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Maternal condition, yolk androgens and offspring performance: a supplemental feeding experiment in the lesser black-backed gull (Larus fuscus)

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It has been proposed that the maternal androgens in avian egg yolk enhance offspring fitness by accelerating growth and improving competitive ability. Because egg quality is strongly influenced by maternal condition, we predicted that females in good condition would produce high-quality eggs with relatively high androgen content. We experimentally enhanced maternal condition by supplementary feeding lesser black-backed gulls (*Larus fuscus*) during egg formation and compared the concentrations of androstenedione (A4), 5α-dihydrotestosterone (DHT) and testosterone (T) in their eggs with those in eggs laid by control females. We also measured circulating levels of T in females immediately after laying. Egg androgens could affect offspring performance directly through chick development and/or indirectly through changes in the competitive ability of a chick relative to its siblings. To avoid confounding these two routes, and to separate effects operating through the egg itself with those operating through experimental changes in parental chick rearing capacity, we fostered eggs from both maternal treatment groups singly into the nests of unmanipulated parents. Contrary to expectation, mothers with experimentally enhanced body condition laid eggs with lower levels of androgens, while exhibiting higher circulating T concentrations post-laying. Despite these lower levels of egg androgen, offspring hatched from eggs laid by mothers in good condition did not show reduced growth or survival when reared in the absence of sibling competition. Our results demonstrate that yolk androgen concentrations vary with the body condition of the female at the time of egg formation and that females in good condition reduced the yolk androgen content of their eggs without altering offspring performance.

Keywords: *Larus fuscus*; maternal condition; offspring performance; plasma testosterone; supplemental feeding; yolk androgens

1. INTRODUCTION

Parents, and mothers in particular, can affect the phenotype of their offspring by mechanisms other than genetic inheritance. These so-called 'maternal effects' mean that the environmental conditions experienced by the parents influence the offspring phenotype, potentially enhancing offspring fitness (Bernardo 1996; Mousseau & Fox 1998). In birds, as in other oviparous species, the egg contents are an important route through which maternal effects can be transmitted. The condition of the mother at the time of egg laying will influence her allocation of resources to egg production and, because developing embryos are completely dependent on these resources, egg quality can have a profound effect on offspring performance (Monaghan & Nager 1997; Metcalfe & Monaghan 2001). Mothers in good condition often produce relatively large eggs, and egg size is positively correlated with hatching probability, early growth and survival of the young (Williams 1994; Christians 2002). In addition, the presence of specific egg components such as lipids (Royle *et al.* 1999; Nager *et al.* 2000), antibodies (Gasparini *et al.* 2001) and carotenoids (Royle *et al.* 2001; Blount *et al.* 2002) may enhance offspring fitness, independent of overall egg size.

There is increasing evidence that relatively minor hormonal differences during early development influence the survival and quality of offspring in a wide range of taxa (see Hews *et al.* 1994; Clark & Galef 1995; Schwabl 1997*a*; McCormick 1998). Variable quantities of maternally derived androgens are present in avian egg yolk (Schwabl 1993) and they are generally believed to have a positive influence on offspring fitness (Schwabl 1996*a*; Lipar & Ketterson 2000; Eising *et al.* 2001; Lipar 2001; Godsave *et al.* 2002), but there may also be some costs (Peters 2000; Sockman & Schwabl 2000). Differential allocation of androgens to eggs within a clutch may enable mothers to influence the pattern of development and the competitive asymmetries within their brood (Schwabl *et al.* 1997). For example, increased androgens in the last egg of asynchronously hatching clutches could compensate for age- and size-related disadvantages of the chick

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hatching from that egg (Winkler 1993; Lipar & Ketterson 2000). In line with this idea, yolk androgen concentrations are often found to increase with the position of an egg in the laying sequence (Schwabl 1993; Lipar *et al.* 1999; Sockman & Schwabl 2000; Royle *et al.* 2001; French *et al.* 2001; Eising *et al.* 2001; Pilz *et al.* 2003).

Yolk androgen levels also differ between females. They have been found to vary with male attractiveness (Gil *et al.* 1999), breeding density (Schwabl 1997*b*; Reed & Vleck 2001; Groothuis & Schwabl 2002), frequency of intrusions into the territory (Whittingham & Schwabl 2002), maternal social status (Müller *et al.* 2002), age and laying date (Pilz *et al.* 2003). It is not yet known, however, whether such hormonal differences in eggs are in fact contingent on variation in the condition of the mother. If mothers adaptively modify yolk androgen concentrations in relation to the environmental conditions they experience, we may expect a link between yolk androgens and maternal state during egg formation, because maternal state will influence the balance of costs and benefits to both mother and offspring of different yolk androgen levels. Specifically, if early exposure to androgens is beneficial to the offspring, we may expect mothers in good condition to produce eggs with relatively high androgen levels.

In this study, we experimentally enhanced maternal condition in lesser black-backed gulls (*Larus fuscus*) by providing extra food during the egg-formation period and examined the effects on androgen levels in the eggs and in the maternal circulation as well as the subsequent effects on offspring development. Lesser black-backed gulls generally lay three eggs, the last egg being smaller and hatching slightly later than the other two, leading to a pronounced size hierarchy within a brood. As a consequence, mortality is highest in third-hatched chicks, although broods of three can be reared successfully by some pairs (see Royle & Hamer 1998). Beneficial effects of egg androgens on chicks could therefore be mediated through direct effects on chick development, and/or indirectly through effects on their competitive ability relative to other siblings. To avoid confounding these two routes, we fostered eggs of control and enhanced-condition females singly into the nests of otherwise unmanipulated females. This allowed us to examine the development of offspring in the absence of sibling competition. The cross-fostering design further removed the possibility of confounding the effects operating through the egg itself with those due to the experimental enhancement of maternal condition during egg formation also influencing the mother's chick rearing ability.

2. MATERIAL AND METHODS

(a) *Experimental treatment*

Fieldwork was done in a large lesser black-backed gull colony on Walney Island, Cumbria, UK. Pairs laying three eggs and breeding in the centre of the colony were randomly allocated to either the control or the enhanced body condition group. Enhanced body condition pairs received a portion of *ca.* 140 g of microwaved hens' eggs beside their nest every night, from three weeks before the start of egg laying until the third and last egg was laid. Egg formation takes *ca.* 10 days in this species and previous studies have shown that food supplementation enhances female body condition and egg production (see, for example, Bolton *et al.* 1993), without affecting the laying date of the clutch (Bolton *et al.* 1992; Nager *et al.* 1999). We studied a total of 48 food supplemented and 56 control pairs. The average clutch initiation date did not differ between these two groups ($F_{1,102} = 1.96$, $p = 0.16$). Between 29 April and 24 May 2000, we collected the eggs of 23 control and 23 food-supplemented pairs. Each egg was collected within 12 h of it being laid, weighed and replaced with a dummy egg to avoid interference with the normal laying pattern. All collected eggs were kept in an incubator (Curfew 747 Professional) for 4 days at 38 °C and then stored at −20 °C. This enabled us to sex the embryo by using molecular techniques (Griffiths *et al.* 1998) and to measure androgen concentrations in the yolk of these eggs. The eggs of the remaining 25 food-supplemented and 33 control clutches were fostered to assess offspring performance (see below).

(b) *Measurement of maternal condition*

Maternal condition was measured in a separate sample of 20 females (11 control and 9 experimental) captured in walk-in traps placed over the nests within 24 h of clutch completion. As a measure of body condition, we used body mass corrected for size (i.e. head–bill length). We also estimated the pectoral muscle lean dry mass, which gives an index of maternal protein reserves, by using the breast profile technique described by Bolton *et al.* (1991). Within 10 min of capture, we collected a blood sample of *ca.* 500 µl from the tarsal vein (under UK Home Office licence). The blood sample was centrifuged at 13 000 r.p.m. for 10 min and plasma was stored at −20 °C until laboratory analyses. A small amount of the blood was also used to confirm the sex of the bird.

(c) *Offspring performance*

We fostered 162 eggs of 58 different females (33 control, 25 enhanced condition) singly into 162 nests of unmanipulated parents that had also laid three eggs and bred in the centre of the colony. Each egg was transferred on the day it was laid and it replaced a freshly laid third egg in the cross-foster nest. To exclude competition in the nest only the fostered egg was allowed to hatch. The first two eggs that had been laid by the foster parents were dipped in mineral oil to prevent embryo development. Nests were checked daily around the expected day of hatching. We recorded the day and time at which the chick first cracked the shell (pipping) as well as the day and time on which it emerged (hatching). Incubation duration was calculated as the number of days from laying to hatching and the duration of the actual hatching process was calculated as the time between pipping and hatching (available for 58 cross-foster nests). On the day of hatching (day 0), we measured the chick body mass and head–bill length and we obtained a blood sample (max. 500 µl) for molecular sex determination and plasma testosterone analysis. To assess the immunocompetence of the hatchlings we injected 0.5 mg of phytohaemagglutinin (PHA-P, Sigma Aldrich, Poole, UK) dissolved in 100 µl of phosphate-buffered saline (PBS) subcutaneously into the left foot web on the day of hatching. PHA causes a local swelling that reflects the strength of the T-cell-mediated immune response (McCorkle *et al.* 1980). As a control, we injected 100 µl of PBS into the right foot web. Immediately before injection, and 24 h (range ± 1 h) later, we measured the thickness of the injection sites to the nearest 0.01 mm using a modified pressure-sensitive micrometer (K50 Tasterform C, Kälfer, Germany). Each measurement was taken three times. Because the repeatability of successive measurements was high ($r = 0.98$, $p < 0.001$), we used the

Table 1. Clutch initiation date, body mass, pectoral muscle lean dry mass and plasma testosterone concentration (mean ± s.e.m.) of control and supplementary fed female lesser black-backed gulls measured within 24 h after clutch completion. (Structural size (i.e. head–bill length) was controlled for in the analyses of body mass and pectoral muscle lean dry mass.)

	control	food supplement	*F*- and *p*-values
number of females	11	9	
laying date	9.8 May ± 2.9 days	6.9 May ± 2.1 days	$F_{1,18} = 0.60$, $p = 0.45$
body mass (g)	727.7 ± 14.0	831.2 ± 38.1	$F_{1,17} = 5.71$, $p = 0.029$
pectoral muscle lean dry mass (g)	19.3 ± 0.3	22.3 ± 1.0	$F_{1,16} = 5.89$, $p = 0.028$[a]
plasma testosterone (pg ml^{-1})	28.1 ± 6.3	60.6 ± 11.7	$F_{1,18} = 6.63$, $p = 0.019$

[a] For one supplemented female we did not obtain an estimate of pectoral muscle lean dry mass.

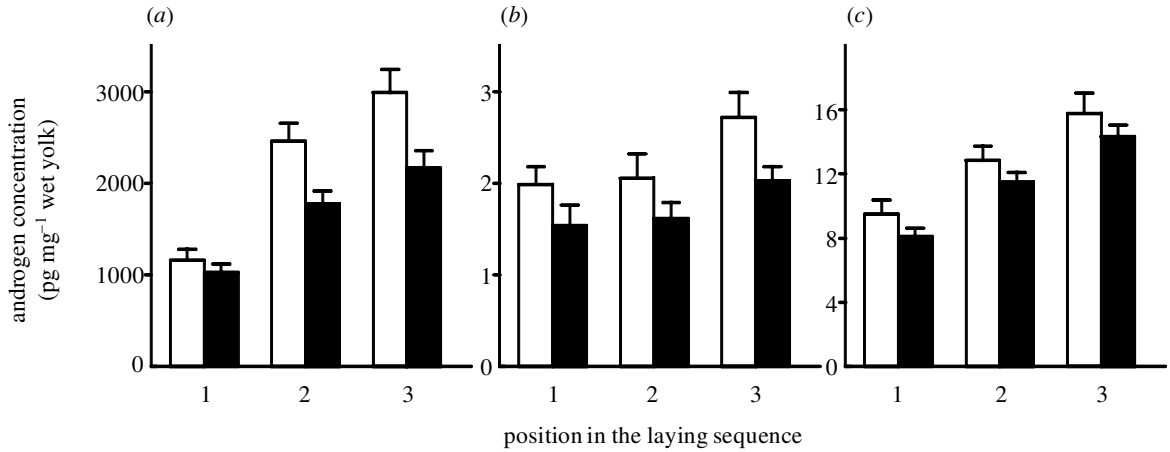

Figure 1. Concentration of (*a*) androstenedione (A4), (*b*) 5α-dihydrotestosterone (DHT) and (*c*) testosterone (T) in the first, second and third egg laid by control (white) and supplementary fed (black) females.

average of the three values. We expressed immune response as the change in thickness of the left foot web minus the change in thickness of the right foot web, although in fact the control injection did not increase the thickness ($t = 1.12$, d.f. = 72, $p = 0.27$).

All the nests under study were surrounded with a mesh wire enclosure, measuring *ca*. 3 m across and 45–75 cm high, to prevent chicks straying far from the nest site and to minimize disturbance when catching them. From day 3 onwards we measured the body mass of the nestlings every 4 days until they reached fledging at an age of 35 days, at which time the fences were removed. Missing chicks that we failed to find inside or outside the enclosures on three consecutive days were assumed to have died. However, we assumed that a missing chick had fledged, if at the previous visit the chick was at least 30 days old and weighed more than 80% of the average asymptotic mass at fledging (695 g for females and 790 g for males).

(d) *Androgen assays*

In domestic fowl, which have a similar incubation duration to lesser black-backed gulls, endogenous androgen production has first been detected after 6–8 days of incubation (Ottinger 1989; Elf & Fivizzani 2002). We therefore assumed that all androgens measured as early as day 4 of incubation were of maternal origin. We measured the concentrations of the androgens androstenedione (A4), 5α-dihydrotestosterone (DHT) and testosterone (T) in egg yolk at Washington State University, using a standard protocol (Schwabl 1993). Briefly, on thawing, shell, albumen and yolk were separated. After recording yolk wet mass, 150 mg of yolk was homogenized with an equal volume of distilled water and extracted twice with 4 ml of petroleum ether/diethyl ether (30 : 70). Before extraction, 2000 c.p.m. titriated hormone was added to each sample to allow calculation of steroid recovery. Extracts were precipitated with 90% ethanol to remove neutral lipids and proteins. Individual steroids were separated from the supernatant by application to a celite chromatography column and elution with 2% (A4), 10% (DHT) and 20% (T) ethyl acetate in iso-octane. We measured hormone concentrations using competitive-binding radioimmunoassay. The samples were analysed in six different assays. Average steroid recovery was 38% for A4, 18% for DHT and 38% for T (samples with recoveries below 10% were not used, $n = 5$) and the intra-assay coefficient of variation (CV) averaged 6.5%, 2.6% and 5.6% for A4, DHT and T, respectively. The cross-reactivities of the antibodies at 1000 pg were A4: Δ5-androstene-3-17-dieone 80%, androstanedione 38.5%, 5α-dihydrotestosterone 5.5%, all others tested less than 3.0%, and T (used for both T and DHT assay): 5α-dihydrotestosterone 59.5%, Δ1-testosterone 4%, 5α-androstan-3α, 17β-diol 18%, Δ5-androsten-3β, 17β-diol 12.5%, all others tested less than 5% (Wien Laboratories, Inc.).

Plasma T concentrations (mothers and hatchlings) were measured by double antibody radioimmunoassay at the University of Glasgow using a modification of an established assay (Sheffield & O'Shaughnessy 1989). Plasma (25–50 μl) and standards (50 μl) were extracted with chloroform (900 μl), dried and resuspended in assay buffer (0.05 M PBS with 0.25% bovine serum albumin) before assay. Column chromatography was not used. Mean sensitivity (two standard deviations of buffer controls) of the assays required for adult ($n = 1$) and hatchlings ($n = 4$) averaged 9.1 pg ml^{-1}. The intra-assay CV values at 55%

and 21% displacement points averaged 27.3 and 13.7%, respectively. The inter-assay CV averaged 15.6%.

(e) *Data analyses*

We analysed variation in yolk androgen concentrations and offspring performance using a mixed model with the identity of the biological mother as a random effect and position of an egg in the laying order in the nest of origin (first, second or last) and treatment (control or food supplement) as fixed effects (PROC MIXED; SAS 2001). To ensure that within- and between-nest patterns of androgen concentrations in eggs and hatchlings were not confounded by inter-assay variation we controlled for assay number in all analyses. We also tested for differences in offspring sex and clutch initiation date. Interactions between variables were included in the model, if prior tests indicated significance. We modelled hatching success, fledging success and sex ratio (proportion of male embryos) with binomial error structure and logit link function (PROC GENMOD; SAS 2001), controlled for the identity of the biological mother. Predated eggs were not included in the analysis of hatching success. As a measure of hatchling condition, we used the residuals of a regression of body mass on head–bill length (body condition index). To investigate postnatal growth we fitted a logistic growth model, $\text{mass} = A/(1 + e^{(-k \times \text{age})})$, through the body mass measurements of each individual chick calculated the maximum growth rate as the slope of the curve at the inflection point ($kA/4$ in g d^{-1}, where A is the asymptotic mass and k is a constant growth rate factor). Only chicks that fledged were included in the analysis of growth rate.

3. RESULTS

(a) *Experimental manipulation of maternal condition*

At clutch completion, females that received a food supplement were significantly heavier for their size and they had significantly larger pectoral muscles than controls (table 1). Supplementary fed females thus finished egg laying in better condition than control females. The average concentration of circulating T in females with experimentally enhanced body condition was more than double that of control females (table 1).

(b) *Maternal condition and yolk androgens*

Yolk androgen concentrations did not change with laying date (effect of clutch initiation date on total androgens: $F_{1,123} = 0.28$, $p = 0.60$, A4: $F_{1,127} = 0.26$, $p = 0.61$, DHT: $F_{1,126} = 0.96$, $p = 0.33$ and T: $F_{1,126} = 2.90$, $p = 0.091$). Androgen concentrations increased with the position of an egg in the laying sequence (figure 1; table 2). All androgens, except A4, also correlated between different eggs laid by the same mother (random factors in table 2). Overall, female identity explained 0.7%, 20.2% and 27.2% of the variation in yolk androgens for A4, DHT and T, respectively. In contrast to our prediction, eggs of supplementary fed females had lower total androgen concentrations than those of control females ($F_{1,124} = 16.74$, $p < 0.001$), with the same pattern being evident for all three androgens (figure 1; table 2). For DHT and T the effect of treatment did not differ between first-, second- and third-laid eggs, as indicated by a non-significant interaction term between laying order and treatment. The difference between the control and the

Table 2. Results of mixed model analyses of yolk androgen concentrations in eggs laid by control and supplementary fed lesser black-backed gulls (Wald Z-tests for random effects and F-tests for fixed effects).
(The analysis includes 137 eggs (A4) and 136 eggs (DHT and T) of 46 different females. n.s., not significant.)

	androstenedione (A4)			5α-dihydrotestosterone (DHT)			testosterone (T)		
	Wald Z/F-statistic	d.f.	p	Wald Z/F-statistic	d.f.	p	Wald Z/F-statistic	d.f.	p
random effect:									
female identity	0.00		n.s.	1.83		0.033	2.41		0.008
fixed effects:									
assay number	3.4	5.128	0.007	30.2	5.127	< 0.001	1.5	5.127	n.s.
laying order	43.4	2.128	< 0.001	10.6	2.127	< 0.001	43.7	2.127	< 0.001
treatment	20.2	1.128	< 0.001	7.5	1.127	< 0.01	3.5	1.127	0.065
treatment × laying order	3.0	2.126	0.056	2.2	2.125	n.s.	0.02	2.125	n.s.

enhanced-condition group tended to increase from the first to the third egg for A4 (table 2).

Neither fresh egg mass, nor yolk wet mass was significantly different between eggs laid by control and enhanced-condition females (fresh egg mass $F_{1,134} = 1.56$, $p = 0.21$, yolk mass $F_{1,134} = 0.08$, $p = 0.78$, controlled for laying order). Because yolk mass varied among females (Wald $Z = 3.04$, $p = 0.001$) and declined with the position of an egg in the laying sequence ($F_{2,134} = 12.3$, $p < 0.001$), we repeated the analyses using the total amount of androgens present in the entire yolk, rather than concentrations. This gave the same results (effect of treatment on total androgens: $F_{1,124} = 14.4$, $p < 0.001$, A4: $F_{1,128} = 17.5$, $p < 0.001$, DHT: $F_{1,127} = 5.8$ $p = 0.018$ and T: $F_{1,127} = 3.1$, $p = 0.079$) and the interactions between treatment and laying order were non-significant ($p > 0.05$ after Bonferroni adjustment).

In total, we sexed the embryos of 129 eggs from 46 different females (nine embryos were too small to be sexed). The sex of the embryos did not differ with the position of an egg in the laying sequence ($F_{2,82} = 0.36$, $p = 0.70$). Moreover, offspring sex ratio was not affected by the enhancement of maternal condition ($F_{1,84} = 0.28$, $p = 0.60$). There was no difference in yolk androgen concentration between eggs containing male or female embryos (corrected for laying order and treatment: total androgens $F_{1,114} = 0.15$, $p = 0.70$, A4: $F_{1,118} = 0.04$, $p = 0.84$, DHT: $F_{1,117} = 0.12$, $p = 0.73$, T: $F_{1,117} = 0.55$, $p = 0.46$).

(c) *Offspring performance*

Eggs laid by control females and females with experimentally enhanced body condition were equally likely to hatch (mean hatching success control group: 0.51 ± 0.053, enhanced-condition group: 0.55 ± 0.059, $\chi^2 = 0.23$, d.f. = 1, $p = 0.63$). Hatching success was not related to the position of an egg in the laying sequence ($\chi^2 = 2.74$, d.f. = 2, $p = 0.25$). The length of the incubation period did not differ between chicks hatching from eggs that were laid first, second or third in their nest of origin (table 3). The hatching process itself was 13.2 h shorter for chicks hatching from first eggs compared with chicks hatching from second or third eggs, but it was not affected by the experimental treatment (table 3). As in other studies of this species (e.g. Nager *et al.* 1999) males were skeletally larger than females at hatching (mean head–bill length males: 49.0 ± 0.3 mm, $n = 36$ and females 47.7 ± 0.3 mm, $n = 44$; table 3). Chicks from first- and second-laid eggs hatched in better condition than chicks from third eggs (average body condition index for chicks from first eggs: 2.0 ± 0.8, $n = 31$, second eggs: 0.3 ± 1.3, $n = 25$ and third eggs: -2.9 ± 1.1, $n = 24$). However, this was mainly an effect of egg mass decreasing from the first to the third egg of the clutch, because when egg mass was added to the model, the position in the laying order was no longer significant ($F_{2,72} = 0.41$, $p = 0.66$). After controlling for the effect of laying order, we observed a seasonal decline in chick body condition at hatching (table 3). However, neither hatchling condition, nor structural size of the hatchlings, was related to maternal treatment group (table 3).

At hatching, chicks from second-laid eggs had significantly higher levels of circulating plasma T than first or

Table 3. The performance of chicks hatched from eggs laid by control females and females with experimentally enhanced body condition.
(Eggs were cross-fostered into the nests of unmanipulated parents for incubation and reared as single chicks. The identity of the biological mother was included as a random effect in all models. Body condition was expressed as body mass corrected for size (head–bill length, $F_{1,73} = 18.49$, $p < 0.0001$) and plasma testosterone concentration was controlled for assay number ($F_{3,25} = 7.21$, $p = 0.001$). For 22 chicks the interval from pipping to hatching was unknown, seven chicks died before foot web swelling could be measured and for four chicks we did not obtain a large enough blood sample to determine the testosterone concentration.)

	number of eggs/females	female	hatching date	sex	laying order	treatment	interactions
duration of incubation period	80/46	$Z = 0$	$F_{1,74} = 0.13$	$F_{1,74} = 0.68$	$F_{2,74} = 0.50$	$F_{1,74} = 1.85$	all $p > 0.16$
duration of hatching process	58/42	$Z = 197.45^{*}$	$F_{1,52} = 0.01$	$F_{1,52} = 0.56$	$F_{2,52} = 5.25^{*}$	$F_{1,52} = 0.57$	all $p > 0.28$
head–bill length	80/46	$Z = 0.36$	$F_{1,74} = 0.66$	$F_{1,74} = 9.27^{**}$	$F_{2,74} = 2.86$	$F_{1,74} = 0.80$	all $p > 0.28$
body condition index	80/46	$Z = 13.7^{**}$	$F_{1,73} = 5.85^{*}$	$F_{1,73} = 0.08$	$F_{2,73} = 5.82^{**}$	$F_{1,73} = 0.30$	all $p > 0.35$
immune response	73/44	$Z = 0$	$F_{1,64} = 16.43^{**}$	$F_{1,64} = 0.01$	$F_{2,64} = 0.69$	$F_{1,64} = 0.13$	treatment × sex: $F_{1,64} = 7.14^{*}$ treatment × laying order: $F_{2,64} = 4.00^{*}$
plasma testosterone	76/44	$Z = 0.81$	$F_{1,67} = 0.56$	$F_{1,67} = 0.13$	$F_{2,67} = 4.06^{*}$	$F_{1,67} = 0.06$	all $p > 0.38$

$^{*}p < 0.05$; $^{**}p < 0.01$.

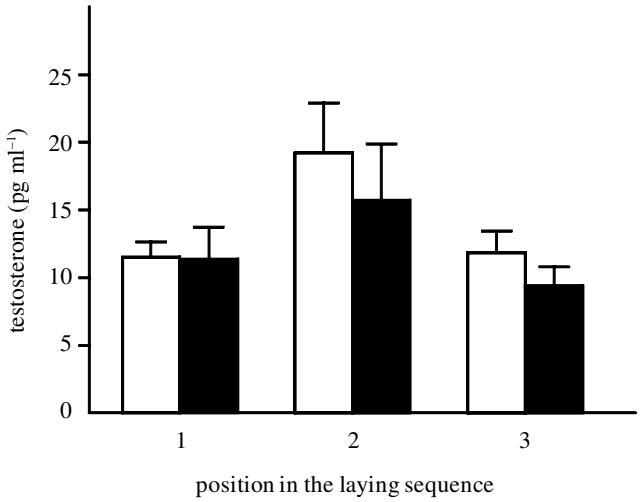

Figure 2. Concentration of circulating testosterone in blood plasma of hatchlings from first-, second- and third-laid eggs of control mothers (white) and mothers with experimentally enhanced body condition (black).

third chicks of the same brood (figure 2; table 3). There was no effect of either maternal treatment, hatching date or offspring sex on hatchling T (table 3). The only aspect in which hatchlings from experimental eggs differed from control eggs was in the thickness of foot web swelling in response to PHA injection, which is a function of the hatchling's immune response. Overall, hatchling immune response declined through the breeding season, irrespective of sex, position in the laying order and treatment. However, although the strength of the immune response of hatchlings varied with sex and the position of the egg in the laying order (table 3; figure 3), the pattern differed between maternal treatment groups. Among female hatchlings, those from first and second eggs laid by enhanced-condition mothers showed a stronger immune response than controls, whereas the third-hatched females did not differ (figure 3*a*). In males on the other hand, the immune response of chicks from control eggs did not differ with laying order, whereas in the experimental treatment, immune response declined with laying order such that third-laid males had a significantly lower response than control males from the same egg position (figure 3*b*). These differences were not due to differences in size or condition at hatching, because, after adding hatchling size or hatchling condition to the model, the sex × treatment interaction and the laying order × treatment interaction remained significant (sex × treatment: $F_{1,63} = 7.0$, $p = 0.01$ and $F_{1,63} = 6.6$, $p = 0.01$, laying order × treatment: $F_{2,63} = 4.1$, $p = 0.02$ and $F_{2,63} = 3.8$, $p = 0.03$).

The maximum growth rate of the nestlings was analysed in a mixed model with the identity of the biological mother as random effect and sex of the chick, hatching date, laying order, experimental treatment and plasma T as fixed effects. As expected from an earlier study (Griffiths 1992) males gained mass faster than females (males 37.4 ± 1.7 and females 30.9 ± 1.1 g d^{-1}, $F_{1,49} = 10.9$, $p = 0.002$). Maximal postnatal growth rate was not related to the experimental manipulation of the condition of the biological mother at the time of egg formation ($F_{1,48} = 0.75$, $p = 0.39$), nor to the position of an egg in the laying order ($F_{2,47} = 1.06$, $p = 0.35$), hatching date ($F_{1,48} = 0.05$, $p = 0.82$) or plasma T concentration of the chicks at hatching ($F_{1,45} = 0.11$, $p = 0.74$). Mean fledging success of chicks hatched from control (0.60 ± 0.1, $n = 43$) and experimental eggs (0.68 ± 0.1, $n = 37$) was not significantly different ($\chi^2 = 0.52$, $p = 0.47$), independent of the position in the laying order, the plasma T concentration at hatching or the sex of the nestlings (all $p > 0.27$).

4. DISCUSSION

The experiment presented here clearly demonstrates that yolk androgen concentrations are directly related to the nutritional state of the female at the time of egg formation. As in several other studies (Schwabl 1993; Lipar *et al.* 1999; Sockman & Schwabl 2000; French *et al.* 2001; Eising *et al.* 2001; Pilz *et al.* 2003), including one on lesser black-backed gulls (Royle *et al.* 2001), we found that yolk androgen concentrations increased with the position of an egg in the laying sequence. Third (last) laid eggs in the lesser black-backed gull had the highest androgen levels, both in terms of total amount and concentration. By the provision of additional food, we successfully enhanced the body condition of females during egg formation. Females in enhanced condition produced clutches showing the same tendency for androgen levels to increase with laying order, but all their eggs had lower yolk androgen levels. This pattern was observed for overall androgen levels, and separately for A4, a precursor of T, as well as DHT, an active metabolite of T and T itself, although in the latter case the effect of feeding treatment was marginally significant. These results are different from what one would predict if high androgen levels were beneficial to chick development and if females in good condition produced high-quality eggs (see also Pilz *et al.* 2003). Clearly, there are several additional factors that need to be considered in assessing the balance of the costs and benefits of egg androgen levels to parent and chick, and these are discussed below.

(a) *Relationship between androgens in maternal circulation and egg yolk*

Studies in which androgen levels in maternal circulation and in egg yolk were measured simultaneously suggest that high androgen levels in eggs are accompanied by high androgen levels in the female circulation at the time of yolk formation. For example, in canaries (*Serinus canaria*), yolk T concentrations were positively correlated with concentrations of circulating T in the mother (Schwabl 1996*b*). In addition, experimental elevation of steroid concentrations in maternal plasma resulted in a corresponding increase in egg yolk (Arcos 1972; Adkins-Regan *et al.* 1995). However, androgens are known to regulate a suite of physiological processes and high levels of circulating T, associated with producing eggs containing high levels of androgens, may be costly to the parent. High levels of T may suppress the immune system (Peters 2000), adversely affect survival (Dufty 1989; Nolan *et al.* 1992), increase energy expenditure (Råberg *et al.* 1998; Buchanan *et al.* 2001) and/or suppress parental behaviour, at least in males (e.g. Ketterson *et al.* 1992; Alonso-Alvarez 2001). If yolk androgen concentrations are indeed correlated with circulating androgen levels in the mother it would only pay mothers to bear the costs of high levels of circulating

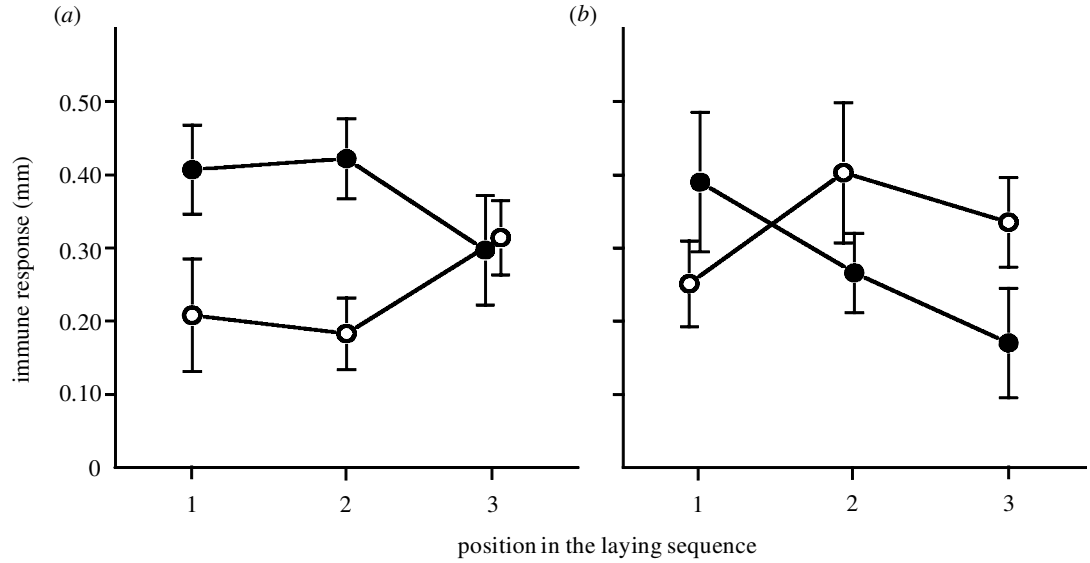

Figure 3. Immune response 24 h after PHA injection for (*a*) female hatchlings and (*b*) male hatchlings from first-, second- and third-laid eggs of control mothers (open circles) and mothers with experimentally enhanced body condition (filled circles).

T if these costs are outweighed by the fitness benefits of elevated androgen concentrations in egg yolk. In fact, this study demonstrates that mothers whose body condition was experimentally enhanced produced eggs with lower androgen levels, but had higher circulating plasma androgen levels themselves. This suggests that a reduction in yolk androgen concentrations is not necessarily associated with a reduction in plasma T in the female, so that females that produce eggs with low androgen concentrations may not benefit by having reduced exposure to circulating androgens.

The lack of a simple correspondence between T levels in the clutch and T levels in the maternal plasma further questions the parsimonious assumption that the process of androgen deposition into egg yolk is a simple passive process. Cells that form the follicular walls around the developing ova are the main source of androgens (Bahr *et al.* 1983). Testosterone may be actively transported from these steroidogenic cells directly into the yolk and thus T levels in blood plasma and egg yolk could vary independently of each other (Doi *et al.* 1980; Hammond *et al.* 1980). We do not have information about maternal T levels during the 10 days of yolk formation; therefore, our data do not allow inferences about the mechanism of androgen transport into the egg. However, the observed opposing patterns in plasma and yolk T suggest that females could independently regulate the deposition of androgens in egg yolk rather than yolk androgen concentrations being merely a reflection of the levels present in maternal circulation.

The question remains, why did females in the enhanced condition group have higher circulating levels of plasma T. The plasma T concentrations reported in this study were low compared with those measured in other gull species before egg laying (see Wingfield *et al.* 1982; Groothuis & Meeuwissen 1992), possibly due to an inhibition of ovarian function associated with the onset of incubation and parental care. The small difference between the enhanced-condition group and the control group may have been functionally insignificant. However, our findings are in line with Duckworth *et al.* (2001) who report a positive correlation between plasma T levels and body condition in male house finches (*Carpodacus mexicanus*). Moreover, T has been found to increase territorial aggressiveness in yellow-legged gulls *Larus cachinnans* (Alonso-Alvarez & Velando 2001). In lesser black-backed gulls, both sexes defend the territory and it is possible that high levels of circulating T result from experimental females defending the acquired food source against neighbouring gulls. High levels of plasma T may also enforce nest defence behaviour and reduce the probability of intraspecific nest predation, an important source of breeding failure in our study colony (Nager *et al.* 2000). Individuals in good condition may be better able to bear the potential costs of increased androgen levels and benefit from lower losses due to predation.

(b) *Adverse effects of yolk androgens on offspring development*

When chicks were raised in a non-competitive situation, we found no differences in growth between chicks hatching from the low androgen eggs laid by females in experimentally enhanced condition and chicks hatching from control eggs with higher yolk androgen levels. Although it is generally believed that androgens have a positive effect on offspring development, the benefits may not always outweigh the costs. For example, faster growth, associated with high androgen levels, may only be beneficial in some circumstances, and it may carry long-term costs (Metcalfe & Monaghan 2001). Few studies have found evidence for costs associated with the presence of androgens in egg yolk. *In vivo* injection of T before incubation had a negative effect on embryonic growth of female domestic chickens *Gallus domesticus* (Henry & Burke 1999) and injection of a cocktail of A4 and T into first-laid eggs of the American kestrel (*Falco sparvarius*) resulted in delayed hatching, reduced growth and lower post-hatching survival (Sockman & Schwabl 2000). In our study, we did not find any negative effects on pre- or post-hatching growth or on offspring survival associated with the higher

androgen levels in eggs laid by control females relative to the experimental females.

An important and widely discussed cost of high levels of androgens is immunosuppression. Androgens modulate immune cell traffic and cytokine synthesis (Roitt *et al.* 1996) and high levels of T may impair immune function (Grossman 1984). Furthermore, increased metabolic rate associated with high androgen levels causes oxidative stress and this might result in suppression of the immune system (Råberg *et al.* 1998). Interestingly, we detected some evidence of a sex-specific effect associated with hatching from eggs with different androgen levels on PHA, a measure of cell-mediated immunity. With the exception of the last-laid egg, female chicks that hatched from eggs from the mothers with enhanced condition had a stronger PHA response. Because these eggs had lower yolk androgen concentrations, it suggests that the presence of large quantities of yolk androgens may not benefit the chick under all circumstances.

(c) *Indirect effects of yolk androgens through sibling competition*

High levels of yolk T have been found to increase a chick's begging behaviour (Schwabl 1996*a*) and to improve its later social status (Schwabl 1993). These effects become evident in a situation where chicks share the nest with other chicks. However, investigating offspring performance in a competitive situation does not allow the separation of direct effects of androgens on chick development from indirect effects through differences in competitive ability. In this study, where chicks were raised singly, we found no correlation between yolk androgen levels and chick performance. This suggests that yolk hormones did not change the intrinsic value of the offspring, but they may play a role when competing for parental resources. However, from our results it can be concluded that the competitive ability of chicks is unlikely to be directly related to circulating T levels in the hatchling's plasma. Despite lower yolk androgen concentrations in experimental eggs, the plasma T levels in hatchlings did not differ between the treatment groups. Interestingly, the plasma T concentrations were higher in hatchlings developing in second eggs, regardless of sex. Since all chicks were raised singly, this cannot be due to social interactions (Ramos-Fernandez *et al.* 2000); rather, it suggests a maternal effect specific to the second egg.

It has been suggested by several authors that females may influence the competitive situation in the nest by allocating different amounts of androgens to different eggs within the clutch (Winkler 1993; Lipar & Ketterson 2000). For example, the growth of first chicks was reduced after an experimental increase of yolk androgens in the last eggs of the clutch (Eising *et al.* 2001). By contrast, we found no evidence that the relative allocation of androgens to different eggs within the clutch was influenced by maternal condition. Similarly Groothuis & Schwabl (2002) found no differences in within-clutch patterns when comparing black-headed gull (*Larus ridibundus*) clutches with overall high or low androgens levels. It seems that, at least in gulls, although mothers are able to alter the overall androgen concentrations, they do not change the relative levels between eggs.

(d) *Variation in other egg components*

In previous experiments, eggs were often injected with androgens so that the effects of androgens on offspring performance could be studied while keeping other egg components constant (Henry & Burke 1999; Sockman & Schwabl 2000; Lipar & Ketterson 2000; Eising *et al.* 2001). In contrast to these studies, we investigated natural variation in yolk androgen concentrations. This means that the androgen concentrations did not reach unnaturally high levels and that females may have adjusted other egg components in conjunction with yolk androgens. This is important because certain yolk components may interact with androgens to reduce or enhance the hormonal effects. For example, opposite patterns of yolk T and antioxidants across the laying order suggest that a trade-off may exist between these egg components (Royle *et al.* 1999). Similarly, Groothuis & Schwabl (2002) suggested that high yolk androgen concentrations in eggs of lighter clutches could compensate for poor nutritional quality of the eggs. Feeding a high-quality diet to pre-laying lesser black-backed gulls affects egg size and egg composition (Bolton *et al.* 1992; Nager *et al.* 1999). Even though supplementary fed females in this study did not increase egg size significantly, they may have been limited by proteins or specific nutrients contained in the food supplement (Bolton *et al.* 1992). Thus, females in enhanced condition may have produced high-quality eggs with low androgen concentrations, but rich in other essential egg components. This suggests that females may be capable of adaptive fine-tuning of various egg components depending on the prevailing environmental conditions.

In conclusion, our experiment shows that female lesser black-backed gulls in good condition deposit less androgens into their eggs without compromising offspring performance in a non-competitive rearing situation. How females differentially allocate androgens to their growing ova remains unclear. A better understanding of this physiological process, as well as insight into the balance of costs and benefits of androgens to offspring and parent, are required for the interpretation of the ecology and evolution of maternal hormonal effects.

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