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Yolk androgens in the barn swallow (*Hirundo rustica*): a test of some adaptive hypotheses

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Abstract

Maternal effects such as androgen in avian eggs can mediate evolutionary responses to selection, allowing manipulation of offspring phenotype and promoting trans-generational adaptive effects. We tested the predictions of two adaptive hypotheses that have been proposed to explain female variation in yolk androgen allocation in birds, using the barn swallow *Hirundo rustica* as a model. We found no support for the first hypothesis proposing that yolk androgen varies as a function of breeding density in order to prepare offspring for different breeding densities. However, we found experimental support for the hypothesis that female yolk androgen allocation depends on mate attractiveness and that it constitutes an example of differential allocation. Females increased the concentration of androgens in their eggs when mated to males with experimentally elongated tails. Female phenotypic quality in terms of arrival date and clutch size was positively related to egg androgen concentration, consistent with the hypothesis that this is a costly investment, constrained by female condition. We found correlative evidence of a direct relationship between egg androgen concentration and performance of offspring as measured by mass increase.

Introduction

The study of maternal effects has experienced an extraordinary development in the last decade (Bernardo, 1996; Mousseau & Fox, 1998). Evolutionary biologists no longer consider maternal effects as 'troublesome sources of environmental resemblance' (Falconer & Mackay, 1996), but rather as mechanisms that can generate adaptive phenotypic plasticity at the heart of evolutionary responses to natural selection (Mousseau & Fox, 1998). One important property of maternal effects is that selection may affect parents and offspring in different ways and in different directions. This implies that one should carefully examine the costs and benefits of a given maternal effect both in parents and offspring to understand its evolutionary dynamics and adaptiveness.

A key problem to consider when tackling the adaptiveness of a maternal effect is to ascertain whether it has truly evolved as an adaptation or if it is a mere physiological constraint with little room for evolutionary modification (Heath & Blouw, 1998). Life history theory predicts that parental investment should be correlated with the expected fitness of a reproductive attempt (Stearns, 1992; Trivers, 1972). Therefore, claims about the adaptiveness of a given maternal effect can be strengthened if it can be shown that parents facultatively vary it as a function of the perceived value of that given reproductive attempt.

Since the discovery of androgens of maternal origin in avian yolk (Schwabl, 1993), numerous studies have dealt with the adaptive properties of this maternal effect (Gil, 2003). Increasing levels of androgens in the yolk positively correlate with nestling growth and begging

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(Eising *et al.*, 2001; Schwabl, 1996), muscle development at hatching (Lipar & Ketterson, 2000), dominance in juveniles and development of sexual characteristics (Schwabl, 1993; Strasser & Schwabl, 2004).

Androgen investment can increase or decrease within a clutch as a function of laying order, depending on the species (Eising *et al.*, 2001; Schwabl *et al.*, 1997) and these patterns have been related to adaptive brood reduction and fine tuning of hatching asynchrony (Eising *et al.*, 2001). However, most intraspecific variance in androgen allocation is found between females rather than within clutches (Reed & Vleck, 2001), raising the question of how this variation is related to fitness.

Two different adaptive patterns have been proposed to account for variation between females in yolk androgen. First, Schwabl (1997) found that testosterone (T) levels in the eggs of house sparrows *Passer domesticus* increased with increasing breeding density. He interpreted these data as an example of a trans-generational maternal effect by which mothers could influence the phenotype of their offspring to prepare them for the environmental conditions that they will encounter (Fox & Mousseau, 1998). Second, females have been found to increase their androgen investment in a clutch when paired with attractive males (Gil *et al.*, 1999,2004a; Tanvez *et al.*, 2004). Such differential allocation is to be expected if the value of a given reproductive attempt is predicted by mate attractiveness (Burley, 1988) and if androgen allocation is costly (Gil *et al.*, 2004b).

These two hypotheses were questioned by Petrie *et al.* (2001), who showed that androgen levels differed in peacock *Pavo cristatus* yolks depending on the sex of the offspring in an egg. They suggested that differences between females in the androgen content of their yolks were an epiphenomenon of sex ratio determination, rather than an adaptation to modify offspring fitness.

The present study was designed to test these adaptive hypotheses of allocation of yolk androgen in the wild, using as a model the barn swallow (*Hirundo rustica*), a species where sexual selection has been extensively studied (Møller, 1994c). We experimentally manipulated male attractiveness and we investigated female response to this manipulation in terms of androgen yolk investment. Further, in a large sample, we measured the correlative relationship between yolk androgen and colony size, female quality, offspring performance and brood sex ratio.

Material and methods

Study area and material

The study was done in spring 2000–2001 in 12 farms of the surroundings of Badajoz (south-western Spain). Farms are scattered in a landscape of open farmlands with maize, grass and cereal crops. The size of the swallow colonies differs greatly among farms, from two to twenty breeding pairs. In 2000, the study was restricted to the two largest farms and only correlative data were collected ($N = 67$ nests). In 2001, male ornament size was manipulated and correlative data were also collected ($N = 77$ nests).

General field procedures

We visited farms every other day from mid February, and captured barn swallows in mist nets as soon as possible after arrival. We marked birds with a unique combination of colour rings and breast markings with a marker pen. We measured wing, tarsus and beak length following standard methods used in previous studies of this species (Møller, 1994c). Tail length was defined as the average length of the two outermost tail feathers, which was measured by sliding a thin plastic ruler between the outermost and the second outermost feather (Møller, 1994c). Weight was recorded with a Pesola spring balance to a precision of 0.1 g. Body condition was defined as the effect of body weight when tarsus length was considered in an analysis of covariance (García-Berthou, 2001). We estimated the intensity of parasitism by chewing lice by counting the number of holes in the tail feathers (Møller, 1994b). Blood samples were taken at the time of capture by puncturing the brachial vein and collecting one or two capillary tubes per bird (75–150 μL). For the analysis of blood parasites and leukocyte counts a drop of blood was smeared on a microscope slide, air-dried, fixed in absolute methanol and stained with Giemsa.

Nests were visited every other day before laying and daily as soon as laying started. All eggs were carefully labelled and we took the first or second egg laid when the female had laid three eggs. Nests were visited again 5 days later to record clutch size and daily from day eleven of incubation to determine hatching day with a precision of one day. All nestlings were ringed, weighed and measured (tarsus and wing lengths) at the age of 13 days (hatching date = day 1). We used wing web swelling in response to an intradermal injection of a plant lectin [phytohemagglutinin (PHA)] to evaluate T-cell mediated immunity (Merino *et al.*, 1999). We measured the wing web thickness of nestlings with a thickness gauge (Mitutoyo) at the age of 13 days and we subsequently injected them with 0.2 mg of PHA (Sigma) dissolved in 0.04 mL of saline (PBS) in their left wing and with the same volume of PBS in their right wing as a control. We measured the injected wing webs again 24 h later and calculated the difference of swelling in response to PHA with respect to PBS. In 2001 we also measured tarsus length and mass of nestlings at the age of 7 days.

Experimental manipulation

We manipulated males that were paired, once nest building had begun but before females had started laying. Males were manipulated on average 13.32 (SE = 2.3) days before laying and we only included

those that were manipulated at least 3 days before laying to allow manipulations sufficient time to affect T content of eggs. We randomly assigned a total of 77 males to the three experimental conditions. Of these 16 had their tail shortened, 17 enlarged and 44 were unmanipulated. We used the same technique as in previous studies (Møller, 1994c). Briefly, we shortened tails by cutting a 20-mm piece of feather 20 mm from the base of the two longest outermost tail feathers and then gluing the apical part to the base. We elongated tails by inserting the 20-mm pieces of feathers from the shortened tails in between the two pieces cut at the same position and gluing them in a similar way. As a control, we simply handled the males without manipulating the tail. Previous studies have shown that this is equivalent to cutting and gluing the feathers without modifying their length (e.g. de Lope & Møller, 1993; Møller, 1994c). Manipulations were successful in establishing differences between the experimental groups in tail length. Means (SE) for the three groups were: shortened: 82.4 mm (1.6), control: 101.2 mm (1.2) and elongated: 122.3 mm (1.6); ($F_{2,74} = 106.9$, $P < 0.001$). Final sample size was reduced to 64 nests, since the nests of some males could not be found on time for egg sampling.

Analysis of blood smears: blood parasites and leukocytes

Each blood smear was randomly divided in two halves. One half was examined under 200× magnification in search for large extra-erythrocytic haematozoa (i.e. *Trypanosoma*) and in the other half 20 microscope fields were scanned under 400× magnification for intraerythrocytic haematozoa (i.e. *Haemoproteus*) (Merino & Potti, 1995). None of the nestling smears inspected contained blood parasites. Of the female blood smears inspected ($N = 35$), 29 were parasite free, 7 contained *Trypanosoma* and 1 *Haemoproteus*. Therefore, we expressed parasitism as a binary variable: absence vs. presence.

After scanning for blood parasites, slides were also used to estimate the total number and the proportion of different types of leukocytes under 1000× magnification with oil immersion. The proportion of different types of leukocytes was assessed on the basis of an examination of 100 leukocytes other than thrombocytes (thrombocytes normally present an irregular, aggregated distribution). The total number was quantified as the number of leukocytes per 50 fields examined. H/L ratio was estimated from the number of heterophils and lymphocytes per 100 leukocytes obtained in these counts (Merino *et al.*, 1999). All leukocyte proportions were Box-Cox transformed to obtain normal distributions (Sokal & Rohlf, 1995).

Hormone assays

Yolk concentrations of T, androstenedione (A_4) and 5α-dihydrotestosterone (DHT) were determined by radioimmunoassay (RIA) at the CEBC laboratory. Yolks were homogenized in 1 mL of distilled water by vortexing, with the aid of some glass beads. We took 100 μL from a further 10× dilution of the sample for steroid extraction, corresponding to an average of 8.0 mg (SE = 0.17). Hormone extraction followed a modification of Schwabl's (1993) method, justified because we extracted only a small fraction of the yolk (Gil *et al.*, 2004a). Extraction by this method provides results highly correlated with those using Schwabl's extraction [linear regression: $F_{1,8} = 29.1$, $P < 0.001$; $R^2 = 0.75$, slope = 0.73 (SE = 0.13)] (Gil *et al.*, 2004a). Briefly, we added 3 mL of diethyl ether to the sample, vortexing for 1 min and centrifuging for 5 min (4 °C, 2000× RPM). The ether phase was decanted after snap-freezing the tube in an alcohol bath at −30 °C and evaporated under a stream of nitrogen. The dried extract was redissolved in 1 mL of phosphate buffer. Tritiated steroids (1000 CPM) (Amersham) were added to the original samples for the calculation of extraction recoveries. Recoveries for all hormones were greater than 89%. Specific steroid antibodies were obtained from P.A.R.I.S. laboratories (France). The rest of the methods follow standard RIA techniques. The intra-assays coefficients of variation for T, A_4 and DHT were 7.0, 5.9 and 6.5%, respectively. The interassay coefficient of variation for A_4 was 6.9%. Only one assay was performed for DHT and T. The lowest detectable concentrations were 1 pg mg^{-1} for the three steroids. Cross-reactivity of A_4 antiserum at 50% binding was 0.9% for DHT, 0.3% for T and <0.1% for other steroids.

Nestling plasma T levels were determined using two ELISA kits according to manufacturer's instructions (Cayman Chemical). Plasma samples of 25 μL diluted in 25 μL of buffer were analysed with reference to a standard curve. According to the supplier, the antibody used was highly specific to T with little cross-reactivity to other steroids (<5%), except 5α-DHT (21%). The obtained values should therefore be interpreted as a relative measure of T. Due to the small quantities of plasma available we did not run duplicate samples. However, intra-assay variation calculated from plasma samples was sufficiently small to produce reliable data: CV = 8.2 ($N = 8$). Interassay CV was 21.2 ($N = 8$). We removed from the data set six samples that fell outside the standard curve (four lower than 8 pg mL^{-1}, two higher than 100 pg mL^{-1}); adding these samples did not change the results.

Yolk hormone sampling

Most species of passerines show high within-clutch repeatability of androgen concentration, with most variation being distributed among rather than within clutches, e.g. great tit *Parus major* (Tschirren *et al.*, 2003), European starling *Sturnus vulgaris* (Pilz *et al.*, 2003) and domestic canary (Gil *et al.*, 2004a). This implies that

assaying a single egg from a nest provides a reliable measure of the average concentration of the clutch. We tested this repeatability in our population by collecting several eggs at random (2–5 eggs) from seven different nests. The repeatability of A_4 was very high (0.72; $F_{18,6} = 10.01$, $P < 0.001$), indicating that variation between clutches was larger than within clutches. Therefore, in the remainder of the study we sampled only one egg per clutch. In order to remove possible laying order effects, we collected the first or second egg laid after the female had laid three eggs.

Molecular sexing

Nestlings were sexed using a molecular method (Griffiths *et al.*, 1998). Briefly, DNA was extracted using Perfect gDNA Blood Isolation kits (Eppendorf). PCR amplifications were performed in an ABI thermocycler (Gene Amp PCR System 9700). Reactions of 10 μL contained 1 μL of genomic DNA, 100 μM of each dNTP, 1.75 mM MgCl2, 0.25 U of Taq DNA polymerase (Qiagen) and 0.2 μM of each of the primers P8.3221 and P2. See Griffiths *et al.* (1998) for details of the procedure. PCR products were visualized in 2% agarose gels stained with ethidium bromide. In each gel run we included adult birds of known sex for comparison.

Statistics

We used mixed linear models (SAS Proc Mixed) for analyses involving several clutches of the same pair or several chicks from the same brood. Broods or nestlings were declared as repeated measurements in a mixed model (Littell *et al.*, 1996). We tested several covariance structures for the repeated data and chose the structure that minimized AIC values (Littell *et al.*, 1996). The effect of clutch (first vs. second) was always included in the first stages of model building, and removed if it was nonsignificant. In all models, we first allowed first-order interactions and removed them if these were not significant. In the Results, only final models with significant terms are presented. Degrees of freedom were calculated using Satterthwaite's approximation (Littell *et al.*, 1996), which takes into account within- and between-subject variance components. Residuals of all models were checked for normality and no significant deviations were found.

For the analysis of sex ratio, we first built a maximal generalized linear mixed model (GLMM) using several factors and covariates that could a priori be important in determining sex ratio. We removed each term in a stepwise fashion from the model, in increasing order of significance and we noted the increase in deviance with the previous model (Wilson & Hardy, 2002). We fitted nest identity as a random factor, although this component provided no significant variance to the model and was subsequently removed.

Results

Androgen concentration in egg yolk

Among the three androgens assayed in a subsample of 20 yolks, A_4 was the most abundant (Table 1). Concentrations of the other two androgens were highly correlated with A_4 (A_4-T: $r = 0.75$, $N = 20$, $P < 0.001$, $R^2 = 0.57$; A_4-DHT: $r = 0.66$, $N = 20$, $P < 0.001$, $R^2 = 0.41$) and, therefore, only A_4 was assayed in the remainder of the yolks. Differences between females in yolk A_4 concentration were large (mean = 20.60 pg mg^{-1}, SE = 0.58, CV = 35.7, $N = 90$). There were no differences between years in A_4 levels (2000: mean 20.63 ± 0.98 SE; 2001: mean 20.73 ± 0.88 SE; $F_{1,101} = 0.01$, $P = 0.91$).

Tail manipulation experiment

There were no differences between males assigned to the three experimental groups in arrival date ($F_{2,74} = 0.69$, $P = 0.52$), natural tail length ($F_{2,74} = 0.06$, $P = 0.92$) or date of manipulation ($F_{2,74} = 0.20$, $P = 0.85$). We tested whether tail manipulation affected the deposition of A_4 in eggs in a mixed ANCOVA model, in which we included male treatment as a main effect and clutch size as a covariate in order to control for female quality. Females paired to males with elongated tails laid eggs with greater concentrations of A_4 than females mated to males with shortened tails ($F_{2,65.9} = 5.91$, $P < 0.01$; Fig. 1). In this model clutch size also showed a significant positive relationship with A_4 [$F_{1,70.7} = 16.43$, $P < 0.001$; estimate 4.99 (1.23 SE)]. The interaction between clutch size and male treatment was not significant, confirming the additive effect of the two variables on egg A_4 concentrations. There were no significant differences between treatments in clutch size ($F_{2,50.9} = 0.88$, $P = 0.40$), egg size ($F_{2,53} = 0.07$, $P = 0.93$), number of fledglings ($F_{2,39.4} = 0.21$, $P = 0.81$) or mass of fledglings at 13 days ($F_{2,48.2} = 2.47$, $P = 0.10$).

Male covariates

In the data set based on unmanipulated birds, there was no significant relationship between yolk A_4 concentration and male tail length ($F_{1,68.1} = 0.14$, $P = 0.60$). There was no significant relationship either with other measures of male quality like body condition ($F_{1,65.3} = 2.44$,

Table 1 Results from assays of the three androgens in a subsample of 20 barn swallow yolks.

	T	DHT	A_4
Mean (SE) yolk concentration (pg mg^{-1} yolk)	7.87 (0.77)	6.89 (1.26)	23.79 (2.06)
Mean steroid in average yolk (ng $yolk^{-1}$)	3.74	3.27	11.30

A_4, androstenedione; T, testosterone; DHT, dihydrotestosterone.

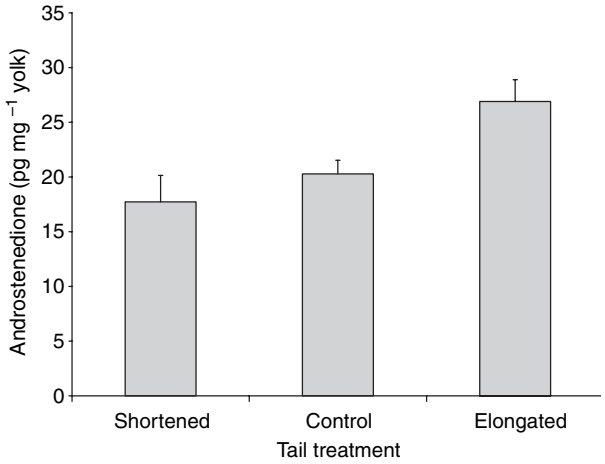

Fig. 1. Concentrations of androstenedione (A_4) (pg mg^{-1} yolk) in the first eggs in clutches of female barn swallows paired to males with shortened, control and elongated tails. Values are least-squares means (+1 SE).

$P = 0.12$) and number of holes in the tail made by chewing lice ($F_{1,70.3} = 0.87$, $P = 0.30$).

Female quality covariates

There was significant, albeit moderate, within-female repeatability between clutches in yolk A_4 concentration (repeatability = 0.22; $F_{1,63} = 1.57$, $P < 0.05$). Second clutches had significantly lower concentrations of A_4 than first clutches (first clutches: mean 22.67 ± 0.83 SE; second clutches: mean 19.17 ± 1.08 SE; $t_{62} = 2.93$, $P < 0.01$).

Clutch size was strongly positively related to yolk A_4 concentration (regression coefficient = 2.08 ± 0.67 SE; $F_{1,162} = 9.54$, $P < 0.01$; Fig. 2). Arrival date was carefully

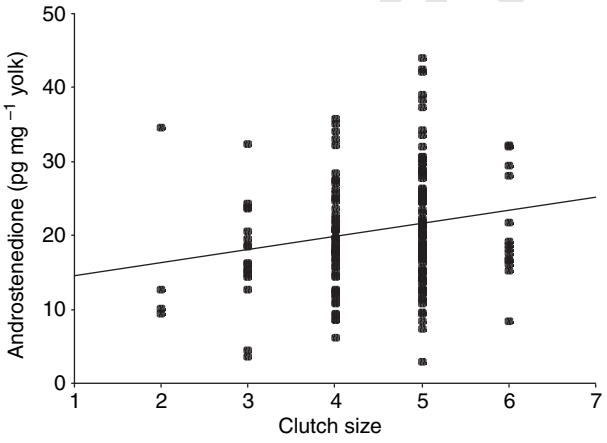

Fig. 2. Relationship between yolk androstenedione (A_4) concentration (pg mg^{-1} yolk) and clutch size.

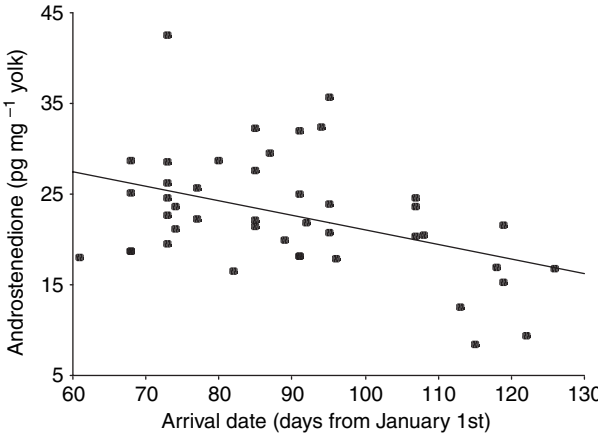

Fig. 3. Relationship between yolk androstenedione (A_4) concentration (pg mg^{-1} yolk) and female arrival date.

recorded in 2000 in a large colony. In that data set, we found that early arriving females had greater concentrations of yolk A_4 than late arriving females (regression coefficient = −0.06 ± 0.01 SE; $F_{1,48} = 13.01$, $P < 0.001$; Fig. 3). Yolk A_4 concentration was not significantly related to other measures of female quality such as body condition ($F_{1,70.9} = 2.49$, $P = 0.12$), number of holes in the tail made by chewing lice ($F_{1,75} = 0.02$, $P = 0.81$) or female age ($F_{1,37.3} = 0.08$, $P = 0.70$). None of the haematological health indices that we analysed in a subsample of females were significantly related to yolk A_4 concentration (lymphocytes: $F_{1,28} = 0.05$, $P = 0.82$; L/H ratio: $F_{1,28} = 0.20$, $P = 0.65$; presence of blood parasites: $F_{1,28} = 1.21$, $P = 0.90$).

Brood sex ratio

Hatchling sex was determined for 109 nests in total. We included in the GLMM several variables that could affect sex ratio (Table 2). We explicitly included yolk A_4 concentration and its square value to test for a nonlinear

Table 2 Summary of the generalized linear mixed model for hatchling sex ratio in 109 broods.

Explanatory term	d.f.	Change in deviance	P
Year	1	0.01	0.92
Clutch (first vs. second)	1	0.46	0.49
Hatching date	1	0.86	0.35
Male tail length	1	1.26	0.26
A_4 concentration (linear and squared terms)	1	1.97	0.16

Changes in deviance correspond to the difference between the deviance of the model after the removal of a given term and the deviance of the previous model. Terms were removed one-by-one starting by the least significant. *P* values correspond to a 1-tailed χ^2 distribution.

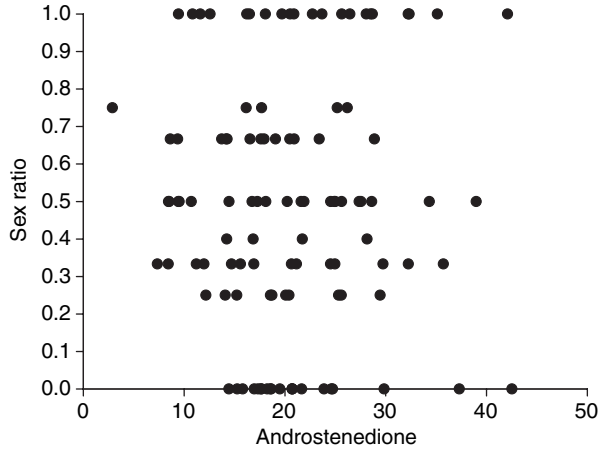

Fig. 4. Relationship between yolk androstenedione (A_4) concentration (pg mg^{-1} yolk) and clutch sex ratio.

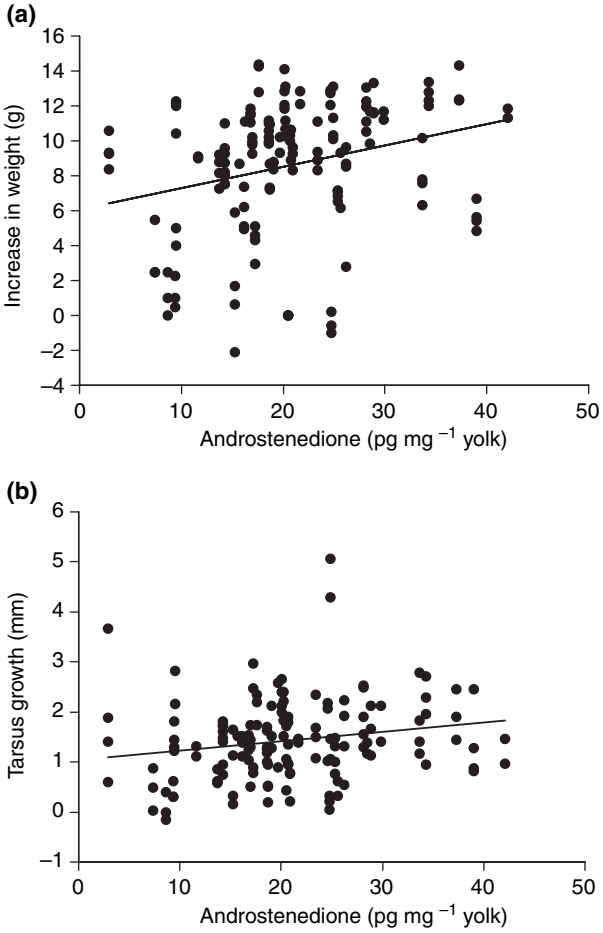

Fig. 5. Relationship between yolk androstenedione (A_4) concentration (pg mg^{-1} yolk) and nestling increase in weight (a) and tarsus length (b).

relationship with sex ratio. None of the terms was significant (Table 2), suggesting that sex ratio was unrelated to any of the proposed explanatory variables. There was no obvious relationship between yolk A_4 concentration and sex ratio (Fig. 4).

Nestling development and growth

Incubation time was positively related to clutch size ($F_{1,146} = 44.6$, $P < 0.001$) and negatively related to laying date ($F_{1,146} = 9.17$, $P < 0.01$). We included these two factors as covariates in a model to test for the relationship with first yolk A_4 concentration. There was no significant relationship between yolk A_4 and incubation time ($F_{1,146} = 0.33$, $P = 0.73$). However, as our measure of incubation time was days rather than hours, this test may be too coarse-grained to detect subtle differences.

All measurements taken in nestlings showed significant within-clutch repeatabilities, ranging from 0.27 to 0.97 (all $P < 0.001$). In the pooled data set, we found no relationship between first yolk A_4 concentration and wing length, tarsus length or mass at 13 days of age (wing length: $F_{1,102} = 1.45$, $P = 0.22$; tarsus length: $F_{1,106} = 1.56$, $P = 0.21$; mass: $F_{1,105} = 0.55$, $P = 0.46$). However, there was a weak positive although nonsignificant relationship between first yolk A_4 concentration and T-cell mediated immune response ($F_{1,86.4} = 3.67$, $P = 0.057$). For the 2001 data set we also measured chicks at 7 days of age, so we could use the differences between ages 7 and 13 as a measure of growth. There was a significant positive relationship of first yolk A_4 concentration with mass increase ($F_{1,43.4} = 8.58$, $P < 0.001$; Fig. 5a) and a nonsignificant tendency with tarsus length increase ($F_{1,46.9} = 3.47$, $P = 0.067$; Fig. 5b). We tested for a possible quadratic relationship in these analyses, but quadratic terms were nonsignificant and not retained in the final model. We also tested whether clutch size, as a measure of female quality, explained variance in growth, but its contribution was nonsignificant and was therefore dropped from the models.

No parasites were found in any of the examined blood smears of nestlings. Yolk A_4 concentration was not significantly related to number of lymphocytes ($F_{1,51.3} = 0.53$, $P = 0.46$), or L/H ratio ($F_{1,51.4} = 0.91$, $P = 0.34$). Blood T levels in nestlings were low (mean = 28.40 pg mL^{-1}, SE = 1.83, CV = 77, $N = 140$). There were no significant differences between male and female nestlings ($F_{1,107} = 0.39$, $P = 0.53$), and T levels were not significantly related to clutch size ($F_{1,38.7} = 0.14$, $P = 0.72$). There was a negative relationship between laying date and blood T levels ($F_{1,42.9} = 7.02$, $P < 0.05$; Fig. 6). T concentration in nestlings was not significantly related to T-cell mediated immune response ($F_{1,128} = 2.24$, $P = 0.12$). Finally, yolk A_4 concentration was not significantly

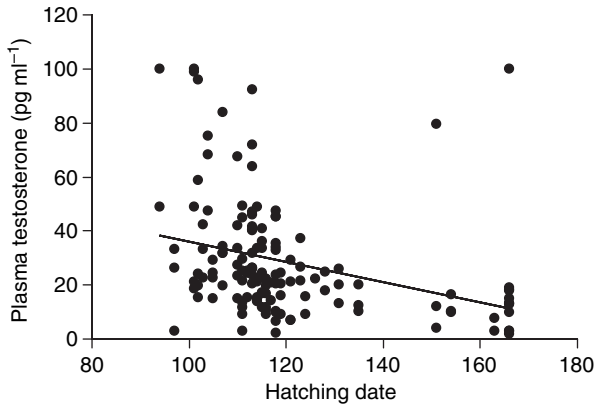

Fig. 6. Relationship between plasma testosterone (T) concentration (pg mL^{-1}) and nestling hatching date.

related to nestling blood T levels ($F_{1,38.9} = 2.58$, $P = 0.11$).

If yolk A_4 concentration increases nestling competition within clutches we might expect an increase in within-brood variance in fitness, and even greater mortality. There was no significant relationship between yolk A_4 concentration and within-brood variance in tarsus length ($F_{1,105} = 0.75$, $P = 0.85$) and mass at 13 days ($F_{1,106} = 1.23$, $P = 0.27$). Neither was there a significant relationship between fledging success for those nests where at least one chick fledged and yolk A_4 concentration ($F_{1,111} = 0.31$, $P = 0.57$).

Colony size

Colonies varied greatly in size, ranging from 2 to 24 breeding pairs (mean = 11.9, SE = 2.0, $N = 12$). There were no significant differences between colonies in average yolk A_4 levels ($F_{11,101} = 0.76$, $P = 0.67$). Average yolk A_4 levels per colony were not significantly related to the number of breeding pairs in the colony ($F_{1,105} = 0.12$, $P = 0.73$; Fig. 7).

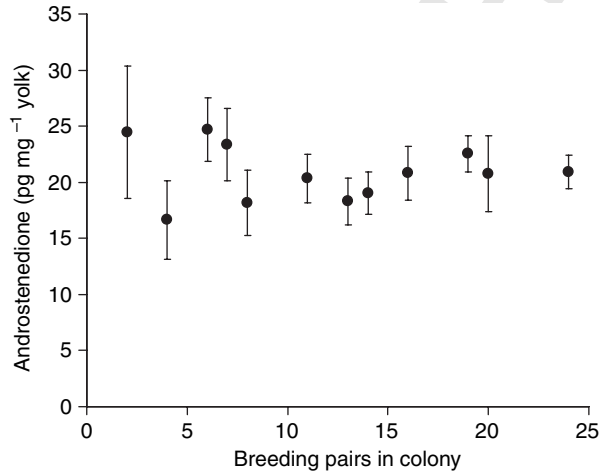

Fig. 7. Differences between colonies in the concentration of androstenedione (A_4) (pg mg^{-1} yolk) in relation to colony size. Values are means (SE).

Discussion

As in previous studies of other species (Eising *et al.*, 2001; Reed & Vleck, 2001), we found large differences between females in yolk androgen concentration (CV = 35.7). Manipulation of male ornament size resulted in a correlated response by females in yolk A_4 concentration. Females paired to males with elongated tails laid eggs with higher A_4 concentrations than those paired to males with shortened tails. This is remarkable since males do not provide their mates with courtship food, not do they defend feeding territories, thereby eliminating any direct way in which males could affect the condition of their mates. This pattern confirms previous results in two different species in captivity (Gil *et al.*, 1999, 2004a; Tanvez *et al.*, 2004), in which females were found to invest higher levels of androgens in the eggs fathered by more attractive males. As androgen in yolks has been shown to boost chick growth and development (Schwabl, 1996; Lipar & Ketterson, 2000; Eising *et al.*, 2001; Strasser & Schwabl, 2004), this finding suggests that females preferentially invest in the offspring of attractive males. An alternative explanation could be that females use yolk androgen levels to manipulate feeding rates by their male partners, or to compensate for the low feeding effort of attractive males (de Lope & Møller, 1993) with faster growth.

The differential allocation hypothesis predicts that a larger investment in the offspring of attractive males should be compensated by the fitness benefits accrued from mating with such males (good-genes benefits) (Burley, 1988). In our study species, it has been previously shown that offspring sired by highly ornamented males have enhanced viability (Møller, 1994a), so the higher investment in the eggs fathered by these males would be selected for.

In the correlative data set, several measures of nestling growth speed were related to yolk androgen concentration after controlling for parental attractiveness. This suggests that nestlings that hatched from eggs with larger androgen concentrations had their development boosted by these hormones. Although these results are correlational and thus potentially caused by a third variable, we tried to control for this possibility by including clutch size in our analysis as a likely surrogate of female parental quality. However, the positive relationship between growth and androgen concentration was maintained, in line with predictions.

For differential deposition of androgen in yolks to qualify as differential allocation, it has to be shown that it is a costly investment for females (Sheldon, 2000; Gil

et al., 2004b). In line with this prediction we found that a positive correlation between androgen concentration in eggs and female quality as measured by arrival date and clutch size. Arrival date in spring from the African winter quarters is a likely surrogate of individual quality (Møller, 1994b; Møller *et al.*, 2004).

Estimates of good genes effects in birds may be biased due to differential investment of hormones by females (Gil *et al.*, 1999). Although the present results strengthen the generality of this caveat (see also Calsbeek & Sinervo, 2002), in our correlative data set unmanipulated male ornament size was not significantly related to female yolk androgen allocation. This suggests that the confounding effect of this variable may not severely affect current estimates of good genes in this species (Møller, 1994a). The disagreement between the results of the experiment and correlative analysis suggests that the correlation analysis did not control for all confounding variables.

Differences between clutches in average androgen concentration may be an epiphenomenon of sex ratio determination (Petrie *et al.*, 2001). Since we did not obtain yolk samples of all eggs, our data do not allow us to test the hypothesis that within-clutch differences in sex ratio are related to egg androgen levels, as suggested by Petrie *et al*. However, a corollary of this hypothesis would predict a positive relationship between average concentration of yolk androgen in a clutch and sex ratio. No such a relationship was found despite the large sample size examined. Although this finding does not allow us to test the hypothesis of a relationship between embryo sex and yolk androgen levels within a clutch, it does suggest however that differential allocation of egg androgen (as measured between-clutches) is not related to brood sex ratio.

Between-brood differences in nestling blood T levels at 13 days of age were unrelated to differences in egg androgen. There are few studies of nestling androgen levels in passerines, and little is known about their role at this stage (Schlinger & Arnold, 1992; Silverin & Sharp, 1996), although a recent study has found a relationship between nestling T levels and levels of sibling competition in zebra finches *Taenyopygia guttata* (Naguib *et al.*, 2004). Since T levels may vary considerably over the nestling period (Silverin & Sharp, 1996), a relationship between yolk and nestling plasma androgen might more likely be detected if blood samples are taken shortly after hatching.

We expected a positive correlation between colony size and androgen levels following previous results in several passerine species (Schwabl, 1997; Whittingham & Schwabl, 2002; Pilz & Smith, 2004). It has been proposed (Schwabl, 1997) that this effect may prepare nestlings for between-colony differences in breeding density and competition. However, we found no differences in yolk androgen concentration between colonies and no correlation with number of nests in the colony. This hypothesis assumes that sons will live in colonies of similar size as that where they were raised; an assumption that has not been tested in the house sparrow. However, there is evidence for such an effect in birds (Brown & Brown, 2000), including the barn swallow (Møller, 2002). Also, androgen levels decreased with increasing arrival data and we would expect the reverse pattern if social environment was determinant for egg androgen levels, since colony size increases as birds arrive to the breeding grounds.

To recapitulate, we tested the predictions of two adaptive hypotheses that explain between-female variation in yolk androgen allocation in birds. We found no support for the first hypothesis that proposed a link between breeding density and yolk androgen deposition (Schwabl, 1997), after failing to find differences in egg androgen across a large range of different colony sizes. This speaks against a general relationship between breeding density and female androgen concentration. However, we found support for the hypothesis that female yolk androgen allocation depends on mate attractiveness and that it constitutes an example of differential allocation (Burley, 1988). First, our experimental manipulation of male ornament size in the wild resulted in a direct response of yolk androgen investment by females, strengthening previous results obtained in two other species in the lab (Gil *et al.*, 1999, 2004a; Tanvez *et al.*, 2004). Second, female quality in terms of arrival date and body condition was positively related to egg androgen concentration, suggesting that this is a costly investment, constrained by female condition (see also: Gil *et al.*, 2004b). And third, there was a direct relationship between egg androgen concentration and performance of offspring as measured by weight gain.

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