Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects

Arild Johnsen^{1*}, Kaspar Delhey¹, Staffan Andersson² and Bart Kempenaers¹

¹Max Planck Research Centre for Ornithology, PO Box 1564, D-82305 Starnberg (Seewiesen), Germany

²Department of Zoology, Göteborg University, PO Box 463, SE-40530 Göteborg, Sweden

Sexual-selection theory assumes that there are costs associated with ornamental plumage coloration. While pigment-based ornaments have repeatedly been shown to be condition dependent, this has been more difficult to demonstrate for structural colours. We present evidence for condition dependence of both types of plumage colour in nestling blue tits (*Parus caeruleus*). Using reflectance spectrometry, we show that blue tit nestlings are sexually dichromatic, with males having more chromatic (more 'saturated') and ultraviolet (UV)-shifted tail coloration and more chromatic yellow breast coloration. The sexual dimorphism in nestling tail coloration is qualitatively similar to that of chick-feeding adults from the same population. By contrast, the breast plumage of adult birds is not sexually dichromatic in terms of chroma. In nestlings, the chroma of both tail and breast feathers is positively associated with condition (body mass on day 14). The UV/blue hue of the tail feathers is influenced by paternally inherited genes, as indicated by a maternal half-sibling comparison. We conclude that the expression of both carotenoid-based and structural coloration seems to be condition dependent in blue tit nestlings, and that there are additional genetic effects on the hue of the UV/blue tail feathers. The signalling or other functions of sexual dichromatism in nestlings remain obscure. Our study shows that nestling blue tits are suitable model organisms for the study of ontogenetic costs and heritability of both carotenoid-based and structural colour in birds.

Keywords: *Parus caeruleus*; structural colour; carotenoids; ultraviolet; maternal half-siblings

1. INTRODUCTION

Sexual dimorphism is common in nature and often attributable to sexual selection (Andersson 1994). This is particularly true for sexual dimorphism in ornaments, such as horns and antlers in mammals and colourful plumages and long tails in birds, where the exaggerated dimorphic traits are used predominantly in the competition over mates (Andersson 1994). Indicator models of sexual selection assume that ornaments are costly to produce or maintain (Zahavi 1975; Andersson 1986; Grafen 1990), and there is extensive empirical evidence for this from many taxa (Andersson 1994).

In birds with ornamental plumage colours, the evidence for production costs differs depending on the type of colour mechanism involved. The development of carotenoid-pigmented plumage (yellow, orange and red plumages) is believed to be costly because birds cannot synthesize these pigments but have to obtain them through the diet (Brush 1981). Furthermore, carotenoids are known to have important functions in many physiological processes (e.g. as antioxidants and as precursors of vitamin A), resulting in a potential trade-off between irreversible feather-pigment deposition and the need for carotenoids elsewhere in the body (Olson & Owens 1998). Accordingly, several avian studies have documented food-limited expression (e.g. Slagsvold & Lifjeld 1985; Hill *et al.* 2002) and condition dependence (e.g. Hill & Montgomerie 1994;

Thompson *et al.* 1997) of carotenoid signals. The costs of producing structural colours (e.g. ultraviolet (UV), violet and blue plumages) are less obvious, but may be found in the ontogenetic costs or constraints on the formation of a regular 'spongy structure', the fine network of air channels separated by keratin rods that is responsible for the coherent scattering of short wavelengths (Dyck 1971; Andersson 1999; Prum *et al.* 2003). A relationship between condition and the expression of structural plumage coloration has so far been found in only two studies (Keyser & Hill 1999; Doucet 2002).

A further requirement of indicator models is that the sexually selected traits show heritable genetic variation (Andersson 1994). Yet, the genetic contribution to sexually selected plumage ornaments is not well documented (see recent review by Merilä & Sheldon (2001)). For bird species in which extra-pair paternity occurs, comparisons of maternal half-siblings can be used to determine whether offspring traits are influenced by paternal genes (Merilä & Sheldon 2001). To our knowledge, only three avian studies have used this approach (Sheldon *et al.* 1997; Sheldon 1999; Johnsen *et al.* 2000). In this study, we investigate the influences of condition and paternal genes on the expression of UV/blue and yellow plumage colours in nestling blue tits (*Parus caeruleus*).

In many bird species with sexual size dimorphism, the difference is already present in the nestling stage (e.g. Smith *et al.* 1989; Merilä & Svensson 1997), where its ontogeny and response to selection has been studied in detail (see Badyaev (2002) for a recent review). The early occurrence and development of sexual dichromatism have

*Author for correspondence (johnsen@erl.ornithol.mpg.de).

rarely been investigated. In tits (*Paridae*), the nestling plumage resembles the adult plumage (Cramp & Perrins 1993), and in the only study of nestling sexual dichromatism to date, Slagsvold & Lifjeld (1985) found that male great tit (*Parus major*) nestlings have higher yellow chroma than female nestlings. Furthermore, it was shown that the yellow plumage pigments derived from ingested green lepidopteran larvae, which contained large amounts of the carotenoids lutein and zeaxanthin (Slagsvold & Lifjeld 1985; Partali *et al.* 1987), thus suggesting a potential nutritional constraint and mediation of signal honesty.

Adult blue tits are sexually dichromatic, with a major part of the sex difference being confined to the human-invisible UV part of the spectrum (Andersson *et al.* 1998; Hunt *et al.* 1998). The structural blue coloration is more UV shifted and more chromatic (i.e. higher chroma or saturation) in males than in females. In addition, Örnborg (2002) recently showed that there are sexual differences in the carotenoid-based yellow coloration of the breast and flank, with males being more chromatically yellow than females. There is experimental evidence that the UV/blue plumage of males is subject to sexual selection through female choice (Hunt *et al.* 1999; Sheldon *et al.* 1999), and the yellow plumage has been suggested to signal male parental abilities (Senar *et al.* 2002). Blue tit nestlings develop yellow breast feathers, and tail and wing feathers that appear blue to the human eye (Cramp & Perrins 1993). There is extensive individual variation in colour, and some of this variation is related to sex. With some experience, it is possible to sex *ca.* 80% of the nestlings by visual inspection of whole broods (Kempenaers *et al.* 1997). We present analyses of the plumage coloration of blue tit nestlings, measured just before fledging (19 days old), and adults captured during chick feeding. Our aims were: (i) to describe objectively the colour variation and sexual dichromatism in blue tit nestlings using reflectance spectrometry; (ii) to investigate whether nestling plumage coloration is condition dependent; and (iii) to test whether there are genetic components in the expression of coloration. While condition dependence is difficult to demonstrate for blue tit adults because the birds are generally not available for measurements during the moult, nestlings are highly suitable as models in investigations of the development of coloration because they can be measured during the time when their colourful plumage is developing.

2. MATERIAL AND METHODS

(a) General methods

The fieldwork was conducted in 2001 in a colour-banded blue tit population breeding in nest-boxes in a mixed deciduous forest at Kolbeterberg, Vienna, Austria (48°13' N, 16°20' E). Adults were caught in the nest-boxes while feeding 9-day-old nestlings, aged (either 1 year or older) and sexed based on the presence or absence of a brood patch, and their plumage colour was measured with a spectroradiometer (see § 2b). On day 14 (day 1 being the day of hatching), nestlings were banded and measured (tarsus length and body mass) and a small blood sample (5–25 µl) was taken for molecular parentage determination and sexing. On day 20, we measured nestling wing length, body mass and coloration (see § 2b), and plucked a tail feather (third from the right). The total length of the tail feather and the lengths of the shaft and vane were measured with a slide calliper.

We sampled 495 nestlings from 51 broods on day 14. The sample was reduced to 365 nestlings from 40 broods on day 20 owing to predation and early fledging.

We used body mass on day 14 as a measure of body condition for the following reasons. First, nestling body mass has been shown to correlate with survival in several bird species, including the blue tit (Nur 1984; Tinbergen & Boerlijst 1990). Second, even though the brood means of body mass on day 14 were correlated with those on day 20 ($n = 40$, $r = 0.68$, $p < 0.001$), we preferred the former because we wanted an estimate of condition during early feather development. Third, by using an absolute measure of body mass, we avoid the statistical problems associated with using residuals (Green 2001), although using relative body mass (residuals from a regression of $\log(\text{body mass})$ on $3 \times \log(\text{tarsus length})$) did not produce qualitatively different results (data not shown).

(b) Colour measurements

Reflectances from the breast and the upper tail were measured in the 300–700 nm spectral range with an Ocean Optics S2000 spectrometer, with illumination by a TOP Sensor Systems deuterium-halogen lamp DH-2000, both connected to the sensor by a bifurcated fibre optics cable. The sensor, providing unidirectional illumination and measurement, was held at 90° to the surface. Reflectance was expressed relative to a WS-2 white reflection standard (TOP Sensor Systems). Scans were taken from five different spots on both the upper breast (two on each side and one in the middle) and on the upper tail (aiming for the most colourful parts), respectively. Based on these measurements, we computed objective indices of the three main dimensions of colour perception: brightness (spectral intensity), hue (spectral location) and chroma (spectral purity or saturation) (Hailman 1977). For both the breast and the tail plumage, we used average reflectance, $R_{320-700}$ as a measure of brightness. For the yellow breast plumage, chroma was estimated by $(R_{700} - R_{450})/R_{700}$ which should directly indicate the influence of carotenoids (since these absorb most strongly at 450 nm). A more general chroma measure, $(R_{\max} - R_{\min})/R_{\text{average}}$ was used for the tail plumage. To estimate the hue of the tail feathers, we used $\lambda(R_{\max})$, the wavelength of peak reflectance. We did not estimate the hue of the yellow breast feathers, because of the double peaked nature of these spectra (Cuthill *et al.* 1999). We focused on the adult-resembling tail feathers because the crown feathers of juveniles are greenish-grey and do not resemble the brilliant blue crown feathers of adults, which have been the focus of most studies on blue tit plumage coloration (e.g. Andersson *et al.* 1998; Hunt *et al.* 1998).

We measured the plumage colour of 352 nestlings (188 males and 164 females) in 38 broods, and 106 adults (50 males and 56 females). The adult sample was balanced with respect to age (males: 25 juveniles and 25 adults; females: 26 juveniles and 30 adults), and we pooled the age classes in analyses of adult sex differences in plumage.

The timetable for feather development in nestling blue tits has been described in detail by Schoppe (1977). Of relevance to this study, the breast feather pins start to appear around days 6–7 and those of the tail appear around days 6–9. The breast feathers then emerge from the pins between days 7 and 10, whereas the tail feathers emerge between days 8 and 10. The colourful parts of the tail feathers appear from around day 15, and can be measured only close to fledging. In our study population, the majority of chicks fledged on day 21. The post-juvenile moult is partial and starts around the age of two months (Cramp & Perrins

1993). All breast feathers are moulted whereas a variable number of juvenile tail feathers are retained in the first breeding plumage (Cramp & Perrins 1993).

(c) *Paternity analysis*

Six polymorphic microsatellite markers (*PC3*, 7 and 9 (Dawson *et al.* 2000), *Pocc1* and 6 (Bensch *et al.* 1996) and *Phtr3* (Fridolfsson *et al.* 1997)) were used to determine the paternity of 505 offspring (480 nestlings and 25 unhatched eggs) in 48 broods (three of the above-mentioned 51 broods had unknown social males and were not included in the paternity analysis). The combined probability of exclusion (Jamieson 1994) for the marker set was more than 0.999. Paternity was excluded if there were mismatches at two or more loci between putative fathers and offspring. In 437 cases, there was no ($n = 429$) or one ($n = 8$) mismatch with the social father. For these offspring, the average $\pm$ s.d. probability of false inclusion (Jeffreys *et al.* 1992) was $9.8 \times 10^{-5} \pm 3.4 \times 10^{-4}$ (range of 4.0×10^{-9} – 4.6×10^{-3}) and we conclude that they were sired by the social male. The remaining 68 offspring showed two or more mismatches with the putative father and were thus sired by extra-pair males. For 49 of these, an alternative male matched the paternal genotype completely, and in one case, there was one mismatch with a putative extra-pair sire. In the latter case, the same male was the unequivocal sire of another offspring in the same nest, and we assign both offspring to this male. The average $\pm$ s.d. probability of false inclusion was $1.2 \times 10^{-4} \pm 5.6 \times 10^{-4}$ (range of 2.6×10^{-7} – 4.0×10^{-3}) for the 50 assigned extra-pair offspring. The remaining 18 extra-pair offspring were sired by unknown males. Thirteen out of the 17 identified extra-pair males had paternity in one extra-pair nest, whereas the remaining four males had paternity in two ($n = 2$), three ($n = 1$) and four nests ($n = 1$). There was one case of reciprocal paternity exchange. The total frequency of extra-pair paternity was 13% (68/505) of offspring in 58% (28/48) of the broods.

(d) *Molecular sexing*

We used the *Phtr3* microsatellite marker, which shows Z-linked inheritance (i.e. females display only one allele; Fridolfsson *et al.* 1997), to sex 83% of the nestlings. To sex the remaining birds, we used the primers *P2* and *P8* (Griffiths *et al.* 1998), which amplify two homologous genes: *CHD1W* on the W chromosome and *CHD1Z* on the Z chromosome.

(e) *Statistical analysis*

We used mixed models (with nest-box as the random effect) to investigate the relationship between body condition and coloration. The length of the tail feather vane was also entered in models where tail feather characteristics were used as response variables, to control for feather development. Restricted maximum likelihood (REML) was used owing to the unbalanced structure of the data. We inspected residual plots and normal probability plots for unequal variance and deviations from normality among residuals to verify the validity of the final model. Tail chroma was log-transformed to normalize the residuals.

We tested for genetic effects on the expression of nestling plumage coloration by comparing maternal half-siblings (Falconer & Mackay 1996; Merilä & Sheldon 2001). Mixed models with REML were performed to test for box (rearing environment and parental effects) and sire effects, and to calculate variance components. Colour parameters were standardized (mean = 0, s.d. = 1) within each sex, so that we could pool the

sexes in the analyses of genetic effects. The sample contained 20 broods with mixed paternity from which we had colour measurements of both categories of offspring. In 16 of these broods, all extra-pair young were sired by a single male. In the remaining four broods, two ($n = 2$) or three ($n = 2$) extra-pair fathers were identified. We pooled extra-pair offspring sired by different males in the analyses. The conclusions remain the same when the analyses are restricted to the 16 broods with only one extra-pair sire (data not shown).

One brood contained only females and was therefore not included in paired *t*-tests of within-brood sex differences.

The analyses were performed using Genstat 6.0 and SPSS 11.0. Results are presented as mean $\pm$ s.e.

3. RESULTS

(a) *Adult plumage characteristics in relation to sex*

Tail coloration in adults resembled the crown (Andersson *et al.* 1998; Hunt *et al.* 1998) in the degree of sexual dichromatism (table 1; figure 1*a*). Males had more chromatic UV-blue coloration with a shorter-wave peak than females (i.e. more UV-shifted). There were no differences in brightness. The yellow breast plumage was not sexually dichromatic in brightness or chroma (table 1; figure 1*b*).

(b) *Nestling characteristics in relation to sex*

There were large sex differences in several of the colour variables within broods of 19-day-old nestlings. Males were more chromatic than females and showed a more UV-shifted hue in the coloration of the tail feathers (table 1; figure 1*a*). Furthermore, the yellow breast plumage was more chromatic in males (table 1; figure 1*b*). In the analyses of nestling colour that follow, we focus on the colour variables that showed significant differences between the sexes (chroma and hue of tail and chroma of breast).

Male offspring were also heavier (males: 11.09 ± 0.14 g; females: 10.50 ± 0.11 g; $n = 39$, $t = 6.90$, $p < 0.001$) and had longer wings (males: 53.7 ± 0.3 mm; females: 52.6 ± 0.3 mm; $n = 39$, $t = 4.45$, $p < 0.001$) and tarsi (measured on day 14 since the tarsi are fully developed at this stage; males: 16.7 ± 0.1 mm; females: 16.2 ± 0.1 mm; $n = 50$, $t = 9.51$, $p < 0.001$) than their female nest-mates, whereas there was no significant difference in tail feather length (males: 36.0 ± 0.4 mm; females: 35.8 ± 0.4 mm; $n = 39$, $t = 0.84$, $p = 0.41$).

(c) *Nestling condition and plumage coloration*

There were significantly positive relationships between the chromas of the tail and breast feathers and body condition, when controlling for offspring sex (and vane length in the case of tail chroma) (table 2; figure 2). Tail hue did not correlate significantly with body condition (table 2). Inspection of residual plots revealed three clear outliers in the tail hue measurements (more than five standard deviations above average). After removing these three data points, there was a weak but significant negative relationship between body condition and the hue of the tail feathers ($\chi^2 = 4.45$, d.f. = 1, $p = 0.035$, effect size $\pm$ s.e. = -1.17 ± 0.56 ; sex and vane length also included in the model). There were no significant interaction effects

Table 1. Sex differences (mean $\pm$ s.e.) in tail and breast coloration in adult and nestling blue tits.

variable	males	<i>n</i>	females	<i>n</i>	<i>t</i>	<i>p</i>
adults						
breast brightness	0.277 $\pm$ 0.006	50	0.270 $\pm$ 0.006	56	0.77	0.44
breast chroma	0.621 $\pm$ 0.008	50	0.622 $\pm$ 0.009	56	-0.08	0.93
tail hue (nm)	382.2 $\pm$ 1.9	49	397.4 $\pm$ 2.2	56	-5.15	< 0.001 ^a
tail brightness	0.206 $\pm$ 0.004	49	0.206 $\pm$ 0.004	56	0.03	0.97
tail chroma	0.81 $\pm$ 0.01	49	0.60 $\pm$ 0.01	56	12.85	< 0.001 ^a
nestlings^b						
breast brightness	0.382 $\pm$ 0.005	37	0.377 $\pm$ 0.006	37	0.67	0.51
breast chroma	0.506 $\pm$ 0.006	37	0.474 $\pm$ 0.007	37	7.71	< 0.001 ^a
tail hue (nm)	325.7 $\pm$ 0.6	36	329.1 $\pm$ 0.8	36	-4.45	< 0.001 ^a
tail brightness	0.182 $\pm$ 0.003	36	0.187 $\pm$ 0.004	36	-1.83	0.076
tail chroma	0.640 $\pm$ 0.010	36	0.483 $\pm$ 0.009	36	16.39	< 0.001 ^a

^a Significant after Bonferroni correction ($\alpha' = 0.01$).

^b Pair-wise comparisons of characteristics of 19-day-old nestling blue tits, using sex-specific brood means.

Figure 1. Average reflectance spectra from the (a) tail feathers and (b) breast feathers of blue tit male (thick solid line) and female (thick dashed line) nestling and male (thin solid line) and female (thin dashed line) adults.

between offspring sex and either body condition or vane length in any of these models (all $p > 0.19$).

Table 2 also shows that, after controlling for differences in body condition between the sexes, males were still substantially more chromatic in both tail and breast coloration, and they had a more UV-shifted tail hue than females.

(d) Genetic effects on nestling coloration

There was a significant effect of paternity (sire) on the hue of the tail feathers (table 3) but not on the other two colour variables (both $p > 0.45$). The largest fraction of the overall tail-hue variance was the result of differences within sib groups (table 3). However, the variance component resulting from sire (between sib groups) was about the same as that resulting from environmental and parental effects (between nests). Residual plots revealed one clear outlier (more than five standard deviations above average), the removal of which resulted in a less strong but still significant sire effect ($\chi^2 = 5.6$, d.f. = 1, $p = 0.018$). Using the raw data (instead of standardized values for each sex) and adding sex as a factor in the models gave similar results (sire effect with the outlier: $p = 0.003$; without the outlier: $p = 0.023$). Furthermore, adding body condition to these models does not influence the conclusion that there are genetic effects on tail hue (data not shown).

Figure 1a shows that the spectral shape of the tail coloration is very different in nestlings and adults, which suggests that their tail hues are not directly comparable. Hence, to explore the relationship between the coloration of nestlings and their foster versus their genetic fathers, we correlated the difference in hue between social and extra-pair fathers with the difference in hue between extra- and within-pair offspring. If tail hue were heritable, we would expect a positive correlation. Using a sample of 14 broods for which we had colour data from both social and extra-pair males, and their respective offspring, we indeed found a positive, albeit not significant ($p = 0.085$), correlation (figure 3).

4. DISCUSSION

Spectrometry revealed that blue tit nestlings are highly sexually dichromatic. Males were more chromatic than females in the tail and breast feathers, and had a more UV-shifted hue in the tail feathers. Chick-feeding adults from the same population showed a qualitatively similar sexual dichromatism in tail colour, but not in breast coloration. Comparisons of the values of colour measurements

Table 2. REML models (with nest-box as a random effect) testing the effects of nestling sex and body condition on the chroma and hue of their tail and the chroma of their breast feathers. Tail feather vane length was also included in the models involving tail feather coloration to control for feather development.

factors	χ^2	d.f.	<i>p</i>	effect $\pm$ s.e.
response variable: tail chroma ^a				
offspring sex	227.14	1	< 0.001	-0.27 ± 0.02
body mass at day 14	10.74	1	0.001	0.047 ± 0.014
vane length	4.53	1	0.033	0.009 ± 0.004
response variable: tail hue				
offspring sex	12.96	1	< 0.001	3.16 ± 0.88
body mass at day 14	1.55	1	0.21	-0.80 ± 0.64
vane length	0.06	1	0.81	-0.05 ± 0.21
response variable: breast chroma				
offspring sex	31.66	1	< 0.001	-0.027 ± 0.005
body mass at day 14	13.99	1	< 0.001	0.013 ± 0.003

^a Log-transformed in order to normalize residuals.

Figure 2. Relationships between nestling body mass and chroma of (a) the tail feathers and (b) the breast feathers. Filled circles: males; open circles: females. The fitted lines represent linear regressions of predicted values from the full REML models (see table 2) on body mass, shown separately for males (solid line) and females (dashed line).

in adults and nestlings (table 1) reveal some clear differences, which are also apparent from the differences in the spectral shapes of the curves (figure 1). Nestlings had a

higher brightness and a lower chroma of the breast plumage and a more UV-shifted hue and lower chroma of the tail plumage. Interestingly, the peak tail reflectances at 326 and 329 nm for male and female nestlings, respectively, are substantially lower than in adult tails (this study) and freshly moulted crown plumage (359–370 nm; Örnberg *et al.* 2002).

We found strong evidence for condition dependence of nestling plumage coloration. Even after controlling for sex and feather development, heavier nestlings were more chromatic in both tail and breast plumage, and tended to have a more UV-shifted tail hue. Since condition was estimated on day 14, which is around the time when breast feathers are becoming fully developed and before the colourful parts of the tail feathers have developed, it is unlikely that our results reflect differential parental investment in colourful nestlings. Thus, there appear to be physiological constraints on the development of both pigimentary and structural coloration. Assuming that similar constraints act on adult plumage development, these results have important implications for the study of avian plumage coloration. First, they bridge the gap between the large number of studies showing that structural colours are important in mate choice (Andersson & Amundsen 1997; Bennett *et al.* 1997; Andersson *et al.* 1998; Johnsen *et al.* 1998; Hunt *et al.* 1999; Siitari *et al.* 2002) and the low number of studies indicating that such signals are costly to produce (Keyser & Hill 1999; Doucet 2002). Second, the positive relationship between body condition and yellow chroma suggests that the carotenoids involved (lutein and zeaxanthin) are limiting resources (via access, uptake or allocation; Olson & Owens 1998), even for birds living in deciduous forests, despite the apparent abundance of these carotenoids in leaf-eating lepidopteran larvae (Partali *et al.* 1987), the main diet also for this blue tit population (B. Kempenaers, unpublished data). This supports previous studies that have indicated environmental constraints on the expression of yellow plumage in birds (Slagsvold & Lifjeld 1985; Hørak *et al.* 2000; Fitze *et al.* 2003). In other words, plumages based on directly deposited yellow carotenoids might also be candidates for honest signalling in birds, although they are less obviously

Table 3. Sources of variance in the tail hue of maternal half-siblings.

source	variance component	% of total variance	d.f.	χ^2	<i>p</i>
nest-box	0.118	11.0	1	7.23	0.007
sire	0.101	9.4	1	8.90	0.003
sib group	0.853	79.6	191	—	—

Figure 3. Relationship between the difference in tail hue between extra-pair male and social male (extra-pair male–social male) and the difference in tail hue between half-siblings (mean of extra-pair offspring–mean of within-pair offspring). Spearman's correlation $r_s = 0.48$, $n = 14$, $p = 0.085$. Nestling tail hue estimates were standardized within each sex so that the sexes could be pooled. The linear regression line is shown.

costly than those based on red metabolically modified carotenoids (Hill 1996).

Maternal half-sibling comparisons revealed that nestling tail feather hue was influenced by paternally inherited genes, whereas none of the other sexually dichromatic traits showed evidence of a genetic component. This result is unlikely to have been confounded by differential allocation (Sheldon 2000), since there is no evidence to suggest that between (i) males can discriminate their own offspring and extra-pair offspring (Kempnaers & Sheldon 1996); or (ii) females are able to allocate resources differentially to eggs sired by different males within a clutch. To our knowledge, this is the first study to demonstrate a genetic effect on the expression of a structural colour. Given the genetic component in nestling tail hue, it seems likely to remain (although perhaps reduced) in adult coloration, but this remains to be shown. The non-significant tendency for a congruent difference in tail hue between extra-pair versus within-pair sires and their respective offspring in the brood (figure 3) also suggests that tail hue is heritable, although a larger sample is required to estimate the heritability of this trait confidently.

Sexual dichromatism in nestling birds has received little attention and its adaptive significance is unknown. Potential functions include signalling to the parents in the nest or outside the nest during the post-fledging period, which may be advantageous if parents adjust their feeding pattern according to the characteristics of their offspring (Lyon *et al.* 1994; Slagsvold *et al.* 1994). Given the low

light levels inside our nest-boxes, with their small entrance holes, a signalling function in the post-fledging period seems more likely. However, there might be sufficient light in natural cavities, the conditions under which the potential signalling has evolved. Alternatively, the juvenile plumage colour might be a status signal used in contest competition over territories during early autumn, or even mate attraction at this time. Finally, sexual dichromatism in the nestling stage might be a result of different developmental pathways for the sexes that have been selected for by plumage-related mating success at the adult stage. Blue tits retain a variable number of tail feathers during the first moult (11–50% retain all juvenile tail feathers whereas 3–30% moult all of them, depending on the population; details in Cramp & Perrins (1993, p. 245)). This implies that the first breeding plumage is in part produced during the nestling stage, which thus might respond to sexually selected dichromatism.

To summarize, the chroma of both the yellow underparts and the UV/blue tail feathers of blue tit nestlings is largely influenced by (or at least covaries with) their condition at the time of feather development, whereas the hue of the tail feathers is relatively more genetically determined. Different components of the colour ornament might therefore give different information to receivers. We conclude that the blue tit nestling is an excellent model organism for studies of the ontogeny of sexual colour ornamentation and the relative importance of environmental and genetic influences on structural as well as carotenoid-based plumage ornamentation.

The authors thank Kim Carter, Karin Hieke, Angelika Poesel and Agnes Türk for field assistance, Emmy Schlicht for tail feather measurements, and Kim Carter for performing the genetic analyses. Donald Blomqvist, Anne Peters and Ben Sheldon contributed much-appreciated input to statistical discussions, and the manuscript was improved by the comments of two anonymous referees. The authors also thank Raphael-Thomas Klumpp and Alfred Fojt from the Institute of Silviculture, Vienna, for providing access to their facilities in the study area, and Hans Winkler from the Konrad Lorenz Institute for Comparative Ethology, Vienna, for generous logistic support. The work received financial support from the Alexander von Humboldt Stiftung, through an AvH research fellowship to A.J.

REFERENCES

- Andersson, M. 1986 Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* **40**, 804–816.
- Andersson, M. 1994 *Sexual selection*. Princeton University Press.
- Andersson, S. 1999 Morphology of UV reflectance in a Whistling-thrush: implications for the study of structural colour signalling in birds. *J. Avian Biol.* **30**, 193–204.

- Andersson, S. & Amundsen, T. 1997 Ultraviolet colour vision and ornamentation in bluethroats. *Proc. R. Soc. Lond. B* **264**, 1587–1591. (DOI 10.1098/rspb.1997.0221.)
- Andersson, S., Örnberg, J. & Andersson, M. 1998 Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proc. R. Soc. Lond. B* **265**, 445–450. (DOI 10.1098/rspb.1998.0315.)
- Badyaev, A. V. 2002 Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol. Evol.* **17**, 369–378.
- Bennett, A. T. D., Cuthill, I. C., Partridge, J. C. & Lunau, K. 1997 Ultraviolet plumage colors predict mate preferences in starlings. *Proc. Natl Acad. Sci. USA* **94**, 8618–8621.
- Bensch, S., Price, T. & Kohn, J. 1996 Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Mol. Ecol.* **5**, 150–151.
- Brush, A. H. 1981 Carotenoids in wild and captive birds. In *Carotenoids as colorants and vitamin A precursors* (ed. J. C. Bauerfeind), pp. 539–562. New York: Academic Press.
- Cramp, S. & Perrins, C. M. 1993 Blue tit. In *The birds of the western Palearctic*, vol. 7 (ed. S. Cramp & C. M. Perrins), pp. 225–248. Oxford University Press.
- Cuthill, I. C., Bennett, A. T. D., Partridge, J. C. & Maier, E. J. 1999 Plumage reflectance and the objective assessment of avian sexual dichromatism. *Am. Nat.* **153**, 183–200.
- Dawson, D. A., Hanotte, O., Greig, C., Stewart, I. R. K. & Burke, T. 2000 Polymorphic microsatellites in the blue tit *Parus caeruleus* and their cross-species utility in 20 songbird families. *Mol. Ecol.* **9**, 1941–1944.
- Doucet, S. M. 2002 Structural plumage coloration, male body size, and condition in the blue-black grassquit. *Condor* **104**, 30–38.
- Dyck, J. 1971 Structure and spectral reflectance of green and blue feathers of the rose-faced lovebird (*Agapornis roseicollis*). *Dans. Vid. Selsk. Biol. Skr.* **18**, 1–67.
- Falconer, D. S. & Mackay, T. F. C. 1996 *Introduction to quantitative genetics*, 4th edn. Harlow: Longman.
- Fitze, P. S., Kölliker, M. & Richner, H. 2003 Effects of common origin and common environment on nestling plumage coloration in the great tit (*Parus major*). *Evolution* **57**, 144–150.
- Fridolfsson, A. K., Gyllenstein, U. B. & Jakobsson, S. 1997 Microsatellite markers for paternity testing in the willow warbler *Phylloscopus trochilus*: high frequency of extra-pair young in an island population. *Hereditas* **126**, 127–132.
- Grafen, A. 1990 Biological signals as handicaps. *J. Theor. Biol.* **144**, 517–546.
- Green, A. J. 2001 Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* **82**, 1473–1483.
- Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. G. 1998 A DNA test to sex most birds. *Mol. Ecol.* **7**, 1071–1075.
- Hailman, J. P. 1977 *Optical signals*. Bloomington: Indiana University Press.
- Hill, G. E. 1996 Redness as a measure of the production cost of ornamental coloration. *Ethol. Ecol. Evol.* **8**, 157–175.
- Hill, G. E. & Montgomerie, R. 1994 Plumage colour signals nutritional condition in the house finch. *Proc. R. Soc. Lond. B* **258**, 47–52.
- Hill, G. E., Inouye, C. Y. & Montgomerie, R. 2002 Dietary carotenoids predict plumage coloration in wild house finches. *Proc. R. Soc. Lond. B* **269**, 1119–1124. (DOI 10.1098/rspb.2002.1980.)
- Hörak, P., Vellau, H., Ots, I. & Möller, A. P. 2000 Growth conditions affect carotenoid-based plumage coloration of great tit nestlings. *Naturwissenschaften* **87**, 460–464.
- Hunt, S., Bennett, A. T. D., Cuthill, I. C. & Griffiths, R. 1998 Blue tits are ultraviolet tits. *Proc. R. Soc. Lond. B* **265**, 451–455. (DOI 10.1098/rspb.1998.0316.)
- Hunt, S., Cuthill, I. C., Bennett, A. T. D. & Griffiths, R. 1999 Preferences for ultraviolet partners in the blue tit. *Anim. Behav.* **58**, 809–815.
- Jamieson, A. 1994 The effectiveness of using co-dominant polymorphic allelic series for (1) checking pedigrees and (2) distinguishing full-sib pair members. *Anim. Genet.* **25**, 37–44.
- Jeffreys, A. J., Allen, M. J., Hagelberg, E. & Sonnberg, A. 1992 Identification of the skeletal remains of Josef Mengele by DNA analysis. *Forensic Sci. Int.* **56**, 65–76.
- Johnsen, A., Andersson, S., Örnberg, J. & Lifjeld, J. T. 1998 Ultraviolet plumage ornamentation affects social mate choice and sperm competition in bluethroats (Aves: *Luscinia s. svecica*): a field experiment. *Proc. R. Soc. Lond. B* **265**, 1313–1318. (DOI 10.1098/rspb.1998.0435.)
- Johnsen, A., Andersen, V., Sunding, C. & Lifjeld, J. T. 2000 Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature* **406**, 296–299.
- Kempnaers, B. & Sheldon, B. C. 1996 Why do male birds not discriminate between their own and extra-pair offspring? *Anim. Behav.* **51**, 1165–1173.
- Kempnaers, B., Verheyen, G. R. & Dhondt, A. A. 1997 Extra-pair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics and offspring quality. *Behav. Ecol.* **8**, 481–492.
- Keyser, A. J. & Hill, G. E. 1999 Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. *Proc. R. Soc. Lond. B* **266**, 771–777. (DOI 10.1098/rspb.1999.0704.)
- Lyon, B. E., Eadie, J. M. & Hamilton, L. D. 1994 Parental choice selects for ornamental plumage in American coot chicks. *Nature* **371**, 240–243.
- Merilä, J. & Sheldon, B. C. 2001 Avian quantitative genetics. In *Current ornithology*, vol. 16 (ed. V. J. Nolan), pp. 179–255. Dordrecht, The Netherlands: Kluwer.
- Merilä, J. & Svensson, E. 1997 Are fat reserves in migratory birds affected by condition in early life? *J. Avian Biol.* **28**, 279–286.
- Nur, N. 1984 The consequences of brood size for breeding in blue tits. II. Nestling weight, offspring survival and optimal brood size. *J. Anim. Ecol.* **53**, 497–517.
- Olson, V. A. & Owens, I. P. F. 1998 Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**, 510–514.
- Örnberg, J. 2002 Ultraviolet coloration and colour communication in blue tits *Parus caeruleus*. PhD dissertation, Göteborg University, Sweden.
- Örnberg, J., Andersson, S., Griffiths, S. C. & Sheldon, B. C. 2002 Sexually selected UV reflectance declines over the breeding season in blue tits, *Parus caeruleus*. *Biol. J. Linn. Soc.* **76**, 237–345.
- Partali, V., Liaaen-Jensen, S., Slagsvold, T. & Lifjeld, J. T. 1987 Carotenoids in food chain studies. II. The food chain of *Parus* spp. monitored by carotenoid analysis. *Comp. Biochem. Physiol. B* **87**, 885–888.
- Prum, R. O., Andersson, S. & Torres, R. H. 2003 Coherent scattering of ultraviolet light by avian feather barbs. *Auk* **120**, 163–170.
- Schoppe, R. 1977 Beiträge zur postembryonalen Gefiederentwicklung bei *Parus* und *Ficedula*. *Die Vogelwelt* **98**, 201–221.
- Senar, J. C., Figuerola, J. & Pascual, J. 2002 Brighter yellow blue tits make better parents. *Proc. R. Soc. Lond. B* **269**, 257–261. (DOI 10.1098/rspb.2001.1882.)
- Sheldon, B. C. 1999 Revealing quantitative genetic relationships in sexual selection using molecular markers. In *Proceedings of the 22nd International Ornithological Congress, Durban, South Africa* (ed. N. J. Adams & R. H. Slotow), pp. 1099–1114. Johannesburg: Birdlife South Africa.

- Sheldon, B. C. 2000 Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* **15**, 397–402.
- Sheldon, B. C., Merilä, J., Quarnström, A., Gustafsson, L. & Ellegren, H. 1997 Paternal genetic contribution to offspring condition predicted by size of male secondary character. *Proc. R. Soc. Lond. B* **264**, 297–302. (DOI 10.1098/rspb.1997.0042.)
- Sheldon, B. C., Andersson, S., Griffith, S. C., Örnborg, J. & Sendecka, J. 1999 Ultraviolet colour variation influences blue tit sex ratios. *Nature* **402**, 874–877.
- Siitari, H., Honkavaara, J., Huhta, E. & Viitala, J. 2002 Ultraviolet reflection and female mate choice in the pied flycatcher, *Ficedula hypoleuca*. *Anim. Behav.* **63**, 97–102.
- Slagsvold, T. & Lifjeld, J. T. 1985 Variation in plumage colour of the great tit *Parus major* in relation to habitat, season and food. *J. Zool. Lond. A* **206**, 321–328.
- Slagsvold, T., Amundsen, T. & Dale, S. 1994 Selection by sexual conflict for evenly spaced offspring in blue tits. *Nature* **370**, 136–138.
- Smith, H. G., Källander, H. & Nilsson, J. A. 1989 The trade-off between offspring number and quality in the great tit *Parus major*. *J. Anim. Ecol.* **58**, 383–401.
- Thompson, C. W., Hillgarth, N., Leu, M. & McClure, H. E. 1997 High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. *Am. Nat.* **149**, 270–294.
- Tinbergen, J. M. & Boerlijst, M. C. 1990 Nestling weight and survival in individual great tits (*Parus major*). *J. Anim. Ecol.* **59**, 1113–1127.
- Zahavi, A. 1975 Mate selection—a selection for a handicap. *J. Theor. Biol.* **53**, 205–214.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.