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ORIGINAL PAPER

Yolk androgens in great tit eggs are related to male attractiveness, breeding density and territory quality

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Abstract Females can adaptively adjust phenotype of their offspring via deposition of various compounds into eggs, including androgens and other hormones. Here, I investigated how egg yolk androgens (testosterone and androstenedione) related to environmental conditions and parental traits in the great tit (*Parus major*) across three breeding seasons. Male and female traits studied included age, condition and multiple feather ornaments, both carotenoid- and melanin-based (carotenoid and UV chroma of yellow breast feathers, area of black breast band and white cheek immaculateness). Yolk mass increased with laying temperature, laying date and area of male black breast band. Concentration of androgens increased with breeding density, territory quality and carotenoid chroma of male yellow breast feathers and was higher in mates of 1 year old as compared to older males. Yolk androgens were not related to any of the female traits analysed. These patterns were thus consistent with (1) social and environmental effects on yolk mass and composition and (2) both positive and negative differential allocation strategies of resource allocation in females. Overall, male traits were the most important predictors of egg yolk characteristics in this socially monogamous songbird.

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Introduction

Parents can significantly affect phenotype and performance of their offspring through non-genetic pathways by modifying prenatal and rearing environments of their young (Badyaev and Uller 2009). Birds are particularly interesting animals in this context because embryo development takes place within a sealed system, the egg, whose contents are fixed by the mother at laying, and no further adjustments of the egg components are possible once the egg is laid. Besides altering egg size (Krist 2010), avian mothers transfer to their eggs many valuable compounds, including antioxidants (Surai 2002), hormones (Groothuis et al. 2005a) and antibodies (Grindstaff et al. 2003). In this way, mothers modify the quality of their eggs and, indirectly, morphology, physiology and behaviour of the offspring.

Androgens in avian eggs can have multiple effects on embryo and the young (Groothuis et al. 2005a) mediated by various potential proximate pathways (Navara and Mendonça 2008). So far, the range of these hormonal effects identified has involved benefits in terms of enhanced growth and competitive ability (Eising et al. 2001; Eising and Groothuis 2003; Groothuis et al. 2005b; Navara et al. 2005; Müller et al. 2009; but see Andersson et al. 2004; Pilz et al. 2004) as well as costs in terms of immunosuppression (Andersson et al. 2004; Groothuis et al. 2005b; Müller et al. 2005; Navara et al. 2005; but see Navara et al. 2006a; Pitala et al. 2009), poor survival (Müller et al. 2009) and elevated metabolic rate (Tobler et al. 2007). These effects are, moreover, often sex-specific (Saino et al. 2006; von Engelhardt et al. 2006; Sockman et al. 2008; Pitala et

al. 2009), environment-dependent (Pilz et al. 2004; Müller et al. 2010) and might not be demonstrated until well later in life (Strasser and Schwabl 2004; Eising et al. 2006; Rubolini et al. 2007; Müller et al. 2009). Thus, there is no simple relationship between yolk androgens and offspring performance, but the outcome of embryonic androgen exposure likely depends on the post-hatching circumstances for the developing offspring such as parasite exposure and the degree of sibling competition.

Deposition of androgens in eggs can be affected by various characteristics of environmental conditions, females and males. Egg yolk androgens were found to be related to food supply (Sandell et al. 2007; Dentressangle et al. 2008), timing of breeding (Gil et al. 2006) and breeding density (Pilz and Smith 2004; Eising et al. 2008; Safran et al. 2010; but see Gil et al. 2006). They were also related to female age (Pilz et al. 2003) and condition (Tobler et al. 2007), female aggressive behaviour (Whittingham and Schwabl 2002), female social status (Müller et al. 2002; Tanvez et al. 2008) and social stress (Mazuc et al. 2003), female immune status (Gil et al. 2006) and exposure to parasites (Tschirren et al. 2004). Females might also positively or negatively differentially allocate yolk androgens in relation to social mate quality and attractiveness (Gil et al. 1999, 2006; Tanvez et al. 2004; Michl et al. 2005; Navara et al. 2006b; Loyau et al. 2007; Dentressangle et al. 2008; Safran et al. 2008; Kingma et al. 2009; Garcia-Fernandez et al. 2010; Ratikainen and Kokko 2010).

Identifying robust correlates of egg yolk androgen deposition is a prerequisite for better understanding of parental investment strategies, potential role of these substances in context-dependent adaptive offspring engineering and the role of environment in modulating patterns of deposition (Gilbert et al. 2005). Here, I investigated correlates of androgen (testosterone and androstenedione) deposition in egg yolk in a wild-ranging population of the great tit *Parus major* in terms of both environmental conditions and female and male quality. Environmental conditions often strongly modulate deposition of egg androgens, either facilitating or constraining it (see above). Thus, (1) I studied how timing of breeding, temperature, breeding density and territory quality predicted yolk mass and deposition of androgens. Females of high quality should allocate more yolk androgens to their eggs (investment hypothesis; Safran et al. 2008). Thus, (2) I studied how female characteristics (feather colouration, condition and age) predicted yolk mass and deposition of yolk androgens. Further, females paired to high quality males might allocate more (positive differential allocation) or less (negative differential allocation, Ratikainen and Kokko 2010) resources to their eggs. Thus, (3) I studied how social male characteristics (feather colouration, condition and age) predicted yolk mass and deposition of yolk androgens.

Methods

General fieldwork

This work was conducted on three adjacent nest-box plots (188 nest-boxes in total) in a deciduous forest near Grygov (49°31′N, 17°19′E, 205 m a.s.l.) in eastern Czech Republic. The forest was dominated by lime *Tilia* and oak *Quercus* with interspersed ash *Fraxinus excelsior*, hornbeam *Carpinus betulus* and alder *Alnus glutinosa*. Nest-boxes were placed about 1.6 m above ground, and besides the great tit were inhabited by collared flycatchers *Ficedula albicollis*, blue tits *Cyanistes caeruleus* and nuthatches *Sitta europea*.

Fieldwork was carried out between 2005 and 2007 from early April until mid-June. Nest-boxes were checked daily to record laying of the first egg and final clutch size. When there were between six and seven eggs laid (i.e. before the incubation started, V. Remeš, unpublished data), the fourth egg was collected. This egg was weighed to the nearest 0.01 g and placed into the freezer under −20°C. Within 1 month after the field season had ended, the eggs were let thaw under room temperature, yolk was separated, weighed and again frozen under −20°C. Yolk androgens were analysed during autumn but no later than in October. I collected only one egg per clutch because of ethical reasons and also because this population is subject to detailed investigation taking place during chick rearing (including capturing the adults). One egg should reasonably represent the whole clutch, because in the great tit, variation in steroid hormone concentrations between clutches is much higher than within clutches (Tschirren et al. 2004).

During feeding of nestlings (median age of young for females=7 days, for males=9 days), parents were captured in the nest-box. Females were captured on all the nests (n=163). However, because of time constraints, males were captured on a subset of nests only (n=101). Tarsus length was measured with a digital calliper (nearest 0.01 mm), and birds were weighed on a spring Pesola balance (nearest 0.125 g). Body condition was calculated as residuals from the regression of body mass on tarsus length. From each bird, 10 to 15 yellow feathers were taken from the upper right part of the breast for later spectrophotometric analysis. The bird's white cheek (right side of the head) and breast were photographed with a digital camera (Panasonic DMC-FZ5). While the picture of the cheek was taken, the bird was held in a standardised position on its left side. While the picture of the breast was taken, the bird was held outstretched by its tarsi and beak and photographed together with a ruler from a standard distance following the protocol of Figuerola and Senar (2000). The age of the birds was determined based on their plumage as 1 year old or older (Svensson 1992).

Breeding density was defined as the number of adjacent nest-boxes (within 2.5 ha centred on the breeding nest-box) occupied by great tit pairs during the formation of the yolk of the sampled egg, i.e. during 7 days preceding egg laying (Perrins 1979). Breeding density varied from zero to nine. The area of 2.5 ha was chosen because this figure lies at the upper end of territory sizes reported for great tit (Wilkin et al. 2006 and references therein). Territory quality was defined as the number of years in which the particular nest-box was occupied by great tit between 2005 and 2009. Territory quality varied between one and five. Laying temperature was defined as the mean of average daytime temperatures (from 0600 to 2000 hours) during 7 days preceding the laying of the sampled egg.

Quantification of feather colouration

The following characteristics of feather colouration were chosen for the analysis: area of the black breast stripe (Norris 1990), carotenoid and UV chroma of yellow breast feathers (Isaksson et al. 2008), and immaculateness of the white cheek (Ferns and Hinsley 2004). Photos of breast and cheek were analysed in Adobe Photoshop CS3 Extended. Quick selection tool was used to roughly delimit the black stripe or the white cheek. Selection was then finished manually so that it was as precise as possible and measured the surface area of the stripe or cheek. A ruler photographed with every bird was used to adjust the scale of each photo and to obtain absolute surface area (in square centimetre) and in the case of the cheek also perimeter (in centimetre). Stripe surface was defined 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 and Senar 2000). Immaculateness of the white cheek was calculated as $4*\pi*$(area per square perimeter), which served as an index to measure regularity of the cheek's borders. It is equivalent to the index used by Ferns and Hinsley (2004), and the value of 1 indicates a perfect circle, whereas lower values (approaching zero) indicate shapes with lower area for a given perimeter, i.e. shapes with irregular borders and thus having lower immaculateness (Ferns and Hinsley 2004). To assess repeatability, a different observer measured a subsample of photos. Repeatability, calculated as the intraclass correlation coefficient (Lessells and Boag 1987), was high for both stripe area (r_i=0.87, p<0.001, n=75) and cheek immaculateness (r_i=0.89, p<0.001, n=75).

Reflectance spectra of yellow feathers sampled from the breast were quantified using standard procedures (Andersson and Prager 2006). From each bird, 10–15 feathers were used, which is enough to obtain reliable values in our study species (Quesada and Senar 2006). The equipment used consisted of 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 feather surface. Five readings from different parts of each set of feathers were taken. Feathers were arranged on a black, non-reflective surface so that they overlapped extensively.

Reflectance (percent) from 320 to 700 nm in 1-nm increments was obtained. Carotenoid chroma was calculated as R700 minus R450, divided by R700, where R700 is reflectance at 700 nm and R450 reflectance at 450 nm. Carotenoid chroma was used because it reflects the amount of yellow carotenoids (lutein and zeaxanthin) in the breast feathers in great tit (Isaksson and Andersson 2008; Isaksson et al. 2008). Hue might be a better measure of carotenoid concentration in saturated carotenoid-based colours (Andersson and Prager 2006, p. 82). However, the reflectance spectra obtained had always reasonable reflectance at 450 nm, where lutein and zeaxanthin absorb maximally (females: mean=14.2%; range, 9.3% to 22.5%; n=128; males: mean=14.7%; range, 7.8% to 24.4%; n=101). This indicated that the great tit carotenoid-based colour was not saturated, and that is why carotenoid chroma was used. UV chroma was calculated as summed reflectance from 320 to 400 nm divided by total reflectance (i.e. summed reflectance from 320 to 700 nm). The average chroma calculated from the five readings from each set of feathers was always used in statistical analyses. To assess repeatability of these measurements, the same procedure was repeated on a subsample of feathers one more time. Repeatability of these two average chroma estimates was calculated as the intraclass correlation coefficient (Lessells and Boag 1987), which was sufficiently high (carotenoid chroma: r_i=0.85, p<0.001, n=55; UV chroma: r_i=0.90, p<0.001, n=55).

Androgens

To determine testosterone (T) and androstenedione (A4) concentrations, a previously published method was used (Hampl 1994). Yolk sample was homogenised in physiological solution (1:2, *w*/*w*). Homogenate was diluted with water (T, 12 µl in 588 µl; A4, 30 µl in 270 µl) and extracted with diethylether (3 ml) for 60 s. The water phase was left frozen in a solid carbon dioxide/ethanol bath, and the organic phase was decanted and evaporated to dryness. The dry residue was dissolved in 80% aqueous solution of methanol (1 ml). *n*-Hexane (1 ml) was then added to remove the excess of lipids. The mixture was extracted and centrifuged to eliminate formed emulsions. The *n*-hexane phase was removed and discarded. Furthermore, *n*-hexane (1 ml) was added, and the procedure was carried out again

as described previously. The methanol phase was then evaporated to dryness. The dry residue was dissolved in 20 mM sodium phosphate-buffered saline pH 7.0 containing 1 mg/ml BSA (T, 600 μl; A4, 300 μl) and used for radioimmunoanalysis.

Radioimmunoassay for T was prepared as follows: [^{125}I] iodo testosterone-3-carboxymethyl-tyrosine methyl ester (8.5 kBq/ml) in 20 mM PBS pH 7.0 as a tracer, polyclonal antibody raised against testosterone-3-*O*-carboxymethyl-BSA conjugate (dilution 1:40,000 in 20 mM PBS pH 7.0) and testosterone (from 0 to 8.0 nM) in 20 mM PBS pH 7.0 as a standard. Analogously, for A4: [1,2,6,7-3 H]-Δ4-androstenedione (6.5 kBq/ml) in 20 mM PBS pH 7.0 as a tracer, polyclonal antibody raised against 6β-hydroxy-Δ4-androstenedione-6β-hemisuccinate-BSA conjugate (dilution 1:20,000 in 20 mM PBS pH 7.0) and Δ^4-androstenedione (from 0 to 34.915 nM) in 20 mM PBS pH 7.0 as a standard.

The content of tubes was mixed and incubated (12 h, 4°C). Dextran-coated charcoal (500 μl) was added to each sample except those for total activity determination. The mixture was mixed briefly, incubated (10 min, 4°C) and centrifuged (3,000×*g*, 10 min, 4°C). The supernatant was decanted and taken for assessment of [^{125}I] radioactivity (T) or [^{3}H] radioactivity (A4). Concentrations of T and A4 were calculated from the log-logit plot and corrected according to previously determined losses.

All samples were assayed in duplicates, and intra-assay coefficients of variation were 8.2% for T and 5.8% for A4. Inter-assay coefficients of variation were 10.7% for T and 11.6% for A4. Recovery rates ranged between 61% and 75%. The cross-reactivity between T and A4 was less than 2%.

Statistical analyses

I was not a priori sure whether the concentration or the amount of yolk androgens is biologically more relevant (Safran et al. 2008). Thus, as dependent variables, I modelled (1) total summed concentration of androgens and (2) total amount of androgens per yolk, obtained by multiplying concentrations by yolk mass. To provide more detailed insight and statistical estimates for potential future meta-analyses, I also modelled testosterone and androstenedione concentrations separately. Besides yolk androgens, I also modelled yolk mass. To use as much data as possible and to avoid overly complex models, three sets of variables were used as predictors: (1) environmental factors (laying date, laying temperature, breeding density and territory quality), (2) female phenotypic traits (UV and carotenoid chroma of yellow breast feathers, area of the black breast band, cheek immaculateness, condition, age and clutch size) and (3) male phenotypic traits (the same as in females except clutch size). To compare relative importance of these three sets of predictors, I compared the models when using the exact same data for which I had all the predictors. I compared the strength of evidence for each of the three models by means of Akaike information criterion (AIC) in Proc Mixed of SAS.

Some females were sampled in more than one season. Seven females were sampled in three seasons, 21 females in two seasons and 100 females in one season only. Female identity was used as a random factor with random intercepts only; general linear mixed models (Proc Mixed of SAS) were always used. No male was sampled in more than one season, which was certainly caused by much lower number of males captured. Denominator degrees of freedom were calculated by the Satterthwaite method.

Concentrations of yolk androgens significantly differed among years (testosterone: $F_{2,160}=134.8$, $p<0.001$; androstenedione: $F_{2,160}=138.1$, $p<0.001$; total androgens: $F_{2,160}=164.8$, $p<0.001$; androgens per yolk: $F_{2,159}=105.6$, $p<0.001$). When year was added to the models as a predictor, its variance inflation factor was ca. 5.5, which indicated potential problems with collinearity of predictors. Thus, I centred the dependent variables (i.e. total concentration of androgens and the amount of androgens per yolk) within years by subtracting every value from the respective yearly mean value. Variance inflation factors in all the models with these centred variables were less than 1.6 for all the predictors, which indicated that the problems with collinearity were solved. Of course, year was not included as a predictor in these latter models. I also centred laying date and laying temperature, which also differed strongly among years (date: $F_{2,160}=54.4$, $p<0.001$; temperature: $F_{2,160}=68.4$, $p<0.001$). Concentrations and the amount of yolk androgens were $\log_{10}$ transformed in all the models.

Ethical note

Standard methods in capturing and handling birds used in the research of cavity-nesting passerines were used. Adults were captured in the nest-box. They were handled for as short time as possible to minimise any distress. The smallest number of feathers possible to obtain reliable results were plucked, which was based on a previous methodological study (Quesada and Senar 2006). This study complies with the current law of the Czech Republic. I had all necessary permits for this study, and it was overseen by the Ethical Committee of Palacky University.

Results

Androgen concentrations and yolk mass

Average egg mass for individual females was 1.66 g (SD=0.13, median=1.66, $n=126$), yolk mass was 0.34 g

(SD=0.03, median=0.34, n=128) and it made on average 20.48% of egg mass (relative yolk mass hereafter, SD=1.58, median=20.42, n=126). Average testosterone concentration for individual females was 55.89 pg/mg (SD=29.04, median=50.12, n=128), androstenedione concentration was 47.87 pg/mg (SD=15.25, median=47.43, n=128) and total androgens per yolk averaged 35.09 pg (SD=13.95, median=33.58, n=128).

Egg and yolk mass were highly positively correlated, whereas egg mass and relative yolk mass were correlated negatively (Table S1 in the Online Resource). Correlations between yolk mass and relative yolk mass, and androgen concentrations were weak, but consistently negative. This resulted in no overall correlation between yolk mass and the amount of androgens per yolk. However, correlations between androgen concentrations and the amount of androgens per yolk were highly positive (Table S1 in the Online Resource). These results suggest that the amount of androgens in yolks was driven more by androgen concentration than by yolk mass. Concentrations of testosterone and androstenedione were highly positively correlated (Table S1 in the Online Resource).

Standardized regression coefficients for the relationships between yolk characteristics and the three sets of predictors (environment and female and male traits) are presented in Figs. 1 and 2.

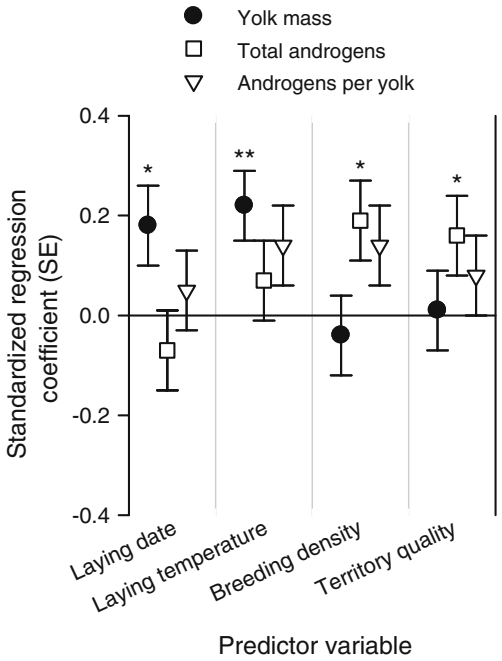

Fig. 1 Summary of the linear mixed models relating yolk mass and composition to the *factors of the environment*. Full results of modelling are available in Table S2 in the Online Resource. *Asterisks* denote significance at $^{**}p<0.01$, $^{*}p<0.05$

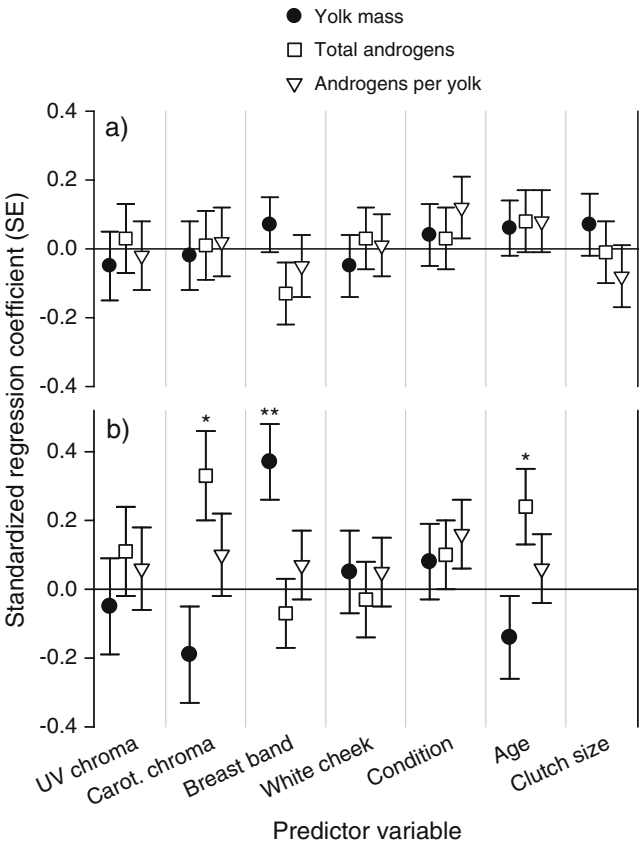

Fig. 2 Summary of the linear mixed models relating yolk mass and composition to (**a**) *female traits* and (**b**) *male traits* (clutch size was not among predictors here). Full results of modelling are available in Table S2 in the Online Resource. *Asterisks* denote significance at $^{**}p<0.01$, $^{*}p<0.05$

Environment

Yolk mass was larger late in the season and increased with laying temperature. Concentration of androgens increased with breeding density and territory quality. When analysed separately, concentration of androstenedione increased with breeding density and territory quality, whereas concentration of testosterone did not (full results of statistical modelling are available in Table S2). Although yolk mass increased with laying temperature, the amount of androgens per yolk did not increase significantly (Fig. 1; Table S2 in the Online Resource).

Female traits

Overall, relationships of yolk characteristics to female traits were weak and not statistically significant (Fig. 2a; Table S2 in the Online Resource).

Male traits

Yolk mass increased with the area of male black breast band. Concentration of androgens increased with carotenoid

chroma of yellow breast feathers of males, which was driven by testosterone. However, the amount of androgens per yolk was not related to carotenoid chroma of males. Androgen concentration was higher in 1-year-old males as compared to older males, which was driven by androstenedione. However, the amount of androgens per yolk was not related to male age. Although yolk mass increased with the area of male black breast band, the amount of androgens per yolk did not increase significantly (Fig. 2b; Table S2 in the Online Resource).

As environmental factors, female traits and male traits were generally not inter-correlated, the results of independent modelling of the effects of these three sets of predictors were genuine and not confounded (Table S3 in the Online Resource). Comparison of AIC values for the three sets of predictors suggested that male traits were the most important set of predictors in explaining variation in egg composition (Table S2 in the Online Resource).

Discussion

Androgen concentrations

Yolk composition in terms of androgen concentrations was similar to values reported by other studies of the great tit. Yolk testosterone concentration in a Swiss population of the great tit was 25.3 pg/mg, whereas concentration of androstenedione was 52.8 pg/mg (Tschirren et al. 2004), values broadly comparable to mine. Concentration of testosterone in my population of the great tit was higher than any species-specific value reported for 36 songbird species (Garamszegi et al. 2007) and for 101 other bird species (Gil et al. 2007). Androstenedione concentration fell within the values reported by Gil et al. (2007) for the same sample of 101 species of bird.

Environment

Yolk mass increased with laying date and temperature, which has been observed previously in another population of the great tit (Lessells et al. 2002). It has been suggested that when temperatures are low, females are either constrained by low activity of insect food during egg formation or they must devote more energy to maintenance metabolism and less is spared to form the eggs. Alternatively, increasing yolk mass with laying date might be an adaptive strategy to provide extra prenatal resources for offspring. Food supply, foraging success (Naef-Daenzer and Keller 1999) and survival prospects of fledging great tits decline with season (Perrins 1979; Naef-Daenzer et al. 2001), and thus, it might be adaptive to boost offspring performance with extra resources (Krist 2010). However, increases of yolk mass with laying date and temperature did not translate into more androgens per yolk due to consistently negative correlations between yolk mass and androgen concentrations (see Table S1 in the Online Resource). Their consistently negative direction might have been enough to weaken the relationship between laying date and temperature and the amount of androgens per yolk.

The concentration of androstenedione increased with breeding density and territory quality. Higher yolk androgen concentrations in denser populations were reported in the European starling *Sturnus vulgaris* (Pilz and Smith 2004; Eising et al. 2008) and the house sparrow *Passer domesticus* (Mazuc et al. 2003), but not in the barn swallow *Hirundo rustica* (Gil et al. 2006; Safran et al. 2010). Females may actively allocate androgens to eggs laid under high density, thus preparing offspring for a difficult breeding situation. Alternatively, high frequency or intensity of aggressive interactions can lead to high yolk androgen concentrations (Whittingham and Schwabl 2002; Hargitai et al. 2009), thus possibly providing an example of physiological constraint which females cannot avoid (Pilz and Smith 2004). Recent evidence suggests that food supply may modify deposition of yolk androgens in relation to laying order (Sandell et al. 2007) or male attractiveness (Dentressangle et al. 2008). If my measure of territory quality (frequency of occupation over 5 years) reflected food supply, more resources would enable females to deposit more androstenedione into eggs. Alternatively, attractive territories might be pre-empted by high-quality females with an intrinsic ability of depositing more androgens into eggs without a direct effect of food supply.

Female traits

Neither yolk mass nor composition was related to any of female phenotypic characteristics. This is quite surprising given the evidence from other great tit populations that feather ornaments are condition-dependent (Senar et al. 2003) and signal individual condition or quality (Hõrak et al. 2001; Ferns and Hinsley 2004, 2008). However, the signal content of individual ornaments might differ between populations. For example, there was a negative relationship between the intensity of yellow feather colour and fledging success in great tits in Estonia (Mänd et al. 2005). In a similar vein, in my population and in a population in Hungary, neither yellow breast feather colour nor the size of the black breast stripe was correlated with and index of condition (Hegyi et al. 2007; Matysioková and Remeš 2010a). This contrasts with a Spanish population where carotenoid-based yellow colour was condition-dependent, whereas the size of the black stripe was not (Senar et al. 2003). Accordingly, in my population, there was no correlation between carotenoid chroma of yellow breast

feathers or the size of their black breast stripe in females and female incubation effort (Matysioková and Remeš 2010b). Similarly, carotenoid chroma of breast feathers did not indicate an ability of females to cope with energetic stress (assessed by a handicapping experiment, Matysioková and Remeš 2010c). All this evidence indicates that feather colouration is not an indicator of female quality in my population of the great tit, and thus the absence of any relationships between yolk mass and composition and female phenotype agrees with other findings from the same population.

Male traits

It has been suggested that females should allocate more resources into offspring when paired with higher-quality males, indicated for instance by their ornaments, a strategy known as positive differential allocation. On the contrary, females might instead boost performance of offspring of lower-quality males, a strategy called negative differential allocation (Harris and Uller 2009; Ratikainen and Kokko 2010). Several studies of yolk androgens in wild birds reported patterns consistent with both positive and negative differential allocation. First, females of the blue tit deposited more yolk androgens for more attractive social partners (Kingma et al. 2009), and females of the grey partridge *Perdix perdix* deposited more androgens for preferred males (Garcia-Fernandez et al. 2010). Second, females in the collared flycatcher laid eggs with higher concentration of yolk testosterone for young as opposed to older males (Michl et al. 2005). Similarly, female house finches *Carpodacus mexicanus* deposited significantly more androgens into eggs sired by less attractive males (Navara et al. 2006b). Finally, there was no relationship of yolk volume or testosterone concentration to the area of a white forehead patch of males in the collared flycatcher (Michl et al. 2005; Török et al. 2007). The complexity of situation is demonstrated by studies on the barn swallow. In a Spanish population, females increased the concentration of androgens in their eggs when mated to males with experimentally elongated tails (Gil et al. 2006). In a US population, androgen concentration increased with male throat colour but did not change with his tale length (Safran et al. 2008). These results hint to possible differences between populations and different male ornaments in female allocation strategies.

Females in my population deposited yolk mass and androgens consistent with both positive and negative differential allocation strategies. First, females laid larger yolks for males with larger black band area, although this did not translate into more androgens per yolk due to negative correlations between yolk mass and androgen concentrations (see Table S1 in the Online Resource). They deposited testosterone in higher concentrations when mated to males with more intense carotenoid chroma of yellow breast feathers, but at the same time laid lighter yolks with the result that the total amount of androgens per yolk did not change. All these patterns are consistent with the positive differential allocation, provided carotenoid- and melanin-based male ornaments in the great tit indicate male quality. Size of the black breast band was positively related to the social status of the bird (Lemel and Wallin 1993) and to the frequency (Norris 1990) and intensity of nest defence (Quesada and Senar 2007). Thus, black stripe signals the ability of the male to win agonistic intraspecific encounters and defend offspring. It may also indicate the ability of the male to provide superior parental care (Norris 1990). Saturation of carotenoid-based colouration might signal the superior foraging ability of individuals in terms of food quality or quantity (foraging performance hypothesis, Møller et al. 2000). In a Swedish population of the great tit, nestling plumage carotenoid chroma was predicted by the chroma of the rearing father (after cross-fostering of the young), indicating that foraging ability of the social mate might have significant effects on offspring phenotype (Isaksson et al. 2006). However, although it has been demonstrated that great tits prefer carotenoid-rich food items in general (Senar et al. 2010), specific tests of the foraging performance hypothesis remain to be carried out.

Second, females laid eggs with higher concentration of yolk androstenedione for 1 year old as opposed to older males, similarly to the situation in the collared flycatcher (Michl et al. 2005). This pattern is consistent with the negative differential allocation and might reflect an attempt by the female to boost the growth of offspring of an inferior father. An alternative explanation is that females try to manipulate their partner's contribution to parental care by allocating yolk androgens that boost offspring solicitation behaviour (Michl et al. 2005). However, this explanation seems unlikely for the great tit because it has been demonstrated that males are not responsive to androgen-mediated solicitation signals in this species (Tschirren and Richner 2008), which is also true in the collared flycatcher (Ruuskanen et al. 2009) and canary *Serinus canaria* (Müller et al. 2010).

Besides direct benefits (offspring feeding and defence), females might gain indirect, genetic benefits from more ornamented males and allocate yolk compounds accordingly. For example, great tit offspring of males with large black band area survived better (Norris 1993). However, revealing allocation based on indirect benefits might be undermined by the occurrence of extra-pair paternity. Then, if most offspring were sired by male(s) other that the social mate, or if social and genetic mates differed systematically in their ornaments, patterns revealed without paying attention to extra-pair paternity could be misleading. However, both of these potential sources of complications

seem to be minimal in the great tit. First, rates of extra-pair paternity are comparatively low in this species, accounting for less than 10% of the young (Verboven and Mateman 1997; Krokene et al. 1998; Strohbach et al. 1998; Lubjuhn et al. 1999, 2001; Otter et al. 2001; Johannessen et al. 2005). Second, social and genetic mates do not differ in feather ornaments (Krokene et al. 1998; Strohbach et al. 1998), and effects of male quality on paternity loss are generally low in this species (Lubjuhn et al. 1999; Otter et al. 2001; Johannessen et al. 2005). The only exception was a Japanese population (a different subspecies, *P. major minor*), where 16.6% of offspring were sired by extra-pair males, and extra-pair sires had larger black breast band than social mates (Kawano et al. 2009).

Conclusions

It is not clear whether the concentration or the total amount of a compound per yolk is more important for developing offspring (Safran et al. 2008). In this study, correlations between yolk mass and androgen concentrations were not significant but overall were slightly negative. This was the reason for the observation that although yolk mass increased with certain factors (laying temperature and male breast band area), total androgens per yolk did not (Figs. 1 and 2). Similarly, although the total androgen concentration increased with male carotenoid chroma, androgen amount per yolk did not because yolk mass correlated slightly negatively with male carotenoid chroma (Fig. 2). These results suggest that offspring may originate from eggs with different combinations of yolk mass, androgen concentrations and total amounts of androgens with possibly different consequences for their performance.

The main findings of this study of yolk androgens in the great tit were as follows. (1) Correlations of androgens with yolk mass were weak. (2) Comparison of Figs. 1 and 2 and AIC values of comparable models (Table S2 in the Online Resource) suggest that male traits were the best predictors of egg characteristics. Further, I conclude that large variation among females in egg yolk androgens suggests that there is a potential for adaptive offspring engineering in relation to environmental and social factors. However, it is important to acknowledge that androgens can have positive as well as negative effects on offspring (see "Introduction" section). Thus, understanding adaptiveness or otherwise of the patterns indentified in this study will require an experimental approach.

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