with lower AIC values than the basic model								
22. $\phi_{a+y}p_{BC+BC^2}$	248.2	6.7	7	233.9	0.5	Stabilizing or disruptive selection on BC	BC: 0.034 ± 0.201 ; BC ² : 0.036 ± 0.203	7–22, $P = 1$
23. $\phi_{a+y}p_{BC}$	246.1	4.6	6	233.9	1.5	Directional selection on BC	0.022 ± 0.192	7–23, $P = 1$
24. $\phi_{a+y}p_{CS+CS^2}$	246.1	4.6	7	231.8	1.6	Stabilizing or disruptive selection on CS	CS: 2.760 ± 2.266 ; CS ² : -2.852 ± 2.240	7–24, $P = 0.35$
25. $\phi_{a+y}p_{CS}$	245.8	4.3	6	233.6	1.7	Directional selection on CS	-0.109 ± 0.194	7–25, $P = 0.58$
26. $\phi_{a+y}p_{HD+HD^2}$	241.6	0.1	7	227.3	14.1	Stabilizing or disruptive selection on HD	HD: 5.724 ± 4.084 ; HD ² : -6.382 ± 4.295	7–26, $P = 0.04$
27. $\phi_{a+y}p_{HD}$	242.0	0.5	6	229.8	11.6	Directional selection on HD	-0.360 ± 0.179	7–27, $P = 0.04$

Table 1 Continued.

Model	QAICc	Δ QAICc	Parameters	Q deviance	QAICc weight (%)	Model explanation	Selection gradient \pm SE (on logit scale)	Models compared, likelihood ratio test test
(F) Models incorporating the covariates body condition (BC), clutch size (CS), and hatching date (HD) as age-dependent effect acting on first year survival								
28. $\phi_{a+y+at,BC+at,1,BC^2}$	247.1	5.6	7	232.8	0.9	Stabilizing or disruptive selection on BC	BC: 0.227 ± 0.251 ; BC ² : -0.092 ± 0.290	7-28, $P = 0.58$
29. $\phi_{a+y+at,BC}$	245.2	3.7	6	232.9	2.4	Directional selection on BC	0.234 ± 0.241	7-29, $P = 0.32$
30. $\phi_{a+y+at,CS+at,1,CS^2}$	245.6	4.1	7	231.3	2.0	Stabilizing or disruptive selection on CS	CS: 3.584 ± 2.682 ; CS ² : -3.722 ± 2.662	7-30, $P = 0.27$
31. ϕ_{a+y+CS}	245.8	4.3	6	233.5	1.8	Directional selection on CS	-0.143 ± 0.232	7-31, $P = 0.53$
32. $\phi_{a+y+at,HD+at,1,HD^2}$	243.7	2.2	7	229.4	5.1	Stabilizing or disruptive selection on HD	HD: 4.808 ± 4.737 ; HD ² : -5.437 ± 4.964	7-32, $P = 0.11$
33. $\phi_{a+y+at,HD}$	242.9	1.4	6	230.7	7.4	Directional selection on HD	-0.392 ± 0.221	7-33, $P = 0.07$
(G) Models incorporating the covariates body condition (BC), clutch size (CS), and hatching date (HD) and the plumage coloration, with lower AIC values than the basic model								
34. $\phi_{a+y+HD+HD^2+PC1^2}$	241.5	0	8	225.1	15.3	Stabilizing or disruptive selection on HD and stabilizing or disruptive selection on PC1	HD: 6.120 ± 4.138 ; HD ² : -6.841 ± 4.354 PC1 ² : -0.362 ± 0.272	7-34, $P = 0.03$ 26-34, $P = 0.14$

Cormack–Jolly–Seber models accounted for overdispersion. Δ QAICc corresponds to the difference in QAICc between the model with the lowest value of QAICc ($\phi_{a+y+HD+HD^2+PC1^2}$) and the model in consideration. The relative likelihood of each model is indicated by QAICc weight. A difference of Δ QAICc ≥ 2 indicates that two models were significantly different (Burnham & Anderson, 1998).

model with the lowest AIC was selected as the best model using AIC-weight and is referred to as the basic model (Table 1B: $\phi_{a+y,p}$). Due to the adjustment of the variance inflation factor the AIC criterion is referred to as QAIC. Likelihood ratio tests were used to confirm the model selection. After selecting the basic model we introduced the parameters describing the nestling’s plumage as covariates (Table 1C) to investigate whether plumage coloration predicts survival. Because H, S and B were inter-correlated (H–S: $F_{1,353} = 10.598$, $P < 0.01$, $R^2 = 0.029$, $\beta = 0.171$; H–B: $F_{1,353} = 60.747$, $P < 0.001$, $R^2 = 0.147$, $\beta = -0.383$; S–B: $F_{1,353} = 19.878$, $P < 0.001$, $R^2 = 0.053$, $\beta = -0.231$) we first conducted a principal component analysis including the three colour variables. The first principal component (PC1) explained 51.1% of the variance and it mainly reflects H and B (factor loadings: H: -0.639 , S: 0.47 , B: 0.608). The second principal component (PC2) explained 28.5% of the variance and reflects S (factor loadings: H: 0.221 , S: 0.870 , B: -0.441) and the third principal component (PC3) again reflects H and B (factor loadings: H: 0.737 , S: 0.147 , B: 0.660). For all three covariates we introduced a linear term to test for directional selection and a quadratic term to test for stabilizing or disruptive selection (Lande & Arnold, 1983; Kingsolver & Smith, 1995) and we run two types of models: models where the covariates affect survival during the entire life (long-term survival, Table 1C,E) and models where the covariates affect survival only during the first year of life (first-year survival, Table 1D,F). We distinguish between these two types of models because juveniles moult their nestling body plumage in autumn after fledging and because the nestling’s plumage coloration is not correlated with the first-year plumage coloration (Fitze *et al.*, 2003a). Thus nestling plumage coloration, if being directly selected for, should predict first-year survival but not necessarily long-term survival.

From previous studies it is known that the nestling’s plumage coloration is correlated with parameters that may influence survival: the nestling’s body condition, brood size (Tschirren *et al.*, 2003), and hatching date (Svensson, 1997; Fitze *et al.*, 2004). We therefore included brood size (the number of hatched offspring), hatching date, and nestling body condition as covariates in the analyses to control for confounding effects. Body condition was defined as the residuals of a linear regression of body weight on tarsus length. Like for the colour variables we modelled brood size, nestling body condition, and hatching date as linear and/or quadratic terms that affected first-year or long-term survival (Table 1E,F). We then selected the best fitting model (lowest QAIC) of the models presented in Table 1E,F and added the three colour covariates. We generated all statistical correct models including at least one of the colour covariates. The models with lower QAIC values than the model selected in Table 1E,F are presented in Table 1G. To indicate the strength and the direction of

the selection we give the selection gradients on a logit scale (Kingsolver & Smith, 1995) for all covariates.

Results

The probability of recruiting locally was both age and year dependent (Table 1B) whereas the recapture probability did not depend on age and year (Table 1A). We therefore selected the model with age and year dependent survival as basic starting model ($\Phi_{\text{age+yearp}}$). This model was subsequently used for the analysis of directional, stabilizing, and disruptive selection on colour PC1, PC2 and PC3. In a first step we tested whether nestling plumage coloration predicts long-term survival (Table 1C). Only the two models including PC1 ($\Phi_{\text{a+y+PC1+PC1}^2}$ and $\Phi_{\text{a+y+PC1}}$) fitted the data similarly well as the basic model, but ΔQAIC (ΔQAIC between the model in focus and the basic model) was 0.1 and 0.3, respectively, indicating that the two models were not significantly better than the basic model. Similarly, the models including plumage coloration as age-dependent effect fitted similarly well or worse than the basic model (Table 1D). Neither brood size nor body condition improved the model (Table 1E,F). However, hatching date was a significantly better predictor of survival than the basic model ($\Delta\text{QAIC} \geq 2.1$; Fig. 1) and it predicted long-term survival better than 1-year survival ($\Delta\text{QAIC} = 2.1$, Table 1E,F). Models including two of the three or all three covariates fitted less well (bigger QAICc values) than the model including hatching date only (QAICc: 244.1–248.2; $\Delta\text{QAIC} = 2.5$ –4.1). Including hatching date and plumage coloration simultaneously into the model and generating all possible and statistically meaningful models revealed that only the model

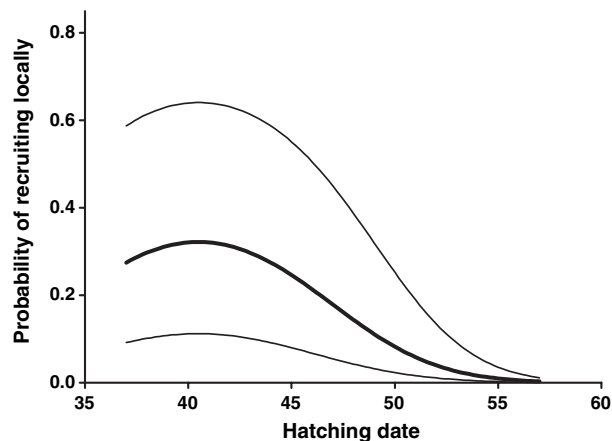


Fig. 1 The probability of recruiting locally (Φ) in relation to the hatching date. The lines represent the estimated local recruitment probabilities of the model $\Phi_{\text{a+y+HD+HD}^2}$ presented in Table 1F. Survival estimations are shown within the natural range of the hatching date. Day 1 corresponds to 1 April (day 40 = 10 May; day 55 = 25 May). 95% confidence intervals are given.

including hatching date as a linear and quadratic term, and PC1 as a quadratic term, showed lower QAIC values than the model including hatching date alone (Table 1G). This model was however not significantly different from the model including hatching date only ($\Delta\text{QAIC} = 0.1$). All models including hatching date and PC2 or PC3 as a covariate fitted less well than the model including hatching date only.

Discussion

Young birds often die at high rates shortly after fledging. In great tits for example 47% of the fledglings die within the first 20 days after leaving the nest and in 65% of the cases predation was the cause of mortality (Naef-Daenzer *et al.*, 2001). This indicates that predation is one of the most important selective pressures acting on young birds after fledging and contributing importantly to the low first-year survival (e.g. 7.4% in our study population; Fitze *et al.*, 2004). Consequently, the nestling's plumage coloration may have evolved as to render a fledgling cryptic (e.g. Butcher & Rohwer, 1989; Götmark & Olsson, 1997). Because the nestling's survival is lowest early after fledging (Naef-Daenzer *et al.*, 2001), because fledglings moult their body feathers in autumn (Svensson, 1992; Jenni & Winkler, 1994) and because their new plumage coloration is not correlated with the nestling's plumage coloration (Fitze *et al.*, 2003a), we might expect that the plumage coloration affects first-year rather than long-term survival. However, our models including the plumage parameters as an age-dependent covariate acting on first-year survival only (Table 1D) did not fit the data better than the basic model and the only model with lower AIC that included the covariates body condition, clutch size and/or hatching date and the colour parameters (Table 1G), was similarly well fitting as the model including the hatching date only. Similarly, models including the original variables hue, saturation and brightness as linear and/or quadratic term were not fitting better than the models including hatching date only (all QAICc ≥ 241.7 , analysis not shown). Thus our results suggest that predation or parental feeding preferences impose currently no or only weak selective pressure on the carotenoid-based nestling coloration of great tits. In contrast to our results, Götmark & Olsson (1997) show in great tits that red coloured compared with control nestlings died at a higher rate post-fledging because of predation. The different results of the two studies may be explained by the red plumage coloration that is more visible than the naturally occurring yellow coloration or by differences in predation pressures between populations and/or study years. However, only specific experiments may disentangle between the different hypotheses.

The fact that we do not find evidence for selection acting on the yellow nestling's plumage coloration neither before (Tschirren *et al.*, 2005) nor after fledging (this study) raises the question why nestlings show this

coloration. One explanation might be that the yellow plumage coloration of the adult great tits is selected and that the nestling's plumage coloration developed as a correlated response (Falconer & Mackay, 1996; Lynch & Walsh, 1998), which is selection neutral and thus maintained until now. However, the nestling's yellow plumage coloration may have evolved because of past selection, which may have led to the optimal plumage coloration, that as a consequence is no longer under selection nowadays (Jennions *et al.*, 2001; Candolin, 2003; for a review). For example, early coloration may have provided nonoptimal camouflage (see discussion on Götmark & Olsson's findings) and predators may have preferentially caught the well-visible birds, thus selecting for cryptic coloration. Plumage coloration may then have adapted to a coloration providing optimal camouflage, which got genetically fixed and is no longer under selection nowadays (e.g. Candolin, 2003 for a review). Although our results provide evidence for these hypothesis we cannot exclude that weak selection may act on the carotenoid-based nestling's plumage coloration after fledging, because detecting weak selection usually requires huge sample sizes ($N \gg 1000$). Further, selection may differ between habitats, geographical regions or it may differ in time. Because the study included two different years and because there were no interactions between the plumage coloration and the study year (all $\Delta\text{QAIC} < 2$) the later hypothesis is unlikely, leaving the possibility open that in other study areas selection on the carotenoid-based nestling plumage coloration might be present.

The results further indicate that in our forest stabilizing selection is acting on hatching date with an optimal hatching date on 10 May. This finding is consistent with earlier studies (e.g. Verhulst & Tinbergen, 1991; Brinkhof *et al.*, 1997; Svensson, 1997) and it is as well consistent with studies showing that the benefits of early hatching are limited by food availability (Drent & Daan, 1980; Van Noordwijk *et al.*, 1995) and with studies showing that late hatching broods are negatively selected for due to reduced food availability (Siikamäki, 1998) and/or increased predation pressure (Naef-Daenzer *et al.*, 2001).

In conclusion, our study reveals that the carotenoid-based nestling plumage coloration of great tits is currently not or only under weak natural selection, since on none of the three colour parameters detectable short- or long-term survival selection was acting. Our study therefore indicates that nowadays neither predators nor parents are imposing strong selective pressures on the nestling's plumage coloration. This suggests that past selection pressures, or correlated selection may have led to its evolution.

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