

SHORT COMMUNICATION

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Growth conditions affect carotenoid-based plumage coloration of great tit nestlings

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Abstract Carotenoid-based integument colour in animals has been hypothesised to signal individual phenotypic quality because it reliably reflects either foraging efficiency or health status. We investigated whether carotenoid-derived yellow plumage coloration of fledgling great tits (*Parus major*) reflects their nestling history. Great tit fledglings reared in a poor year (1998) or in the urban habitat were less yellow than those reared in a good year (1999) or in the forest. The origin of nestlings also affected their coloration since nestlings from a city population did not improve their coloration when transferred to the forest. Brood size manipulation affected fledgling colour, but only in the rural population, where nestlings from reduced broods developed more yellow coloration than nestlings from increased and control broods. Effect of brood size manipulation on fledgling plumage colour was independent of the body mass, indicating that growth environment affects fledgling body mass and plumage colour by different pathways.

Introduction

Carotenoids are terpenic pigments produced by plants, algae and fungi. Higher animals cannot synthesise them *de novo*, but carotenoid-based colours are very common components of animal signals (Goodwin et al. 1984). It has been hypothesised that carotenoid-based

ornaments may signal either foraging efficiency (Endler 1983; Hill et al. 1994) or superior health, because individuals face a trade-off between allocation of carotenoids to colour signals and the use of carotenoids for other activities such as immune function and free-radical scavenging (reviews in Bendich 1989; Lozano 1994; Møller et al. 2000). There are reasons to believe that nestling birds (and offspring of other animals) may be in particular need of carotenoids. Nestlings have a naive immune system that has been exposed to a limited number of antigens, but they are also forced to stay in a nest from which they cannot escape attacks from parasites. Furthermore, offspring are characterised by a rapid burst of growth that results in a high rate of production of free radicals. To study these potentially important functions of carotenoids (and carotenoid-based signals) in growing animals, more knowledge about relationships between growth conditions and carotenoid-based integument coloration is needed.

The aim of this study was to test whether carotenoid-based plumage coloration of great tit nestlings depends on their growth conditions. To address this question we performed two experiments. In the first, partial cross fostering experiment hatchlings (approximately half of nestlings in each experimental dyad) were swapped between broods of great tits breeding in two contrasting (urban and rural) habitats in order to test for the presence of genetic and environmental components of plumage colour of fledglings. Based on the assumption that the rural habitat provides a richer source of dietary carotenoids, we also predicted that nestlings of urban origin, which were reared in the rural study area, would become more yellow than their siblings reared in the original habitat and vice versa. In the second experiment, we manipulated the number of hatchlings in order to produce experimental broods differing by ± 2 nestlings from the original clutch size. The aim of this experiment was to modify the growth conditions of nestlings by reducing or increasing the amount of food available for individual nestlings. We predicted that if the ability of nestlings to develop exaggerated carote-

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noid-based plumage reflects individual quality (modified by parental provisioning rates), then 'yellowness' of plumage should increase from increased to control and to reduced broods. Additionally, we examined the effect of brood size manipulation on fledgling mass and analysed the relationships between nestling body mass and plumage colour.

Methods

The study was carried out in 1998–1999 in two neighbouring (urban and rural) great tit populations breeding in nest boxes in and around Tartu (58°22' N, 26°43' E; human population about 100,000), south-east Estonia. The study areas are described by Hõrak and Lebreton (1998). Only data from first clutches were used. Reproductive parameters of breeding great tits were recorded by regular inspection of nestboxes. On days 8 and 15, nestlings were weighed with a Pesola spring balance with a precision of 0.1 g. For the brood size manipulations, 2-day-old (day 0 = day of hatching) nestlings were transferred between nests to create experimental brood sizes differing from the original clutch size by –2, 0 or 2 nestlings. Experimental and control broods did not differ significantly with respect to clutch size ($P=0.30$), while the hatching date of control broods was slightly later than that of reduced and increased broods ($F_{2,121}=3.7$, $P=0.029$). To adjust for this effect, hatching date was included in all models testing for an effect of brood size manipulation on nestlings.

In the cross-fostering experiment, approximately half of the nestlings (3–5) from each experimental nest were swapped between pairs of broods in the urban and the rural study area at the age of 2 days. Experimental broods in a dyad belonged to the same clutch size categories and brood size manipulation classes in both habitats. Due to lack of power of the statistical test of a full-sib analysis, we addressed the question of the presence of additive genetic and environmental components of colour in a correlation analysis. In this case, the correlation coefficient between average trait values of siblings reared in their own and the foster nests equals the additive genetic variance (Falconer 1989), while the correlation between average trait values of original and foster nestlings equals the environmental variance. In these analyses, data were pooled over the 2 years and colour was standardised within each year to a mean value of zero and a variance of unity.

Lutein is the main carotenoid component of ventral plumage colour in great tit nestlings, juveniles and adults with some additional effects of zeaxanthin (Partali et al. 1987; Stradi 1995). Analyses of plumage colour (see Hõrak et al. 2000 for details) were performed on two feathers, plucked from a standard position on the breast. Colour was measured in an area of the visible surface

of the feather, of approximately 1 mm², using a portable spectroradiometer (Ocean Optics Europe). Since lutein absorbance peaks at 450 nm (Stradi et al. 1995), absorbance due to this carotenoid was calculated from mean transmittance in the interval 445–455 nm, as $-\log_{10}$ (transmittance value), and used in the subsequent analyses. Repeatability (Lessells and Boag 1987) of lutein absorbance was 0.71 in 1998 and 0.60 in 1999 (all $P<0.0001$).

Effects of manipulation were tested in ANCOVAs, using type III sums of squares. All significance levels refer to two-tailed tests. Subscripts used in connection with F -tests refer to degrees of freedom. Values are means (SD).

Results

Background data

Breeding conditions for great tits were generally more favourable in 1998 than in 1999. In both study sites great tits laid larger clutches, had heavier nestlings on day 8, and higher fledgling success in 1998 than in 1999 (Table 1). However, mean fledgling mass did not differ between years.

In both habitats, nestlings were more yellow in 1998 than in 1999 (Table 1). In 1998 lutein absorbance of fledglings increased with hatching date in both habitats ($r_s=0.55$, $n=26$, $P=0.004$ for the rural area, $r_s=0.33$, $n=41$, $P=0.033$ for the urban area). In 1999, this trend was significant only in the urban area ($r_s=0.69$, $n=51$, $P<0.001$).

Heritable and environmental components of plumage colour

The additive genetic component of fledgling colour was small and non-significant, as the correlation between average lutein absorbance of siblings reared in own and foster nests (in different habitat) was not significant ($r=0.26$, $n=28$, $P=0.18$). However, the power of this test was low (27%).

Unlike the genetic component, the environmental component of fledgling colour was significant: Average lutein absorbance of home-reared nestlings correlated to that of foster nestlings (from a different habitat)

Table 1 Comparison of reproductive parameters and fledgling plumage colour (lutein absorbance of yellow breast feathers) of great tits between 1998 and 1999. Statistical analyses are based on brood means

Study site	Trait	1998 mean \pm SD (<i>n</i>)	1999 mean \pm SD (<i>n</i>)	<i>P</i>
Rural	Clutch size	11.2 \pm 1.2 (29)	10.7 \pm 1.1 (52)	0.040 ^a
	Fledgling success ^c	0.87 \pm 0.20 (28)	0.58 \pm 0.31 (45)	<0.001 ^b
	Nestling weight on day 8	12.4 \pm 1.3 (29)	10.8 \pm 1.6 (52)	<0.001 ^a
	Nestling weight on day 15	16.9 \pm 1.1 (28)	16.6 \pm 1.5 (43)	0.218 ^a
	Lutein absorbance	0.81 \pm 0.04 (26)	0.70 \pm 0.07 (43)	<0.001 ^a
Urban	Clutch size	9.4 \pm 1.4 (80)	8.7 \pm 1.3 (69)	0.002 ^a
	Fledgling success ^c	0.75 \pm 0.31 (79)	0.64 \pm 0.32 (65)	0.030 ^b
	Nestling weight on day 8	11.6 \pm 1.6 (80)	10.4 \pm 2.0 (67)	0.001 ^a
	Nestling weight on day 15	15.8 \pm 1.8 (73)	16.0 \pm 1.5 (58)	0.544 ^a
	Lutein absorbance	0.79 \pm 0.04 (41)	0.66 \pm 0.08 (51)	<0.001 ^a

^a *t*-test

^b Kruskal-Wallis test

^c Fledgling success = number of fledglings/(number of hatchlings + number of added or removed nestlings)

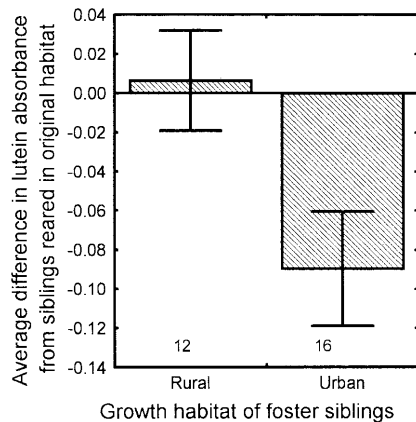


Fig. 1 Colour (lutein absorbance of yellow breast feathers) of great tit nestlings in relation to their rearing environment. Means are average differences in colour between siblings reared in different habitats. Lines represent standard errors and numbers are sample sizes (of pairs of broods)

reared in the same nest ($r=0.51$, $n=35$, $P=0.002$). The effect of the growth environment on nestling colour was especially strong in the urban habitat: nestlings from the rural study area, which grew up in the town, developed significantly less yellow plumage colour than their siblings reared in the original habitat (Fig. 1; $t=-2.33$, $P=0.034$). However, nestlings of urban origin, which were reared in the rural habitat, were not more yellow than their siblings reared in the town (Fig. 1; $t=0.18$, $P=0.90$).

Effects of brood size manipulation on fledgling plumage colour and body mass

Both fledgling mass ($F_{2,121}=7.5$, $P=0.001$) and colour ($F_{2,121}=3.4$, $P=0.035$) were affected by the brood size manipulations in a pooled data set (Fig. 2). The model for colour also included significant habitat \times manipulation interaction term ($F_{2,121}=6.3$, $P=0.003$), implying that the effect of brood size manipulation was different for rural and urban study areas. Fig. 2 reveals that this was due to the highest values of lutein absorbance in reduced broods in the rural habitat. Both models also contained a significant main effect of habitat ($F_{1,121}=13.9$, $P<0.001$ for body mass; $F_{1,121}=8.7$, $P=0.004$ for colour). This effect was due to generally higher body mass and plumage lutein absorbance in the rural study area (Fig. 2). The interaction between manipulation and year was not significant ($F_{2,121}=0.24$, $P=0.8$ for both fledgling colour and body mass), indicating that brood size manipulation affected colour and mass in a similar manner in a poor and a good year.

When both year and habitat categories were analysed separately, the effect of brood size manipulation on fledgling body mass was not significant (all $P>0.1$). However, the effect of brood size manipulation on plumage colour of nestlings still persisted for rural great

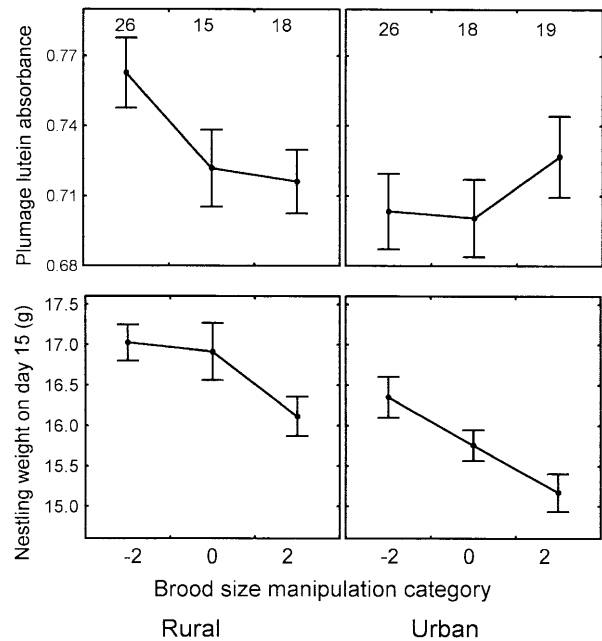


Fig. 2 Colour and fledgling weight in relation to brood size manipulation score. Bars are standard errors and numbers at bars denote sample sizes (number of broods). Both colour and body mass were measured in the same set of individuals. See text for significance of manipulation effects

tit ($F_{2,21}=5.93$, $P=0.011$ in 1998; $F_{2,36}=5.86$, $P=0.007$ in 1999). In both years nestlings from reduced broods appeared more yellow [lutein absorbance = 0.82 (0.04) in 1998 and 0.73 (0.08) in 1999] than those reared in increased [0.79 (0.02) in 1998 and 0.68 (0.05) in 1999] and control broods [0.78 (0.03) in 1998 and 0.67 (0.05) in 1999].

Relationships between body mass and plumage colour

In 1999 plumage yellowness correlated positively with nestling body mass on day 8. This correlation held for both individual nestlings ($r_s=0.11$, $n=507$, $P=0.017$) and brood mean values ($r_s=0.25$, $n=94$, $P=0.017$). However, fledgling body mass was not significantly associated with plumage colour, neither for individual nestlings nor for brood mean values (both $P=0.4$). None of the correlations between body mass and colour was significant in 1998 (all $P>0.1$). Since nestling body mass on day 8 was positively associated with plumage colour, we investigated whether the effect of brood size manipulation acted on colour via general nutritional condition of nestlings. In this case we expected that adding the nestling body mass as a predictor variable to the model would cancel the effect of brood size manipulation on plumage colour. However, that was not the case: the main effect of brood size manipulation ($F_{2,121}=4.22$, $P=0.017$) and its interaction with habitat ($F_{2,121}=6.49$, $P=0.002$) remained both significant predictors of fledgling colour, while the effect

of nestling body mass on day 8 was not significant ($F_{1,121}=0.20$, $P=0.6$). Hence, the brood size manipulation affected the colour of fledglings independent of nestling body mass.

Discussion

Carotenoid-based plumage coloration of great tit fledglings varied both between breeding habitats of different quality and within habitats in response to experimental manipulation of brood size. Additionally, we found that in both study areas, great tit fledglings developed more yellow plumage colour in 1998 which was a more favourable year for breeding than 1999, as shown by the superior breeding performance. Finally, the yellowness of fledgling plumage increased seasonally. The latter result is consistent with that of Slagsvold and Lifjeld (1985), who also found between-year variation in the yellow plumage colour of great tit nestlings and a similar seasonal increase in coloration. Such annual and seasonal variation in pigmentation may either be related to dietary factors, e.g. the frequency of large-sized lepidopteran larvae may have increased during the breeding season, or the use of carotenoids for immune function and/or free radical scavenging may have varied systematically with the season. The finding that great tit nestlings reared in the rural study area developed more yellow plumage colour and obtained higher fledgling mass than conspecifics reared in the urban study area is also compatible with those of Slagsvold and Lifjeld (1985) and Eeva et al. (1998), who found that plumage colour of great tit nestlings was related to the abundance of carotenoid-rich food items in the rearing habitat. Availability of carotenoid-rich food during moult has been found to affect directly plumage coloration also in (adult) house finches (*Carpodacus mexicanus*; Hill 1992) and northern cardinals (*Cardinalis cardinalis*; Linville and Breitwisch 1997). Hence, one may conclude that the quality and quantity of food during feather growth partly determine the expression of carotenoid-based plumage coloration.

The joint role of quality and quantity of carotenoid-rich food in formation of plumage colour is further supported by our result that brood size manipulation consistently affected fledgling colour only in the rural study area but not in the town. Lack of effect of brood size manipulation in the town suggests that relaxing food competition among nestlings is not a sufficient precondition for increasing the yellowness of the plumage. This conclusion is further supported by the result that the effect of brood size manipulation on fledgling plumage colour was independent of the body mass. These results suggest the growth environment affects fledgling body mass and plumage colour by different pathways. This implies that in case if fledgling plumage colour bears a signalling function (e.g., in social dominance interactions in winter flocks), then inter-individual differences in nestling health state that lead to variation in

plumage colour may have a persistent effect on the future performance of individuals.

Siblings reared in the home and foster broods (in different habitats) did not significantly resemble each other with respect to plumage colour. However, the power of this test was low (27%), implying that no firm conclusions about a genetic component in fledgling colour can be made. Unlike the genetic component, the environmental component in fledgling colour was statistically significant: plumage colour of nestlings reared in their original nest resembled that of foster nestlings reared in the same nest. Interestingly, the effect of rearing environment on fledgling plumage colour differed between habitats. The cross-fostering experiment showed that the colour of nestlings of rural origin could be suppressed by rearing them in town (Fig. 1), which appeared to be a poorer growth habitat for great tits (most likely due to more sparse vegetation). However, the colour of nestlings of urban origin could not be enhanced in the rural environment. This result indicates that availability of carotenoid-based food items in the growth environment is not the sole factor affecting plumage colour of great tit nestlings. One possible explanation would be that nestlings of urban origin, which were transferred to the rural habitat, originally started out with a small amount of carotenoids deposited in the yolk by their mothers, and they therefore had to spend more carotenoids on maintenance. In such a case, the increased demand for carotenoids for detoxification and immuno-modulation processes would have reduced the amount of carotenoids available for developing a yellow plumage.

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