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Biol. Lett. 2005 **1**, 78-81
doi: 10.1098/rsbl.2004.0233

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Balancing between costs and benefits of maternal hormone deposition in avian eggs

Ton G. G. Groothuis*, Corine M. Eising, Cor Dijkstra and Wendt Müller

Department of Animal Behaviour, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

*Author for correspondence (t.groothuis@biol.rug.nl)

Avian eggs contain substantial amounts of maternal androgens, and several studies have indicated that these are beneficial for the chick. Nevertheless, there is a large and systematic variation in maternal hormone concentrations both within and between clutches. If maternal androgens also involve costs, this might explain why not all mothers put high levels of androgens in their clutches. However, the simultaneous occurrence of both benefits and costs has not yet been convincingly demonstrated. We show experimentally that yolk androgens suppress immune function and simultaneously stimulate growth in black-headed gull chicks. Thus, mothers face a trade-off between these costs and benefits and may tune hormone deposition to prevailing conditions that influence chick survival.

Keywords: maternal hormones; androgens; testosterone; maternal effects; immune function; growth

1. INTRODUCTION

Embryonic exposure to steroid hormones can have important developmental effects. In vertebrates, the embryo is not only exposed to its own hormones, but also to those of its mother (Clark & Galef 1995). In birds, mothers deposit considerable amounts of maternal androgens in their eggs and the adaptive significance of this deposition currently receives considerable attention. In many bird species androgen levels systematically increase or decrease over the laying sequence of the clutch, which has been interpreted, respectively, as mitigating and enhancing the effect of hatching asynchrony on survival of the last-hatched chicks. This would enable the mother to adjust her brood size to the prevailing food conditions (Schwabl 1993; Schwabl *et al.* 1997; Groothuis & Schwabl 2002). Systematic variation in maternal androgens among clutches has also been found and several adaptive explanations have been proposed for this. Higher clutch levels correlate with greater social density (Reed & Vleck 2001), perhaps preparing the chick for a higher level of competition in this area. They correlate with mate attractiveness, perhaps reflecting higher maternal investment in the offspring

of good quality fathers (Gil *et al.* 1999), and with the rearing capacities of the mother (Müller *et al.* 2002; Verboven *et al.* 2003).

All these hypotheses assume (reviewed in Groothuis *et al.* 2005) that enhanced exposure to maternal androgens is beneficial for the chick. Indeed, experimental evidence indicates that yolk androgens stimulate begging behaviour (Schwabl 1993; Eising & Groothuis 2003), and the growth of the neck muscle (Lipar & Ketterson 2000) involved in begging, and so both may be causally involved in the finding that such chicks obtain a greater share of food (Eising & Groothuis 2003) and faster growth (Schwabl 1993; Eising *et al.* 2001; Pilz *et al.* 2004).

These benefits should lead to both high levels of maternal androgens in all clutches and pronounced within-clutch variation in yolk androgens in species with hatching asynchrony. However, the considerable and systematic variation in yolk androgen levels both between and within clutches of a species suggests that the elevation of these hormone levels are constrained either by direct costs for the mother or for the chick, and that these costs are outweighed by benefits in certain situations only. Such context-dependent costs might also explain why the experimental elevation of yolk androgens can be detrimental for the chick in some cases (Sockman & Schwabl 2000), and beneficial in others (see above; see also Gil *et al.* 1999). However, until now the cause of such detrimental effects has not yet been convincingly demonstrated. The suppression of immune function by androgens, an important topic in the framework of sexual selection (Duffy *et al.* 2000; Peters 2000), might be one of these costs.

We therefore studied the relationship between yolk androgen levels and early immune function in the black-headed gull, a species in which beneficial effects of maternal androgens on hatching time, begging and growth have been demonstrated (Eising *et al.* 2001; Eising & Groothuis 2003). Yolk androgen levels increase considerably from the first to the third and last egg (Eising *et al.* 2001; Groothuis & Schwabl 2002; figure 1*a*), while in newly hatched chicks the T-cell-mediated immune response to a standard challenge substantially decreases over the laying sequence (Müller *et al.* 2003; figure 1*b*). This suggests that elevated levels of maternal androgens in the egg suppress early T-cell-mediated immune function. Early immune function is especially important in colonial breeding species (Tella 2002) such as gulls, where early chick mortality is likely to be influenced by infectious diseases (Harjo & Rudbäck 1999). Furthermore, T-cell-mediated immune defence is of great importance for young birds since the chick's humoral immune system does not yet function during the first weeks after hatching (Apanius 1998). Indeed, T-cell-mediated immunity (CMI) correlates positively with early survival in our species (Müller *et al.* 2003).

However, the decrease in immune response with hatching order may be caused by egg components other than maternal androgens that also change with laying order, such as carotenoids that influence immune function (Blount *et al.* 2002; Saino *et al.*

2003). Therefore, we experimentally manipulated yolk androgen levels to test the effect on the CMI of the young chick.

2. MATERIAL AND METHODS

(a) Design and treatment

Laying and hatching order were determined in nests of two colonies along the north coast of The Netherlands by at least daily checks of nests. Egg injections of either 50 μ l oil or a combination of 7.5 μ g androstenedione and 0.09 μ g testosterone dissolved in oil were carried out on the day of clutch completion (for details see Eising *et al.* (2001)). We used only first-laid eggs containing relatively low levels of androgens. The dose of hormones was similar to the upper range of the difference in yolk content of both androgens between first and third eggs. Oil- and androgen-eggs were matched for egg mass and estimated time to hatching at the pipping stage. These pairs of eggs were then swapped with eggs of other nests in approximately the same stage. CMI (14 pairs) and tarsus growth (8 pairs) were measured in the first week after hatching.

(b) Immunological challenge

We challenged the chick's cellular immune system by injection of 0.04 ml of 1 mg ml⁻¹ phytohaemagglutinin-P (PHA, Sigma), dissolved in phosphate buffered saline (PBS). PHA is a mitogen that produces a local swelling due to prominent perivascular accumulation of T-lymphocytes and macrophage infiltration. This challenge technique is widely used to test the CMI in birds (Lochmiller & Deerenberg 2000; Norris & Evans 2000). We injected the solution in the ball of the left foot between two and four days after hatching (no effect of age on CMI). Chicks of one nest were injected at the same age. Just before (initial measurement) and 24 h after injection (final measurement) we measured the height of the ball twice or thrice with a calliper to the nearest 0.05 mm (see Müller *et al.* 2003). The response, being the difference between the mean initial and the mean final measurement, was used as the CMI.

3. RESULTS

(a) Immune response

Chicks from androgen-injected eggs had a lower CMI than their age-matched controls from oil-injected eggs in the same nest (figure 2; Wilcoxon matched pairs comparison, $T=81$, $n=14$, $p=0.010$). This indicates that prenatal exposure to yolk androgens within the physiological range suppresses immune function early after hatching. The average CMI in oil-eggs was 0.67, almost the same as in first-laid eggs of natural clutches (figure 1*b*), indicating that our measurements were reliable since both groups of eggs were first-laid eggs with unmanipulated hormone levels. The CMI in androgen-eggs was 0.09, less than in last-laid eggs of natural clutches (figure 1*b*). This is consistent with the fact that the dose of androgens injected was at the upper range of natural levels in last-laid eggs.

(b) Growth

Tarsus growth over the period one day prior and after the challenge was stronger in chicks from androgen-eggs than from oil-eggs (mean and s.e.m. in millimetres: 1.68 ± 0.13 , respectively, 1.20 ± 0.22 ; paired t -test: $t_{1,7} = -2.531$, $p=0.041$).

4. DISCUSSION

Exposure to enhanced levels of yolk androgens within the physiological range suppressed immune function and increased growth. The latter is consistent with results of an earlier study in which we found enhanced growth of both tarsus and body mass in gull chicks

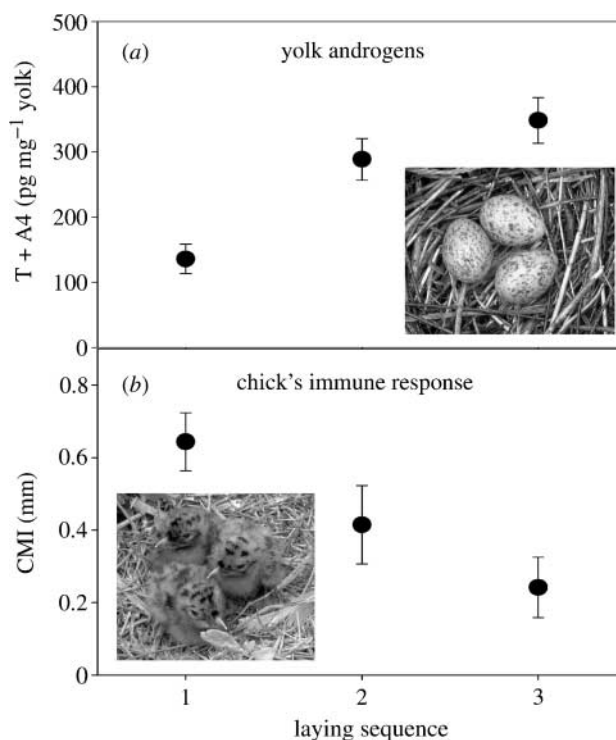


Figure 1. (a) Increase in androgen concentration (mean and s.e.m.) in relation to laying order in 10 clutches of black-headed gulls (repeated ANOVA, $F_{2,18}=21.119$, $p<0.001$; data from Groothuis & Schwabl 2002). (b) Decrease in T-cell-mediated immune response (mean and s.e.m.) for chicks from first, second and third eggs in 24 clutches (repeated measures ANOVA, $F_{2,46}=5.692$, $p=0.006$; data from Müller *et al.* 2003).

from androgen-treated eggs (Eising *et al.* 2001). Nevertheless, the data suggested that the treatment suppressed immune function in fast growing birds. Increased growth might be especially important for last-hatched chicks (that hatch from eggs with elevated levels of androgens) to catch up with their older siblings.

Counterbalancing this beneficial effect, the lower immune response constitutes a cost for the chick as it relates to lower survival (Müller *et al.* 2003). The inverse relationship between growth and immune function is consistent with the interpretation that the immune system, or even mounting an immune response, is energetically costly (reviewed by Sheldon & Verhulst 1996; Lochmiller & Deerenberg 2000; Soler *et al.* 2002; Martin *et al.* 2003). Whether androgens induce immune suppression by affecting energy allocation to growth or by acting directly on androgen receptors in the thymus and bursa needs further study.

One recent similar study with quail failed to find a convincing effect of testosterone injections *in ovo* on the chick's immune function, nor on growth (Andersson *et al.* 2004). However, the dose used in that study was not scaled to the physiological range of that species, while the test of immune function was conducted at an older age. A recent study on lizards found a higher growth in offspring of females treated with dihydrotestosterone compared with those of untreated females (in line with our study), while

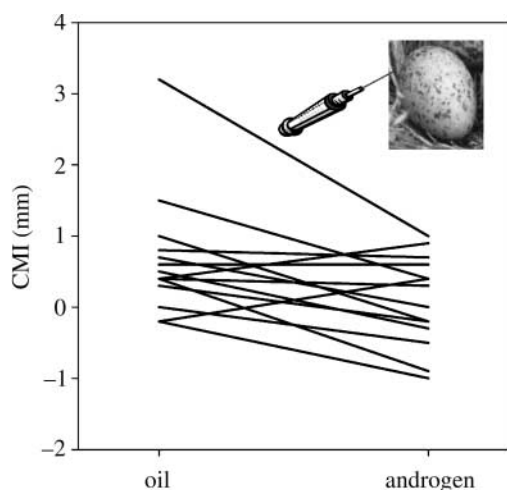


Figure 2. T-cell-mediated immune response of chicks hatched from eggs injected with oil (oil) or androgens dissolved in oil (androgen). Lines connect matched pairs of chicks ($n=14$) raised in the same foster nest.

growth was impaired by exposure to ectoparasites only in the former (Uller & Olsson 2003). However, these authors did not find an effect of maternal treatment on parasite exposure, nor on immunological parameters. Unfortunately, the study did not include a proper (sham-treated) control group and lacked indications of the treatment on androgen and other hormone levels of the mother or offspring.

Clearly, mothers that deposit androgens in their eggs face a trade-off between enhancing growth and suppressing immune function in their offspring. The optimal solution is likely to depend on factors influencing the growth perspective for the chick and its risk on infections. The first is influenced by factors such as food availability, parental quality and hatching order. The second is influenced, among others, by nest density and carotenoid levels in the food (see §1). Most of these factors have been shown to influence yolk levels of maternal androgens (see §1), indicating that females may use these environmental factors as cues for their trade-off. Such a framework can explain the pronounced variation in yolk androgen levels found within species. It may also help to understand why experimental manipulation of the androgen contents in an egg can have beneficial, but sometimes also detrimental, effects on the chick. Future studies should take environmental and parental conditions into consideration when assessing the effects of yolk androgens on chick development, since optimal hormone allocation may differ between parents and between environmental contexts.

We thank Ruedi Nager for advice about, and Bernd Riedstra for help with, the PHA challenge. Ruedi Nager and Serge Daan commented on an earlier draft.

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