

Ectoparasite-modulated deposition of maternal androgens in great tit eggs

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Maternal yolk androgens can promote growth and competitive abilities of nestling birds but are also suggested to increase susceptibility to parasites or suppress immune function. We tested the hypothesis that females exposed to ectoparasites during egg formation will adjust the content of androgens in the yolk. We predicted that when anticipating high levels of parasitism, females deposit (i) less androgens into all eggs of their clutch and (ii) smaller amounts of androgens in eggs late in the laying sequence to facilitate brood reduction.

In a field experiment we exposed female great tits (*Parus major*) to hen fleas (*Ceratophyllus gallinae*), or kept them free of ectoparasites prior to egg laying. We collected the eggs and measured yolk concentrations of androstenedione (A4), testosterone (T) and 5 α -dihydrotestosterone (DHT) by radioimmunoassay. Among clutches, eggs of ectoparasite-exposed females contained significantly less A4 and tended to contain less T, whereas DHT content was unaffected. Within clutches, content of A4 and T increased significantly with laying order whereas DHT content significantly decreased. These patterns were unaffected by ectoparasites.

In summary, our results provide no evidence for hormone-based facilitation of brood reduction under ectoparasite exposure but support the hypothesis that females exposed to ectoparasites reduce levels of T and its precursor A4 in yolk and might thereby reduce the negative effects of parasites on offspring.

Keywords: *Ceratophyllus gallinae*; host–parasite interactions; maternal effects; maternal hormones; parental investment; *Parus major*

1. INTRODUCTION

Non-genetic maternal resources are essential for the modification of the offspring's phenotype, but they are also a costly parental investment (Mousseau & Fox 1998). Females are thus expected to invest maternal resources adaptively in their young to optimize the offspring's development and quality. In birds, maternally derived androgenic hormones in the yolk can have profound effects on embryo development and nestling behaviour. In canaries (*Serinus canaria*), for example, high concentrations of yolk testosterone (T) promote postnatal growth and competitive abilities and influence an individual's social status after fledging (Schwabl 1993, 1996a). Among species, the quantity of maternal androgens in the yolks varies largely within and among clutches. Within-clutch variation in androgen levels may either mitigate the effects of a size hierarchy and thus competitive asynchrony among siblings caused by asynchronous hatching (increasing androgen levels with laying order; e.g. Eising *et al.* 2001; Pilz *et al.* 2003) or enhance these effects to facilitate brood reduction when food is limited (decreasing androgen levels with laying order; reviewed in Schwabl *et al.* 1997). Among-clutch variation, however, is little understood. As high levels of maternal androgens generally are beneficial for the offspring in terms of higher growth rates or increased competitive abilities (Schwabl 1993, 1996a; Lipar & Ketterson 2000; Eising *et al.* 2001; but see

Sockman & Schwabl 2000), among-clutch variation may result from physiological limitations of the female in allocating high levels of androgens to the eggs. Alternatively, differential allocation of maternal androgens might reflect an adaptive strategy of the female (Mousseau & Fox 1998). In an experiment on zebra finches (Gil *et al.* 1999), females were mated to attractive and unattractive males, respectively, and deposited significantly more androgens in the eggs when mated with the attractive male, suggesting that the reproductive value of the offspring influences the allocation of maternal androgens.

Parasites are strong evolutionary forces as they often reduce the host's fitness (reviewed in Clayton & Moore 1997). Natural selection thus favours physiological, behavioural or immunological host responses that reduce the negative impact of parasites. Yolk androgens do not only promote growth or competitive ability, but can also suppress the immune system and thus increase an individual's susceptibility to infection and parasites as shown in the common lizard (Uller & Olson 2003; see Martin (2000) for modulation of immune function by prenatal steroid exposure in mammals). Females might thus trade off the costs and benefits of deposition of high levels of maternal androgens to their offspring according to environmental conditions and adaptively allocate less androgen in the presence of parasites to promote the development and efficiency of the offspring's immune system.

In addition to this hypothesized effect on among-clutch allocation of maternal androgens, parasites might also modulate the within-clutch deposition of steroids.

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Parasites often increase the nutritional demand of their hosts and thus raise parental investment when offspring are parasitized (Tripet & Richner 1997b). When parental investment exceeds the reproductive value of the nestlings or the nutritional demand of the brood is higher than food abundance, a brood reduction strategy of the parents rearing a parasitized brood might be beneficial (Lack 1947). One might thus predict lower levels of maternal androgens in eggs laid late in the laying sequence, a pattern that serves to augment effects of asynchronous hatching within a parasitized brood and thus facilitate brood reduction (Schwabl *et al.* 1997). Parasite-modulated deposition of less maternal androgens to eggs laid late in the laying order might then reflect an adaptive strategy to adjust parental investment to reproductive value of the offspring.

We tested the two predictions that (i) females deposit less androgens into all their eggs when high levels of parasitism are anticipated and, under parasite exposure (ii) females deposit relatively lower levels of androgens in the late-laid eggs of their clutch to facilitate brood reduction.

We tested these predictions in an experimental field study on great tits (*Parus major*). Great tits are small, hole-nesting passerine birds and one of the main hosts of the ectoparasitic hen flea (*Ceratophyllus gallinae*; Tripet & Richner 1997a, 1999). Hen fleas hibernate in the old nesting material or immigrate into the new nests in early spring. They are nest-based parasites that suck blood from their host and produce two overlapping flea generations between the host's egg-laying period and fledging of the young birds. Hen fleas have been shown to decrease host fitness by reducing both offspring quality and survival (Richner *et al.* 1993). We experimentally exposed female great tits to hen fleas, or kept them free of ectoparasites prior to egg laying. We collected eggs and analysed yolk concentrations of the T precursor androstenedione (A4), T and its metabolite 5 α -dihydrotestosterone (DHT) by radioimmunoassay.

2. MATERIAL AND METHODS

(a) Parasite treatment and egg collection

The study was performed in 2002 in a great tit population breeding in nest-boxes in the Forst, a forest near Bern, Switzerland (46°54' N 7°17' E/46°57' N 7°21' E). The nest-boxes were cleaned during winter to remove old nesting material and hibernating parasites, and were regularly visited from the beginning of the breeding season onwards to determine the start of nest building. After construction of the nest cup (7.7 \pm 0.6 days before the female laid the first egg), nests were heat-treated in a microwave oven (Richner *et al.* 1993) to kill nest-based parasites that immigrated into the boxes during nest building. The nests were then randomly assigned to be infested with 25 female and 15 male hen fleas or to remain free of parasites. The hen fleas that were used for the infestation were extracted from old nests collected in the study area at the start of the breeding season. After parasite treatment, nests were visited daily to determine the start of egg laying. We removed the first, third, sixth and then every other egg plus the last egg of the laying sequence on the day that they were laid and replaced them with an artificial egg (e.g. in a clutch of 10 eggs we replaced egg numbers 1, 3, 6, 8 and 10). Eggs from 27 parasitized and 27 parasite-free nests were collected. In six parasite-infested and six parasite-free nests all eggs were replaced. The eggs were taken into the

laboratory and the yolks were separated from the albumen and frozen at -20 °C until hormone analysis.

(b) Steroid hormone assay

Yolks were thawed and homogenized with an equal (1 μ l per mg of yolk) amount of distilled water. Aliquots of this yolk-water emulsion (250 mg) were used for the hormone analysis. The extraction and following radioimmunoassays were according to published methods and protocols (Schwabl 1993).

Samples were randomly distributed with respect to parasite treatment across five assays and all eggs from a clutch were analysed in the same assay. Recoveries were 60.8 \pm 5.6% for A4, 55.0 \pm 3.6% for T and 24.3 \pm 4.5% for DHT. Interassay coefficient of variation was 15.6% for A4, 9.5% for T and 29.9% for DHT. Yolk contents of 17 β -oestradiol and corticosterone were analysed in a subsample of the eggs. The contents of these yolk hormones were very low or undetectable and thus not further analysed in this study.

(c) Statistical analyses

Statistical analyses based on steroid content per yolk (ng of steroid per yolk) and concentration of steroids (pg steroid per mg yolk) gave similar results. Yolk mass (mean: 353.5 \pm 2.0 mg, range: 222–468 mg) was significantly different between clutches ($F_{52,245} = 5.994$, $p < 0.0001$), but not different between parasite-infested and parasite-free nests ($F_{1,52} = 0.072$, $p = 0.790$). There was also no significant change of yolk mass with laying order ($F_{1,245} = 0.420$, $p = 0.518$). Thus, only the results of steroid content are presented.

Right skew in the distribution of yolk androgen contents led to non-normal distribution of the residuals of the statistical model. We therefore used log-transformed data of steroid content to allow for parametric testing. We used nested ANCOVA to analyse effects of ectoparasites on the within- and among-clutch contents of maternal androgens in yolk. Parasite treatment was included in the model as a factor (fixed effect) and female (random effect) as a nested factor, nested within parasite treatment. Laying order, the quadratic term of laying order and clutch size were covariates. Non-significant interactions were backward eliminated. All tests were two-tailed with a significance level set at $p = 0.05$. Residuals of the model were tested for normality and homoscedasticity (Sokal & Rohlf 1981). Means \pm 1 s.e. are given. Statistical analyses were performed using JMP IN v. 4.0 (Sall & Lehmann 1996).

3. RESULTS

(a) Among-clutch allocation of maternal androgens

Hormone content in yolks differed significantly among females for A4 ($F_{52,240} = 9.814$, $p < 0.0001$, mean: 18.51 \pm 0.60 ng per yolk, range: 2.73–60.75 ng per yolk; concentration: 52.75 \pm 1.77 pg per mg of yolk), T ($F_{52,240} = 8.908$, $p < 0.0001$, mean: 8.87 \pm 0.21 ng per yolk, range: 1.66–26.36 ng per yolk; concentration: 25.33 \pm 0.63 pg per mg of yolk) and DHT ($F_{52,240} = 13.498$, $p < 0.0001$, mean: 8.82 \pm 0.15 ng per yolk, range: 3.57–19.89 ng per yolk; concentration: 25.04 \pm 0.42 pg per mg of yolk). The yolks of larger clutches contained significantly more A4 ($F_{1,52} = 5.204$, $p = 0.027$) and T ($F_{1,52} = 4.182$, $p = 0.046$) but tended to contain less DHT ($F_{1,52} = 3.167$, $p = 0.081$) than yolks of small clutches. Neither clutch size (parasite-infested:

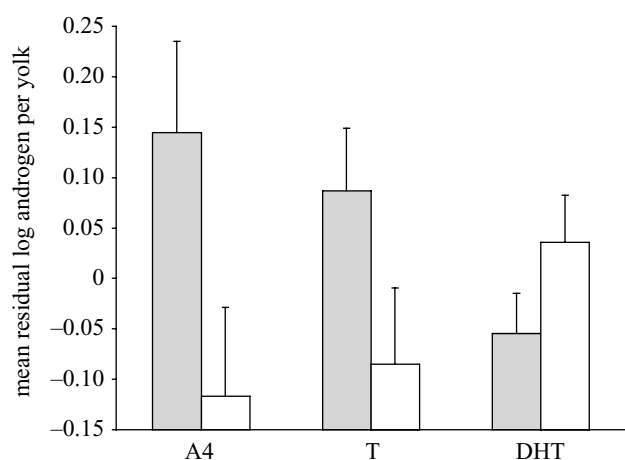


Figure 1. Mean residual content of A4, T and DHT (+ 1 s.e.) of eggs laid by females that were not exposed ($n = 27$ clutches) or exposed ($n = 27$ clutches) to nest-based ectoparasites prior to egg laying. The y -axis reflects residuals of the statistical model (see § 2) without including the parasite treatment as a factor. Filled bars, no parasites; open bars, parasites.

9.4 ± 0.1 eggs, parasite-free nests: 9.0 ± 0.1 eggs, $F_{1,52} = 2.095$, $p = 0.154$) nor laying date ($F_{1,52} = 0.936$, $p = 0.338$) was significantly different between parasite-infested and parasite-free females. Ectoparasites significantly influenced the deposition of A4 into the eggs ($F_{1,52} = 4.387$, $p = 0.041$). Females that were exposed to parasites prior to egg laying deposited significantly less A4 into the yolks than females laying their eggs in a parasite-free nest (figure 1). A similar trend was found for T ($F_{1,52} = 3.459$, $p = 0.069$; figure 1). No significant difference in yolk DHT content was found in eggs laid by infested and parasite-free females, respectively ($F_{1,52} = 1.986$, $p = 0.165$; figure 1). There was no significant interaction between clutch size and parasite treatment (A4: $F_{1,239} = 1.263$, $p = 0.262$; T: $F_{1,239} = 1.116$, $p = 0.292$; DHT: $F_{1,239} = 0.038$, $p = 0.845$).

(b) Within-clutch allocation of maternal androgens

The yolk contents of A4 ($F_{1,240} = 83.925$, $p < 0.0001$) and T ($F_{1,240} = 45.858$, $p < 0.0001$) significantly increased with laying order (figure 2), whereas DHT content significantly decreased ($F_{1,240} = 35.495$, $p < 0.0001$; figure 2). The within-nest allocation of androgens followed a quadratic rather than a linear relationship with laying order as indicated by a significant quadratic term of laying order in A4 ($F_{1,240} = 10.471$, $p = 0.001$), T ($F_{1,240} = 15.017$, $p = 0.0001$) and DHT ($F_{1,240} = 6.563$, $p = 0.011$). The increase of androgen content between the first and the tenth egg was +73.4% for A4, +39.5% for T and -25.3% for DHT (figure 2).

We did not find a significant interaction between parasite treatment and laying order or the quadratic term of laying order in the yolk contents of A4 (parasite treatment \times laying order: $F_{1,238} = 1.200$, $p = 0.274$, parasite treatment \times laying order²: $F_{1,238} = 0.054$, $p = 0.817$), T (parasite treatment \times laying order: $F_{1,238} = 1.223$, $p = 0.270$, parasite treatment \times laying order²: $F_{1,238} = 0.077$, $p = 0.782$) or DHT (parasite treatment \times laying order: $F_{1,238} = 0.286$, $p = 0.594$, parasite treatment \times laying

order²: $F_{1,238} = 2.182$, $p = 0.141$), indicating that females did not alter the deposition of androgens with laying order depending on parasite abundance.

4. DISCUSSION

Our study demonstrates that ectoparasites can modulate the deposition of maternal androgens into avian eggs. Females that were exposed to hen fleas prior to egg laying deposited significantly less A4 and tended to deposit less T into their eggs than females that were not exposed to parasites during egg formation. There was no difference in yolk DHT content between eggs of exposed and unexposed females. However, recoveries of DHT were very low compared to A4 and T, and the intersassay coefficient of variation was high. This higher variability of the DHT assay might reduce the validity of the results found for this androgen.

Hen fleas are nest-based ectoparasites that suck blood from nestlings and reduce nestling growth and survival (Richner *et al.* 1993; Oppliger *et al.* 1994). The development of an efficient immune system might be of crucial importance for nestlings growing up in parasite-infested nests, because immunological host responses can provide the hosts with some protection from the parasite (Allen 1994). Although it is not known if in passerines physiological concentrations of androgens in the yolk jeopardize or reduce the development of immune functions or increase the susceptibility to parasites, there is evidence for this in galliforms. In chicken, *in ovo* administration of androgens inhibited the development of the bursa of Fabricius, an immunological organ in birds, and reduced antibody production (e.g. Glick & Sadler 1961; Schuurs *et al.* 1992). The high doses of injected androgens often used in poultry studies may not be comparable to the effect of yolk androgens on immune function under natural situations. However, immune function was also reduced when chickens were exposed to physiological levels of T shortly after hatching (Gause & Marsh 1986). Further, high levels of androgens during embryonic development resulted in higher postnatal susceptibility to parasites in common lizards (*Lacerta vivipara*) (Uller & Olson 2003). In our study, females reduced levels of androgens in the eggs in the presence of hen fleas. This adjustment might promote the development of the offspring's immune system (Martin 2000) and lower the negative impact of parasites on the offspring after hatching. Two previous studies demonstrated maternal effects that reduce negative impact of hen fleas on great tit nestlings (Heeb *et al.* 1998; Buechler *et al.* 2002). Hen fleas inflicted less negative effects on nestling body mass when their mother was exposed to ectoparasites prior to egg laying. Maternally transferred antibodies, deposited in higher concentrations in eggs when exposed to ectoparasites (Buechler *et al.* 2002), could explain this finding. Our results offer a non-exclusive alternative explanation, differential allocation of maternal androgens, which may directly benefit the nestlings by facilitating development of the immune system or indirectly by promoting the production, deposition or functional properties of maternal antibodies that are transferred into the eggs. Alternatively, androgen and immunoglobulin production may partly require the same resources and thus create an investment trade-off that

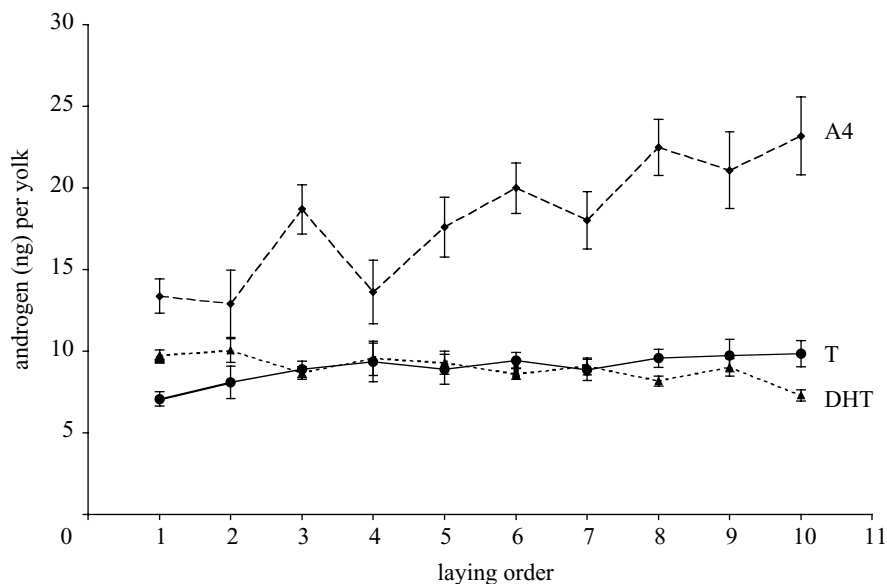


Figure 2. Mean yolk content (± 1 s.e.) of A4 (diamonds), T (circles) and DHT (triangles) in relation to laying order of the eggs ($n = 54$ clutches). Data of parasite-infested and parasite-free nests were pooled because the within-clutch allocation of androgens was not influenced by the parasite treatment (see § 3).

forces females of infested nests to allocate more immunoglobulins to the eggs at the expense of androgens.

Life-history theory predicts a trade-off between current and future reproduction for iteroparous species (Roff 1992; Stearns 1992). We therefore expect adjustment of parental investment to the reproductive value of offspring (e.g. Schaffer 1974; Møller 1997). Differential allocation of maternal androgens depending on the reproductive value of offspring was suggested for zebra finches because females allocated more androgens to their eggs when mated to an attractive male (Gil *et al.* 1999). Thus, adjustment of parental investment seems to include maternal steroid hormones in addition to direct parental care or allocation of nutritional resources (e.g. increased egg size, Price 1998). The lower levels of androgens in the presence of ectoparasites could therefore be a strategic female decision to conserve energy or metabolites for future reproductive events, as reproductive value of the current, parasitized brood is low.

Steroid hormone concentrations in the yolk might reflect levels in female circulation. This assumption is based on a study in canaries showing a positive correlation of concentrations of yolk and circulating androgen in the female (excreted in faeces) during egg formation (Schwabl 1996b). However, Mazuc *et al.* (2003) report a negative correlation between female and yolk T levels, and Verboven *et al.* (2003) report opposite effects of food supplementation on yolk and plasma T concentrations.

Under the assumption that enhanced production of yolk androgens at the same time increases levels of circulating androgens, we expect effects on the female's own physiology, for example an interference with her immune functions (Duffy *et al.* 2000). Although the regulatory roles of androgens in female physiology are not well understood, androgens probably have costs, including immunological, for the female (Ketterson *et al.* 1991). If so, females exposed to parasites might reduce androgen production to promote their own immune defence. Alternatively, lower

levels of androgens in the eggs of parasite-exposed females might not be adaptive but represent a physiological constraint. During the egg laying period, female great tits often roost in their nest-box (B. Tschirren, personal observations) and hen fleas might directly impair their condition or health. Females exposed to hen fleas might thus be unable to produce and allocate high concentrations of androgens owing to the direct negative effect of the ectoparasite on the female.

Within clutches, A4 and T significantly increased whereas DHT significantly decreased following a quadratic relationship with laying order. This pattern might reflect a strategy of the female to compensate for or mitigate effects of a moderate hatching asynchrony of up to 5 days between hatching of the first and last nestling (Glutz von Blotzheim & Bauer 1993) by allocating more A4 and T to eggs in the middle and at the end of the laying sequence. In the presence of blood-sucking hen fleas, the nutritional demand of a brood is increased (Christe *et al.* 1996; Triplet & Richner 1997b) and facilitation of brood reduction might be beneficial. Relatively less androgen in eggs laid late in the laying sequence would thus be expected in clutches infested with parasites. Our data do not support this hypothesis as parasites did not significantly influence the within-clutch allocation of androgens.

The opposite allocation patterns of A4 and T compared with DHT with laying order and in relation to parasite treatment show that measurement of T alone might not fully reflect maternal hormonal investment. Measurements of different maternal hormones should thus be considered in studies investigating differential maternal hormone allocation to their offspring. In summary, our study demonstrates that ectoparasites modulate the among-clutch but not the within-clutch content of maternal yolk androgens in a passerine bird. This suggests either direct benefits of lower androgen levels to the growing nestlings or indirect benefits of reduced androgen production to the female when exposed to ectoparasites.

Further studies will have to address the proximate mechanisms and fitness consequences of maternal androgens in eggs in relation to parasites as selection factors.

The authors thank V. Saladin, B. Holzer and M. Suter for help in the field. They also thank the Endocrinology Group of the Kinderklinik, Inselspital Bern, for preliminary analyses and technical advice, and two anonymous reviewers for providing valuable criticism on the manuscript. The experiment was financially supported by the Swiss National Science Foundation (grant no. 31-53956.98 to H.R.) and conducted under a licence provided by the Ethical Committee of the Office of Agriculture of the Canton of Bern, Switzerland.

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