Avian sex allocation and ornamental coloration
Korsten, Peter

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

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Citation for published version (APA):

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Rapid changes in maternal yolk hormone deposition in response to manipulated male attractiveness

Sjouke Anne Kingma, Jan Komdeur, Oscar Vedder, Nikolaus von Engelhardt, Peter Korsten & Ton G. G. Groothuis
ABSTRACT

Avian eggs contain substantial amounts of androgens of maternal origin that can have profound effects on chick development. Experiments on captive birds have shown that females deposit higher concentrations of androgens in the yolk of their eggs when mated with an attractive male. However, studies in wild bird populations have yielded less clear results with contradictory findings between correlational and experimental studies. We conducted a similar study in a wild bird population, using a correlational and experimental approach in the same individuals, and we applied a within-female design to control for possible between-female variation that could have confounded the results in previous field studies. We manipulated the sexually selected UV coloration of the blue crown feathers of male blue tits (Parus caeruleus) on the day their female had laid the second egg of the clutch, and subsequently measured the effect on androgen concentrations (testosterone and androstenedione) in the fifth, seventh, and ninth egg. We also measured androgen levels of the second egg that was laid before application of the treatment, both as a baseline measure for each clutch and to investigate the correlation between yolk androgen levels and natural male crown coloration. The concentration of testosterone, but not of androstenedione, in eggs laid after the manipulation was significantly higher in control (attractive) than in UV-reduced (unattractive) pairs. The effect diminished over the egg sequence, coinciding with the recovery of crown UV coloration after manipulation. This suggests that females are capable of very rapid adjustments of yolk testosterone levels in response to changes in an ornamental plumage character of their mate. However, we could not detect any correlation between the androgen concentration in the second egg and pre-treatment male crown coloration. We discuss this discrepancy and we suggest that the results of experimental studies on the relationship between yolk androgen deposition and male attractiveness should be treated with caution.
INTRODUCTION

Maternal effects may represent adaptive transgenerational phenotypic plasticity allowing organisms to optimally adjust offspring phenotype to the environment they are encountering (Mousseau & Fox 1998). Over the last decade, maternally derived hormones in the yolk of avian eggs – in particular androgens – have started to attract much attention as potential mediators of such adaptive maternal effects. Avian mothers deposit variable amounts of androgens in the yolk of their eggs that can have important effects on offspring development (reviewed in Groothuis et al. 2005a). These effects include an increase in begging behaviour, suppression of immune function, and both positive and negative effects on growth and survival (e.g. Schwabl 1996; Sockman & Schwabl 2000; Eising et al. 2001, 2003; Pilz et al. 2003; Groothuis et al. 2005b; Müller et al. 2005).

Although most studies have concentrated on explaining within-clutch variation in yolk androgen concentrations, which may be related to female strategies of brood reduction or compensation of hatching asynchrony, between-clutch variation is often even larger (Groothuis et al. 2005a; Reed & Vleck, 2001). One of the factors that have been indicated to induce this variation is the sexual attractiveness of a female’s mate (Gil et al. 1999). Several Lab-based studies have demonstrated that females increase the deposition of androgens to the yolk of their eggs when paired with a more attractive male (see Table 5.1). In captive zebra finches (Taeniopygia guttata), females paired with males wearing ‘attractive’ red leg rings deposited higher concentrations of yolk androgens, than females with males wearing ‘unattractive’ green rings (Gil et al. 1999; but see Rutstein et al. 2004a). Captive zebra finch females also deposited higher levels of yolk androgens when paired to a mate that was found to be preferred in a preceding mate choice trial (von Engelhardt 2004). Furthermore, captive female canaries (Serinus canaria) deposited higher levels of androgens in their eggs when exposed to more attractive male song (Gil et al. 2004; Tanvez et al. 2004). The increase in female androgen deposition in response to male attractiveness found in these studies has been interpreted as a form of increased investment in offspring of which the mother expects higher fitness returns (e.g. Gil et al. 1999; 2004), as predicted by the differential allocation hypothesis (Burley 1988, Sheldon 2000). However, this interpretation assumes that enhanced hormone allocation to eggs is costly for the mother, for which no convincing evidence exists to date. Moreover, this view ignores the fact that increased yolk androgen levels can also have negative effects on the offspring, such as reduced immune function (Groothuis et al. 2005b) and survival (Sockman & Schwabl 2000). An alternative hypothesis is that females use yolk androgens to manipulate their male’s feeding rate to the offspring. For example, if a female anticipates her male to feed her offspring at a low rate, she may increase the male’s feeding rate through a stimulation of offspring begging, which could be induced by increased yolk androgen deposition (Navara et al. 2006). Positive relationships between yolk androgen deposition and...
Table 5.1 Overview of studies on the effect of mate attractiveness on yolk androgen deposition, including which androgens were investigated (testosterone [T], androstendione [A4] and dihydrotestosterone [DHT]) and the relationship found. It is indicated whether studies were correlational (Corr) or experimental (Exp), and whether studies were conducted in wild or captive birds.

<table>
<thead>
<tr>
<th>Species</th>
<th>Male characteristic</th>
<th>Effect</th>
<th>Androgens</th>
<th>Captive/Wild</th>
<th>Corr/Exp</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canary Serinus canaria</td>
<td>Song quality</td>
<td>Positive</td>
<td>T</td>
<td>Captive</td>
<td>Exp</td>
<td>Gil et al. 2004</td>
</tr>
<tr>
<td>Canary</td>
<td>Song quality</td>
<td>Positive</td>
<td>T, DHT (total)</td>
<td>Captive</td>
<td>Exp</td>
<td>Tanvez et al. 2004</td>
</tr>
<tr>
<td>Zebra finch Taeniopygia guttata</td>
<td>Ring colour</td>
<td>Positive</td>
<td>T</td>
<td>Captive</td>
<td>Exp</td>
<td>Gil et al. 1999</td>
</tr>
<tr>
<td>Zebra finch</td>
<td>Ring colour</td>
<td>Zero</td>
<td>T</td>
<td>Captive</td>
<td>Exp</td>
<td>Rutstein et al. 2004a</td>
</tr>
<tr>
<td>Zebra finch</td>
<td>Mate preference</td>
<td>Positive</td>
<td>total androgen level</td>
<td>Captive</td>
<td>Exp</td>
<td>v. Engelhardt et al. 2004</td>
</tr>
<tr>
<td>Barn swallow Hirundo rustica</td>
<td>Tail length</td>
<td>Positive</td>
<td>A4</td>
<td>Wild</td>
<td>Exp</td>
<td>Gil et al. 2006</td>
</tr>
<tr>
<td>Barn swallow</td>
<td>Tail length</td>
<td>Zero</td>
<td>A4</td>
<td>Wild</td>
<td>Exp</td>
<td>Saino et al. 2006</td>
</tr>
<tr>
<td>Collared flycatcher Ficedula albicollis</td>
<td>Forehead patch</td>
<td>Zero</td>
<td>T</td>
<td>Wild</td>
<td>Corr</td>
<td>Michl et al. 2005</td>
</tr>
<tr>
<td>House finch Carpodacus mexicanus</td>
<td>Plumage coloration</td>
<td>Negative</td>
<td>T, A4, DHT (total)</td>
<td>Wild</td>
<td>Corr</td>
<td>Navara et al. 2006</td>
</tr>
<tr>
<td>House sparrow Passer domesticus</td>
<td>Testosterone level</td>
<td>Zero</td>
<td>T</td>
<td>Wild</td>
<td>Exp</td>
<td>Mazuc et al. 2003</td>
</tr>
</tbody>
</table>
male attractiveness are then to be expected, because in some species attractive males tend to feed less (Groothuis et al. 2005a).

In wild bird populations, however, the overall direction of the relationship between yolk androgen levels and male attractiveness appears less straightforward (Table 5.1). Experimentally manipulated length of the sexually-selected tail streamers of barn swallows had a positive effect on maternal androgen deposition in one population (Hirundo rustica; Gil et al. 2006), but not in another (Saino et al. 2006). In a study on wild house sparrows (Passer domesticus) no effect of male testosterone implantation (which was assumed to increase male attractiveness) was found on yolk androgen levels (Mazuc et al. 2003). A correlational study on collared flycatchers (Ficedula albicollis) also found no correlation between yolk androgen levels and male attractiveness (size of the forehead patch; Michl et al. 2005). Finally, in a population of house finches (Capodacus mexicanus) a negative correlation between yolk androgen levels and male ornamental plumage was found (Navara et al. 2006). Since in this species unattractive males show lower paternal feeding rates, this result would be consistent with the hypothesis that avian mothers increase paternal feeding rate via androgen deposition in the egg if they anticipate a low male feeding rate.

The greater inconsistency in the results of field studies compared to lab studies may be caused by the fact that in addition to the single tested aspect of male attractiveness several other factors affect yolk androgen levels in the field. This may also explain why correlational studies, taking only one aspect of male quality into account, do not yield clear results. Furthermore, differences between studies could be due to differences in timing of the experimental treatments relative to egg laying. Therefore we conducted a field study which had the following aims: 1) analysing the effect of an experimental manipulation of mate attractiveness on egg androgen concentrations using a within-female design, which controlled for the confounding effect of between-female variation; 2) comparing correlational and experimental data in the same population; and 3) investigating on what time scale females adjust their hormone deposition in response to male quality.

The blue tit (Parus caeruleus) is an excellent model species to experimentally test the effect of male attractiveness on patterns of female yolk androgen deposition. Blue tits have sexually selected UV-reflecting crown feathers (Andersson et al. 1998; Hunt et al. 1998; Delhey et al. 2003) and the UV coloration of the crown plumage plays a role in female mate choice (Andersson et al. 1998; Delhey et al. 2003; Hunt et al. 1998) and may serve as male viability indicator (Griffith et al. 2003; Sheldon et al. 1999; but see Delhey & Kempenaers 2006). It has recently been found that female blue tits decrease their reproductive investment (in terms of nestling food provisioning) in response to experimentally reduced crown UV reflectance of males and consequently these females fledge significantly smaller chicks (Limbourg et al. 2004). In addition, yolk androgens have been suggested to be involved in avian primary sex ratio control (Petrie et al. 2001; but see Pilz et al. 2005) and female blue
tits were found to modify the primary sex ratio in response to manipulation of male crown UV reflectance (Sheldon et al. 1999, Korsten et al. 2006).

We manipulated UV coloration of the crown feathers of male blue tits and subsequently measured the effect on yolk androgens in their females’ eggs. UV reflectance of males was first measured and thereafter manipulated on the day their female laid the second egg. The UV-reduction treatment was non-permanent, and UV reflectance is known to recover within days after treatment (Limbourg et al. 2004; Chapter 3). Therefore we collected the fifth, seventh, and ninth laid eggs, enabling us to test whether and at what time scale females adjusted yolk androgen levels to changes in male attractiveness. We also measured androgen levels of the second egg, which was laid before application of the treatment, in order to obtain a baseline measurement for the two treatment groups. Finally, we correlated androgen levels of the second unmanipulated egg to pre-manipulation crown coloration of males.

METHODS

Study area, bird handling and sample sizes
The experiment was carried out in the breeding season of 2005 (7 April–5 June) in a population of blue tits breeding in nestboxes at the ‘Vosbergen’ estate (ca. 50 ha; 53°08’ N, 06°35’ E), near Groningen, The Netherlands. This population has been intensively studied since 2001. The study area consists of patches of mixed deciduous and coniferous forest interspersed by patches of open grassland. Nestboxes were checked daily for presence of the first-laid egg.

Males (n = 36) were caught in front of occupied nestboxes on the day the second egg had been laid, using a mistnet and a decoy (a mounted male blue tit) with song playback. Males were subsequently transported in a dark bird bag to the nearby field station, where their age, body mass (to the nearest 0.1 g using a 30 gram spring balance), tarsus (to the nearest 0.1 mm using sliding callipers) and natural crown reflectance were measured. We determined age (1 year or >1 year) based on the colour of the primary coverts following Svensson (1992). Thereafter, we manipulated the males’ crown UV coloration (see below). Birds were released in their own territory after treatment.

We caught the females of experimental pairs (n = 30; three females were not caught) in their nestboxes during chick feeding (6–10 days after hatching) using a spring trap, and we determined their age and measured their body mass, tarsus length and crown reflectance following the protocol described above. During the same period we also captured the males of all occupied nestboxes in the study area. Three males included in our experiment were also caught at another nestbox, indicating these males to be polygynous. All other males in the experiment were re-captured in the same territory as where they were initially caught, indicating that in all
territories we manipulated the resident male. The three polygynous males were excluded from further analyses, yielding a final sample size of 33 experimental clutches (16 controls and 17 UV-reduced). Of these, we could calculate the correlations between yolk androgen levels and natural male crown colour for 32 broods as the measurement of natural crown reflectance before manipulation failed in one male.

**Crown reflectance measurements**

Before the manipulation of the crown UV reflectance, the spectral reflectance of the crown feathers was measured with an USB-2000 spectrophotometer with illumination by a DH-2000 deuterium-halogen light source (both Avantes, Eerbeek, The Netherlands). The measuring probe was held at a right angle against the plumage, i.e. both illumination and recording were at 90° to the feathers. During each crown reflectance measurement we took 5 replicate readings of the same spot and smoothed each of these reflectance spectra by calculating the running mean over 10 nm intervals. Following previous studies of UV colour signalling in blue tits (Andersson et al. 1998; Sheldon et al. 1999; Griffith et al. 2003; Delhey et al. 2003; Korsten et al. 2006) we calculated three indices describing the variation in crown coloration – ‘brightness’, ‘hue’, and ‘UV chroma’ – from each reflectance spectrum, and averaged these across the 5 replicate spectra. ‘Brightness’ was the sum of reflectance between 320–700 nm (R<sub>320-700</sub>), which corresponds to the spectral range visible to blue tits (Hart et al. 2000). ‘Hue’ was the wavelength of maximum reflectance (R<sub>max</sub>). ‘UV chroma’ was the sum of reflectance between 320–400 nm divided by the sum of reflectance between 320–700 nm (R<sub>320-400</sub> / R<sub>320-700</sub>). Both the ‘hue’ and ‘UV chroma’ indices have previously been identified as important predictors of male attractiveness and viability in blue tits (Andersson et al. 1998; Sheldon et al. 1999; Delhey et al. 2003; Griffith et al. 2003).

Males were captured within a relatively short period (10–21 April) leading to little variation in crown feather wear (Örnborg et al. 2002; Delhey et al. 2006), and consequently crown coloration was not significantly related to the date of capture (brightness: r = −0.253, P = 0.16; hue: r = 0.250, P = 0.17; UV chroma: r = −0.319, P = 0.08; all n = 32).

**Crown UV manipulation**

UV reflectance was reduced with a mixture of duck preen gland fat and UV blocking chemicals (50% Parsol 1789 and 50% Parsol MCX [by volume]; Roche, Basel, Switzerland) as used successfully in previous studies of wild blue tits (e.g.; Sheldon et al. 1999; Limbourg et al. 2004; Korsten et al. 2006). Control males were treated with the duck preen gland fat only. This treatment was smeared on the crown feathers and to measure its effect, three replicate crown reflectance measures were taken directly after the manipulation following the protocol described above. Males were assigned sequentially to either the UV-reduced or control treatment.
**Egg collection**

Nestboxes were visited daily and newly laid eggs were marked with non-toxic markers until the last egg was laid and clutch size was determined. We collected eggs 2, 5, 7 and 9 from each brood, on the day they were laid. Collected eggs were replaced with plastic dummy eggs. Collected eggs were incubated for 72 hours in an incubator at 35°C to induce embryonic development for DNA extraction to be used for molecular sexing. However, for unknown reasons incubation failed and eggs contained no embryos, so that eggs could not be sexed. After incubation, eggs were stored at –20°C until androgen analyses were conducted.

**Androgen quantification**

Androgens (testosterone [T] and androstenedione [A4]) were measured by radioimmunoassay (RIA) after extracting them from the yolk with ether and on celite columns (Wingfield & Farner 1975; Schwabl 1993). The whole yolk was removed from the eggs when still frozen and weighed to the nearest 0.001 gram using an analytical balance. A weighed amount of yolk (150–300 mg) was homogenized in 200 µl of distilled water by vigorous mixing on a vortex facilitated by the addition of a few glass beads. A known amount of radioactive T and A4 (ca. 2000 counts per minute) was added to a weighed subsample (150–280 mg) of the homogenate to assess extraction efficiency, and samples were kept for 1 h at 37°C for equilibration. Batch 1 of samples was extracted three times with 3 ml of petroluemether: diethylether, 30:70 (vol:vol), batch 2 and 3 were three times extracted with 3 ml of diethylether (both methods extract T and A4 from yolk and yielded similar recoveries, which were on average 56% en 50% for T and A4 respectively). The three ether fractions were decanted from the snap-frozen egg yolk/water phase, combined, and dried under a stream of nitrogen. The dried extract was re-dissolved in 1 ml of 90% ethanol, stored overnight at –20°C and then centrifuged. The supernatant was dried under nitrogen, re-dissolved in 1 ml 2% ethylacetate in isooctane and transferred to diatomaceous earth chromatographic columns (Kieselgur, pro-analyisi, Merck). Steroids were eluted with 4 ml of pure isoctane (discarded), 4.0 ml of 2% ethylacetate in isoctane and transferred to diatomaceous earth chromatographic columns (Kieselgur, pro-analyisi, Merck). Steroids were eluted with 4 ml of pure isoctane (discarded), 4.0 ml of 2% ethylacetate in isoctane (eluate containing A4), 4.5 ml of 10% ethylacetate in isoctane (discarded) and 4.5 ml of 20% ethylacetate in isoctane (eluate containing T). The eluates were dried and re-dissolved in 200 µl of Tris-Buffer. T and A4 levels were measured in duplicates of 50 µl of sample using DSL (Diagnostic System Laboratories, USA) radioimmunoassay kits.

**Statistical analyses**

To test the effect of UV treatment on yolk testosterone levels, we used a multilevel model that included a random effect for female identity to account for the non-independence of the eggs produced by a single female. We calculated the relative change of T and A4 levels after the treatment was applied by dividing the levels of egg 5, 7 and 9 by the androgen levels of egg 2, the baseline level for each clutch before
treatment. We also tested the effect of the interaction of UV treatment and laying sequence on the relative change of yolk androgen concentration, to take into account a potentially diminishing treatment effect due to recovery of the UV reflectance with time after application of the UV reduction treatment. Significance was assessed using the increase in deviance ($\Delta$ deviance, which follows a $\chi^2$ distribution) when a parameter was removed from the model.

Since egg 2 was not affected by male UV manipulation, we related androgen levels of egg 2 to natural male crown coloration as measured before manipulation to investigate the natural correlation between yolk androgen levels and male crown coloration. To account for possible confounding factors we additionally carried out two separate multiple regression analyses using a stepwise backward selection procedure to explain the variation in T and A4. In the first class of models, we entered the three male crown colour indices (brightness, hue, UV chroma) together with male age, body mass and tarsus length as predictors of either T or A4 levels ($n = 32$). In the second class of models, we entered the three male crown colour indices as well as female characteristics – female crown colour indices (brightness, hue, UV chroma), age, body mass, tarsus length and lay date – as predictors of either T or A4 levels ($n = 29$). We chose not to run a single analysis including all male and female predictor variables at the same time, to avoid the risk of over-parameterisation of our models given the limited sample size. For the same reason we did not include interaction effects in the models. Analyses were conducted using MLwiN 2.02 for multilevel models and SPSS 12.0 for all other statistical tests.

**RESULTS**

Date of capture, body size (mass and tarsus), and crown coloration before manipulation did not differ between UV-reduced and control-treated males (Table 5.2). The UV-reduction treatment caused a large decrease in the UV reflectance of the crown plumage directly after manipulation (Figure 5.1). All three indices of crown coloration were significantly different between the two treatment groups directly after manipulation (Table 5.3). Clutch size did not differ between UV-reduced and control-treated pairs (Mean ± SE, UV-reduced: $11.8 \pm 0.15$, control treatment: $12.1 \pm 0.25; \ t = 0.826, \ df = 31, \ P = 0.42$).

T and A4 levels of eggs were correlated ($r = 0.378, P < 0.001, n = 138$). There were significant negative effects of UV treatment on the change of yolk T concentrations ($\Delta$ deviance = 4.11, $df = 1, P = 0.043$) and also of the position in the laying sequence (egg number 7–9) ($\Delta$ deviance = 4.32, $df = 1, P = 0.038$), as well as a significant interaction effect of UV treatment x position in laying sequence ($\Delta$ deviance = 4.04, $df = 1, P = 0.045$; Figure 5.2). The data indicate that after treatment yolk testosterone levels were initially higher in the control group than in the UV-reduced group, which difference subsequently decreased over the laying sequence (Figure 81).
Table 5.2 Pre-treatment characteristics of UV-reduced and control-treated male blue tits.

<table>
<thead>
<tr>
<th></th>
<th>UV-reduced (n = 17)</th>
<th>Control (n = 16)</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SE</td>
<td>Mean  SE</td>
<td>t</td>
</tr>
<tr>
<td>Capture date (April days)</td>
<td>16.35  0.69</td>
<td>16.25  0.81</td>
<td>0.10</td>
</tr>
<tr>
<td>Tarsus length (mm)</td>
<td>16.99  0.08</td>
<td>17.03  0.12</td>
<td>0.33</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>11.28  0.13</td>
<td>11.08  0.14</td>
<td>1.04</td>
</tr>
<tr>
<td>Brightness1</td>
<td>75.86  3.38</td>
<td>79.06  2.82</td>
<td>0.72</td>
</tr>
<tr>
<td>Hue (nm)1</td>
<td>386.2  2.12</td>
<td>387.4  2.59</td>
<td>0.35</td>
</tr>
<tr>
<td>UV Chroma1</td>
<td>0.29   0.004</td>
<td>0.29   0.005</td>
<td>0.20</td>
</tr>
</tbody>
</table>

1 Pre-treatment crown coloration was measured for 15 males only in the control-treated group.

Figure 5.1 Mean reflectance spectra (± SE at 20 nm intervals) of crown plumage of male blue tits before treatment, and after UV-reduction or control treatment (the pre-treatment measurement of one control-male failed).

Table 5.3 Indices of male crown coloration after UV-reduced and control treatment.

<table>
<thead>
<tr>
<th></th>
<th>UV-reduced (n = 17)</th>
<th>Control (n = 16)</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SE</td>
<td>Mean  SE</td>
<td>t</td>
</tr>
<tr>
<td>Brightness</td>
<td>65.07  2.82</td>
<td>85.27  2.46</td>
<td>5.371</td>
</tr>
<tr>
<td>Hue (nm)</td>
<td>416.2  0.67</td>
<td>363.7  3.58</td>
<td>0.002</td>
</tr>
<tr>
<td>UV Chroma</td>
<td>0.18   0.004</td>
<td>0.29   0.004</td>
<td>19.601</td>
</tr>
</tbody>
</table>

1 t-test
2 Mann-Whitney U test
Relative yolk A₄ concentrations did not differ with respect to treatment (Δ deviance = 0.002, \( P = 0.97 \)) or laying sequence (Δ deviance = 0.58, \( P = 0.45 \)). Also the interaction between treatment \( \times \) laying sequence was not significant (Δ deviance = 2.28, \( P = 0.13 \)).

Baseline concentrations of T and A₄ measured in the second egg did not correlate with natural male crown reflectance before manipulation (T, brightness: \( r = 0.103, n = 32, P = 0.58 \); hue: \( r = -0.036, n = 32, P = 0.84 \); UV chroma: \( r = 0.140, n = 32, P = 0.44 \); A₄, brightness: \( r = 0.164, n = 32, P = 0.40 \); hue: \( r = -0.079, n = 32, P = 0.69 \); UV chroma: \( r = 0.108, n = 32, P = 0.58 \)). Likewise, none of the multiple regression analyses in which we used male and/or female characteristics in addition to male crown colour indices to explain the variation in either yolk T or A₄ concentrations in the second egg yielded a significant model (all \( P \) values > 0.05).

**DISCUSSION**

Maternal androgens in avian eggs represent an intriguing example of hormone-mediated maternal effects. One of the most frequently cited factors that may explain variation in androgen concentrations among clutches is male attractiveness. However, especially results of field studies are inconsistent (Table 5.1). The inconsistency among field studies may be due to a greater influence of confounding factors in the field compared to the controlled laboratory situation. Another reason for the inconsistency could be the differences between studies in the timing of experimental treatment or the measurement of attractiveness relative to egg laying. All studies until to date used a between-female experimental design. We used a more
sensitive within-female design, in which we manipulated male attractiveness after the second egg was laid, comparing the hormone concentrations of subsequently laid eggs with the second egg. We found that wild female blue tits quickly changed the deposition of testosterone, but not androstenedione, in the yolk of their eggs in response to manipulation of male crown UV coloration, a sexually selected trait.

Relative to the second egg, concentrations of testosterone were significantly lower in the subsequent eggs in clutches of UV-reduced – unattractive – males compared to males that received a control treatment. This effect diminished over the laying sequence, and had disappeared in the ninth egg. Possibly, the diminishing treatment effect was due to a rapid female response to recovery of male crown coloration after a few days in UV-reduced males (Chapter 3). To test this idea, one would need to compare the yolk testosterone pattern in clutches of singly UV-reduced males to males in which the UV reduction treatment is re-applied after some days. In any case, the fact that we observed a treatment effect from the fifth egg onwards demonstrates that female blue tits can adjust the level of androgen deposition in their eggs very rapidly in response to external stimuli.

There are several hypotheses that predict adjustment of female yolk androgen deposition to the characteristics of her mate. According to the differential allocation hypothesis females are expected to invest more in a current reproductive event at the cost of future reproduction when paired with an attractive or high-quality male (Sheldon 2000). In this scenario increased yolk androgen deposition by females in response to the mate’s attractiveness could be viewed as greater maternal investment in the offspring (e.g. Gil et al. 1999, 2004, 2006). According to this idea maternal yolk androgens are viewed as a limited resource for the offspring. Indeed, increased maternal yolk hormones can have several beneficial effects on the offspring, including increased nestling growth (Groothuis et al. 2005a). But yolk androgens may also have negative effects on the offspring, such as reduction of immune function (Groothuis et al. 2005b). Moreover, it remains unclear whether the deposition of yolk androgens is costly to the female herself (Groothuis et al. 2005a).

Alternatively, yolk androgens may not act as limited resources, but as signals to the offspring, which fine-tune the balance of different trade-offs in the offspring (e.g. investing in growth versus immune function; Groothuis et al. 2005b), thereby maximising the fitness of the offspring depending on the prevailing circumstances. Females may, for example, change the yolk hormone deposition to adjust offspring begging behaviour to the parental care they expect from their male (Navara et al. 2006). If for example male food provisioning is correlated with his attractiveness or some other characteristic (either positively or negatively), then females could anticipate, and extract maximum parental investment from her male by influencing offspring growth and begging through adjustment of maternal hormones in the eggs. Consistent with this idea, female house finches (Carpodacus mexicanus) deposit higher levels of androgens in their eggs when paired to unattractive males, which
show reduced nestling feeding. Whether one of these adaptive explanations applies to our results in the blue tit remains an open question. Females decreased yolk testosterone deposition in response to an experimental reduction of male attractiveness (crown UV coloration), which seems consistent with the differential allocation hypothesis, but it remains to be demonstrated that such increased androgen levels benefit the offspring and are costly to produce for the female.

Although we found a significant effect of the male UV manipulation on yolk testosterone levels, there was no significant correlation between androgen levels in the second, base-line egg, and male pre-treatment coloration. The inclusion of additional male characteristics (age, body mass, tarsus) as explanatory variables in a multiple regression analysis, which accounted for the possibility that female androgen deposition was dependent on multiple male cues, did not reveal any significant effect either. Nor did the inclusion of female characteristics (age, body mass, tarsus, laying date). The UV reduction caused by our treatment is large and even reduces crown UV reflectance below the natural range (Chapter 3). Possibly, in the natural situation blue tit females only modify yolk androgen deposition if the UV reflectance of the crown plumage of their male shows a sudden and dramatic decrease, for example caused by damage due to disease or severe fighting. The magnitude of the manipulations of the male sexual signals applied in the studies listed in Table 5.1 was probably also large. It should be carefully considered to what extent the results of such studies indicate adaptive maternal androgen deposition in response to male attractiveness.

ACKNOWLEDGEMENTS

We thank the ‘Kraus-Groeneveld’ foundation for permission to work at estate ‘De Vosbergen’. Maarten Bleeker, Sandra Bouwhuis, Oege Dijk, Thomas Dijkstra, Gerlind Hoogcarspel, Marije Oostindjer, István Szentirmai, Marlien de Voogd and Linda Wester assisted in the fieldwork. Maarten Lasthuizen and Bonnie de Vries helped in the lab with the hormonal essays. This research was financially supported by the Netherlands Organisation for Scientific Research (NWO; ALW grant 810.67.022 to JK). Our experimental procedures were approved by the Animal Experimental Committee (DEC) of the University of Groningen.