

University of Groningen

## Maternal androgens in black-headed gull (*Larus ridibundus*) eggs

Eising, C.M; Eikenaar, C.; Schwabl, H; Groothuis, A.G.G.

*Published in:*

Proceedings of the Royal Society of London. Series B, Biological Sciences

*DOI:*

[10.1098/rspb.2001.1594](https://doi.org/10.1098/rspb.2001.1594)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2001

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Eising, C. M., Eikenaar, C., Schwabl, H., & Groothuis, A. G. G. (2001). Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: Consequences for chick development. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, 268(1469), 839-846.  
<https://doi.org/10.1098/rspb.2001.1594>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development

Corine M. Eising<sup>1\*</sup>, Cas Eikenaar<sup>1</sup>, Hubert Schwabl<sup>2</sup> and Ton G. G. Groothuis<sup>1</sup>

<sup>1</sup>*Department of Animal Behaviour, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands*

<sup>2</sup>*School of Biological Sciences, Center for Reproductive Biology, Washington State University, Pullman, WA 99164-4236, USA*

We tested the hypothesis that mother birds counterbalance the negative effects of hatching asynchrony for later-hatched chicks by increasing the yolk androgen concentrations in consecutive eggs of their clutch. In doing so, they may adaptively tune each offspring's competitive ability and, thus, growth and survival. However, evidence in support of this hypothesis is contradictory. The yolk concentrations of maternal androgens in the eggs of black-headed gulls increase significantly with the laying order of the eggs in a clutch. We experimentally tested the functional consequences of this increase on chick development under natural conditions by injecting eggs with either an oil or androgen solution. We created experimental clutches in which androgen levels either stayed constant or increased with laying order while controlling for differences in egg quality by using only first-laid eggs. We then compared development, growth and survival between these broods. Androgen treatment enhanced embryonic development because androgen-treated eggs hatched half a day earlier than controls, while their size at hatching was similar to oil-treated controls. Androgen treatment did not increase chick survival, but it enhanced growth. Androgen-treated, third-hatched chicks had a higher body mass and longer legs than third-hatched chicks that hatched from oil-treated eggs. At the same time, growth of first chicks (which were all oil treated) was reduced by the presence of two androgen-treated siblings, suggesting that yolk androgens enhance the competitive ability of later-hatched chicks. Our results support the hypothesis that transfer of different amounts of androgens to the eggs of a clutch is a mechanism by which mothers maximize their reproductive output.

**Keywords:** yolk androgens; hatching asynchrony; sibling competition; parental investment; chick growth; maternal hormones

## 1. INTRODUCTION

The number of studies that are applying endocrinology to the investigation of adaptive behavioural strategies is increasing rapidly. Recent studies in vertebrates have shown that hormones of maternal origin are transferred to the offspring via the egg yolk, and may influence several developmental processes (Feist *et al.* 1990; Adkins-Regan *et al.* 1995; Conley *et al.* 1997; Janzen *et al.* 1998; McCormick 1999). It has been shown in birds that yolk steroid hormones are of maternal origin, correlate with the hormone levels of the mothers and differ with laying sequence within the clutch (Schwabl 1993; Schwabl *et al.* 1997; Gil *et al.* 1999; Lipar *et al.* 1999; Sockman & Schwabl 2000; French *et al.* 2001; Royle *et al.* 2001) and with environmental conditions (Schwabl 1996a). Between-species levels may increase or decrease with laying order, suggesting that there are species-specific strategies of hormone allocation by the mother.

It is hypothesized that differential allocation of maternal androgens to consecutive eggs of the same clutch has a function in modifying the size hierarchy that arises among chicks of a brood as a result of hatching asynchrony. Hatching asynchrony occurs in a wide variety of bird species and several hypotheses for its adaptive value have been proposed (Clark & Wilson 1981; Hahn 1981; Graves *et al.* 1984), of which Lack's (1947)

'brood reduction hypothesis' is the most prominent. This hypothesis proposes that, when food supply is poor during the nestling phase, the established size hierarchy among the chicks facilitates the reduction of the brood to the adequate size. In cattle egrets *Bubulcus ibis*, a facultative siblicidal species, higher yolk androgen levels in first-hatched chicks may enable them to eliminate younger siblings (sibs) swiftly when food availability does not allow for raising a full brood (Schwabl *et al.* 1997). However, the size hierarchy and accompanying competitive asymmetry within the brood may result in brood reduction when food is abundant, thereby reducing fitness (Forbes 1994). The combination of hatching asynchrony and differential androgen allocation will allow mothers to tune the survival probabilities of their individual offspring adaptively according to the circumstances prevailing at the time of egg laying (Schwabl 1996a; Eising *et al.* 2001). This may give mothers the upper hand in parent-offspring conflict (Winkler 1993). Yet experimental proof is scarce. A study on domesticated canaries revealed increased growth rate, begging vigour (Schwabl 1996b) and aggression (Schwabl 1993) in chicks hatching from eggs with higher maternal androgen concentrations. Although the increased competitive ability of these chicks could not fully compensate for the disadvantageous effect of the sibling size hierarchy, these results support the idea that differential allocation of maternal androgens may adaptively modify the competitive hierarchy within a brood. The only study to date that tested this hypothesis under natural conditions was conducted in the American

\*Author for correspondence (c.m.eising@biol.rug.nl).

kestrel, in which yolk androgens are also higher in later-laid eggs (Sockman & Schwabl 2000). The study failed to support the hypothesis because, in contrast to what was expected, chicks from eggs with experimentally increased androgen levels hatched later and showed reduced growth and lower survival.

We conducted a field experiment in which we manipulated the yolk hormone concentrations of black-headed gull eggs by mimicking the normal increase in yolk androgens from egg to egg while controlling for egg quality by using only first-laid eggs. Black-headed gulls lay a modal clutch of three eggs that hatch asynchronously. Androgen levels in female black-headed gulls are high during the egg-laying phase (Malickiene 1999), and the concentrations of testosterone and androstenedione in the yolk increase significantly with laying order (Groothuis & Schwabl 2001; present study). Black-headed gull parents feed their chicks by regurgitating food onto the ground, over which the chicks then have to compete. Elevated levels of plasma androgens after hatching not only enhance the aggressiveness of chicks of this species in the short term (Groothuis & Meeuwissen 1992), but also have long-term effects on aggressiveness (Ros 1997). Therefore, it is likely that chicks hatching from later-laid eggs, in which they were exposed to high androgen levels during embryonic development, may be better competitors when competing for food with older siblings.

We measured and manipulated testosterone and androstenedione concentrations in the eggs of black-headed gulls in order to determine whether increasing yolk androgen levels in relation to laying order enhance the growth and survival of chicks. We hypothesized that chicks hatching later within a brood benefit from elevated androgen levels through an increase in their competitive ability, resulting in enhanced growth and survival.

## 2. METHODS

### (a) *Study area and species*

Eggs were collected and the experiment was conducted in two black-headed gull colonies (*c.a.* 1250 and 500 breeding pairs, respectively) on the north coast of The Netherlands. Black-headed gulls are monogamous colony breeders and defend small breeding territories on the salt marsh. Clutches typically contain three eggs (Cramp & Simmons 1983), which are laid over a three- to five-day period (average three and a half days). On average, the eggs are incubated for 23 days and hatch over a one- to four-day period (average two days). Young typically fledge at the age of 26–28 days (Glutz von Blotzheim & Bauer 1982). The nest is situated on the ground. Both males and females incubate the eggs and participate in chick rearing. Food consists mainly of shrimps, worms, crabs and small fish.

### (b) *Egg collection and androgen assays*

In 1998, empty nests were marked with sticks and checked every day for egg laying. Freshly laid eggs were marked with a non-toxic marker and collected for androgen analyses. The eggs were stored at  $-20^{\circ}\text{C}$  upon arrival at the housing facilities. Collected eggs were replaced with similar looking, marked, substitution eggs in order to prevent desertion of the nests. When three eggs of matching size and coloration were laid within a period of five days we were sure we had the first-, second- and

third-laid eggs of the same female and only those clutches ( $n = 41$ ) were used in the analyses.

Within seven months of collection, frozen eggs were thawed for 1 h, after which the yolk and albumen were separated. Yolks were weighed and homogenized with an equal (1 ml per gram of yolk) amount of water. Approximately 300 mg of this mixture was used for analysis. The extraction and radioimmunoassay procedures were as according to Schwabl (1993). We measured testosterone and androstenedione concentrations in single radioimmunoassays with interassay coefficients of variation of 3.37 and 2.47% for androstenedione and testosterone, respectively. Dihydrotestosterone and oestradiol levels were found to be low in a previous study (Groothuis & Schwabl 2001) and were therefore not measured or manipulated in this study.

### (c) *Experimental design*

In spring 1999, we created two types of nests containing three eggs. Since it is very probable that, besides differences in yolk androgen content, the eggs of a clutch also differ with laying order in mass, protein and water content and vitamins (e.g. in the common tern *Sterna hirundo*) (Heaney *et al.* 1998; Royle *et al.* 2001), we used only first-laid eggs for composing the experimental clutches. Since first-laid eggs have the lowest maternal androgen concentration, we were able to manipulate the hormone level by injections without exceeding the levels normally found in later-laid eggs.

Although they were all first-laid A eggs, the three eggs will be referred to as A, B and C eggs, A being the first- and C the last-hatched egg. Chicks hatching from A, B and C eggs will be regarded as 'siblings'. The experimental nests consisted of either three oil-treated eggs (A oil, B oil and C oil, which will be referred to as oil nests) or one oil- (A egg) and two androgen (H)-treated eggs, with doses increasing with hatching order in order to mimic the natural situation (A H<sub>0</sub>, B H<sub>1</sub> and C H<sub>2</sub>, which will be referred to as H nests). Note that in these clutches the A egg was always oil injected. The eggs of the experimental clutches were matched for egg weight. Hatching interval was fixed at one day by placing the eggs into the clutches at 24 h intervals.

In order to ensure that an egg was a first-laid egg, colonies were visited daily and all single eggs were marked as '1' using a non-toxic, water-resistant marker. Measurements of mass, width and length were taken using a balance and callipers, and laying date was noted. If a nest had gained an additional egg upon a next visit, this egg was marked as '2' and the next egg as '3'. When a nest was completed with three eggs, egg 1 was considered to be the first-laid egg and was used in an experimental clutch. If any of the eggs in a nest deviated in shape, colour or pattern, this nest was excluded from the experiment since this may have indicated that the nest contained eggs of more than one female and, therefore, that the laying order was not unambiguously ascertained.

After clutch completion, the yolk of the first-laid egg was injected with either 50  $\mu\text{l}$  of vehicle (sterile sesame oil for A, B and C eggs in oil nests and A eggs in H nests) or 50  $\mu\text{l}$  of androgen solution (B and C eggs in H nests). Androgen treatment consisted of injection of a mixture of 0.09  $\mu\text{g}$  of testosterone (4-androsten-17 $\beta$ -ol-3-one) (Sigma, Germany) and 7.5  $\mu\text{g}$  of androstenedione (5 $\alpha$ -androstane-3, 17-dione) (Sigma, Germany) per 50  $\mu\text{l}$  of vehicle for B eggs and 0.12  $\mu\text{g}$  of testosterone and 10.0  $\mu\text{g}$  of androstenedione per 50  $\mu\text{l}$  of vehicle for C eggs. Because of the variable concentrations found in first-laid eggs, the total androgen concentrations after injection may have been high in

some eggs, but were probably within the physiological range found for second- and third-laid eggs (present study). The injections were made with a 25 G needle. The hole in the shell was then sealed with a tiny strip of flexible wound dressing (Opsite, Smith & Nephew, Hull, UK). All eggs remained in their original nest for incubation until the pipping stage. In order to ensure that none of the chicks were raised by their own parents, all experimental eggs were relocated to foster nests at this stage. In order to produce equal hatching intervals in all nests, we placed an A egg into an experimental nest on day  $X$ , a matching B egg on day  $X + 1$  and a matching C egg on day  $X + 2$ .

All experimental nests were situated within enclosures (wire mesh covered with cloth of 40–50 cm height) that varied in size from 30 to 100 m<sup>2</sup>. Enclosures always contained from 10 up to 25 nests for both oil and H nests. After hatching, all chicks were marked with a small numbered plastic band for individual identification.

#### (d) *Experimental nests*

We injected 595 eggs within a two-week period. We were able to create 46 nests (22 oil nests and 24 H nests) from the 231 eggs that hatched, which each contained three chicks that hatched from eggs with equal egg mass and with the desired degree of hatching asynchrony. The average hatch date did not differ for oil- and H-treated clutches (two-sample  $t$ -test,  $t_{55} = 0.852$  and  $p = 0.398$ ).

#### (e) *Survival and growth*

Survival of chicks was assessed by recording the presence of each chick in the enclosures at least every other day. During their first two weeks after hatching, chicks were considered dead when found as such or when not found again during the rest of the experiment. When older than two weeks, missing chicks as well as their sibs were excluded from the survival analyses when they were not found dead because they may have managed to escape from the enclosures. Their sibs were also excluded from the analyses because this 'brood reduction' had altered the competitive position of the remaining chick(s).

We measured body mass and tarsus plus toe (leg) length of all chicks every second day in order to determine whether chicks hatching from androgen-treated eggs grew faster than chicks hatching from oil-treated eggs and whether the effects of androgen on growth depended on hatching order. Body mass was measured beginning on the day of hatching, and leg length was first measured when chicks were one day old. Chick body mass was measured to the nearest 0.5 g using a Pesola spring balance and leg length was scored to the nearest 1 mm with a ruler. The data set of the 46 three-chick nests consisted of 665 body mass and 538 leg length measurements. It should be noted that the sample size decreased with age from 46 to 9 nests as nests in which one or more chicks died or disappeared were withdrawn from the analyses. We followed chick development in the field until they were at least 30 days old, after which we brought them into the laboratory in order to monitor development until maturity. The birds were considered to have fledged at an age of 30 days.

#### (f) *Statistical procedures*

The data on androgen levels in natural clutches and time until hatching of experimental eggs are presented as means and standard errors (s.e.). The relationship between androgen levels and laying order was tested using a repeated-measures ANOVA. Survival was tested using the Wilcoxon Gehan statistic in the

Life Tables option of SPSS and the  $\chi^2$ -statistic. The effects of treatment and hatching order on size at hatching were tested using parametric statistics (Pearson correlations and one-way ANOVAs). The data on body mass and leg length were analysed using hierarchical linear modelling (Bryk & Raudenbush 1992; Loonen *et al.* 1999) on the raw data using the MLWin Program. This method accommodates unbalanced data and allows analyses of variances and covariances, while simultaneously taking into account the nested relationships between nest mates and repeated measures of the same chick and controls for multiple (independent) variables. A growth curve was fitted for each model with age and age<sup>2</sup> as independent variables until the point where the curve began to level off (14 days for leg length and 21 days for body mass). The effect of age, age<sup>2</sup>, nest treatment, hatching order and possible interactions were tested. Age contributed significantly ( $\alpha = 0.05$ ) to the model in all tests and, therefore, these statistics are not presented. Significance was based on a two-tailed  $t$ -test. Only variables that contributed significantly ( $\alpha = 0.05$ ) to the model were retained. Only these  $p$ -values are presented in the text. A full description of the model and the complete output can be found in electronic Appendix A. Colony had no effect on any of the dependent variables and was therefore excluded from the analyses. *Post hoc* analyses were performed using the same test for subsamples and sequential Bonferroni correction. For optimal graphical representation of the growth data, we normalized the mass and leg length measurements for all chicks at all ages by subtracting the means of B oil chicks (the intermediate group) from each nestling's body mass or leg length. Each point in the figure 2 graphs represents the difference in means between the groups.

### 3. RESULTS

#### (a) *Androgen concentrations in natural clutches*

The testosterone and androstenedione concentrations measured in the first-, second- and third-laid eggs of 41 nests are shown in table 1.

The repeated-measures ANOVAs indicated that the concentrations increased significantly with laying order for both testosterone ( $F_{2,39} = 9.335$  and  $p < 0.001$ ) and androstenedione ( $F_{2,37} = 7.096$  and  $p < 0.005$ ). *Post hoc* contrasts indicated that first-laid eggs had a lower androstenedione concentration than second and third eggs, whereas the testosterone concentration was significantly different for all eggs. We used this information for calculating the dosages in the experimental clutches in which we tested the effects of maternal androgens. We injected dosages of testosterone and androstenedione into first-laid eggs that mimicked those in first-, second- and third-laid eggs in natural clutches.

#### (b) *Hatching success and time to hatch*

The hatching success and incubation times of the eggs in experimental clutches are presented in table 2.

Hatching success did not differ between the three treatments ( $\chi^2 = 4.46$ , d.f. = 5 and n.s.). However, time to hatch was significantly affected by treatment (one-way ANOVA,  $F_{2,184} = 0.80$  and  $p < 0.05$ ). *Post hoc* contrasts indicated that eggs treated with the highest androgen concentration (C H<sub>2</sub> eggs) hatched sooner than B H<sub>1</sub> or oil-treated eggs. This result is very interesting in itself but it does not influence the interpretation of the present experiment for the following reasons. First, our experimental clutches were set



Table 1. Maternal androgen concentrations ( $\mu\text{g per mg of yolk}$ ) in yolks of first-, second- and third-laid eggs

egg	mean testosterone ( $\pm$ s.e.)	range of testosterone	mean androstenedione ( $\pm$ s.e.)	range of androstenedione
first	10.96 $\pm$ 0.62	1.9–21.5	607.01 $\pm$ 42.96	118.3–1261.6
second	13.17 $\pm$ 0.63	5.7–24.0	810.30 $\pm$ 48.68	148.7–1761.1
third	15.08 $\pm$ 0.79	6.1–26.2	825.58 $\pm$ 42.06	248.8–1423.7

Table 2. Number and percentage of eggs that hatched, failed to hatch or were predated upon for eggs injected with vehicle (the A, B and C eggs in an oil clutch and the A eggs in H clutches) low androgen concentration (B eggs in H clutches) and high androgen concentration (C eggs in H clutches)

(The time to hatch is also presented for these eggs.)

treatment	number hatched	number failed	number predated	total	number of days until hatching ( $\pm$ s.e.)
oil	137 (38.9%)	138 (39.2%)	77 (21.9%)	352	24.7 $\pm$ 0.11
B in H	56 (45.2%)	42 (33.9%)	26 (20.9%)	124	24.9 $\pm$ 0.18
C in H	38 (32.0%)	53 (44.5%)	28 (23.5%)	119	24.2 $\pm$ 0.27

up in order to control the hatch interval between subsequent eggs of a clutch (always one day apart) and, second, these earlier-hatching chicks had the same size at hatching (see below). Thus, only a direct effect of androgens in the egg on competitive abilities or androgen-induced earlier hatching accounts for the enhanced growth rates that are reported below.

### (c) Survival

Survival up to the age of 30 days could be accurately recorded in 19 oil and 15 H nests that contained three chicks. Survival of first-hatched chicks was highest and survival of third-hatched chicks was lowest in both oil (figure 1a) and H (figure 1b) nests. The timing of brood reduction was also similar in both types of nests. The fraction of surviving chicks decreased significantly with hatching order in both oil (Wilcoxon Gehan statistic = 13.354 and  $p < 0.002$ ) and H nests (Wilcoxon Gehan statistic = 9.678 and  $p < 0.01$ ). Androgen treatment did not affect the survival chances of second- (Wilcoxon Gehan statistic = 0.628 and  $p = 0.43$ ) or third-hatched chicks (Wilcoxon Gehan statistic = 0.125 and  $p = 0.72$ ). The survival chances of first-hatched chicks, which were oil injected in both types of nests, were also not influenced by the presence of treated or non-treated sibs (Wilcoxon Gehan statistic = 0.449 and  $p = 0.50$ ). On average, 1.26 ( $\pm 0.27$ ) chicks fledged from oil nests and 1.67 ( $\pm 0.25$ ) chicks fledged from H nests. These numbers were not significantly different ( $t_{32} = -1.058$  and  $p = 0.298$ ).

### (d) Growth

#### (i) Body mass

The hatching mass of the chicks was strongly correlated with egg weight ( $r = 0.411$ ,  $n = 152$  and  $p < 0.01$ ) and did not vary between the two types of nests ( $F_{1,139} = 0.007$  and  $p = 0.93$ ) or with hatching order ( $F_{2,139} = 0.806$  and  $p = 0.45$ ). However, in the following weeks A chicks in H nests tended to be lighter (figure 2a) and C chicks in H nests tended to be heavier (figure 2c) compared with their

oil-treated counterparts. Indeed, the overall effect of nest treatment in the model was not significant ( $p = 0.139$ ).

There was a significant effect of hatching order on mass ( $p = 0.001$ ). Regardless of treatment, first-hatched chicks were heavier than second-hatched chicks, which were heavier than last-hatched chicks. However, the effect of hatch order on chick mass depended on treatment, as indicated by a significant treatment  $\times$  order interaction term ( $p = 0.001$ ). A chicks in oil nests that had to compete with two oil-treated sibs had a higher body mass than A chicks in H nests that had to compete with two androgen-treated sibs (figure 2a). *Post hoc* analyses of this difference in mass over the first three weeks showed that it was significant ( $p = 0.010$ ). In contrast, over the same period, B chicks in H nests were significantly heavier ( $p = 0.046$ ) than B chicks in oil nests (figure 2b). This was also true for last-hatched chicks (figure 2c), with C chicks in H nests being significantly heavier than C chicks in oil nests ( $p = 0.001$ ). Thus, the mass hierarchy among chicks was less pronounced in androgen-treated nests than in oil-treated nests. After day 21, body mass differences between the groups fluctuated much more, probably because of the small sample sizes.

#### (ii) Size

Leg length, which is a good measure of size, did not vary with nest treatment at the age of one day ( $F_{1,143} = 0.003$  and  $p = 0.96$ ). However, leg length varied significantly with hatching order ( $F_{2,143} = 4.25$  and  $p = 0.02$ ). The interaction effect of both factors was not significant ( $F_{2,143} = 1.05$  and  $p = 0.35$ ). The measurements of leg length seem to support the idea that there is a relation between androgen treatment and growth (figure 2d–f). Both B and C chicks in H nests appeared to have longer legs than B and C chicks in oil nests. In contrast, A chicks in oil nests competing with two oil-treated sibs had longer legs than A chicks that had to compete with androgen-treated sibs. Statistical analysis revealed no overall treatment effect ( $p = 0.825$ ) on leg length, but a

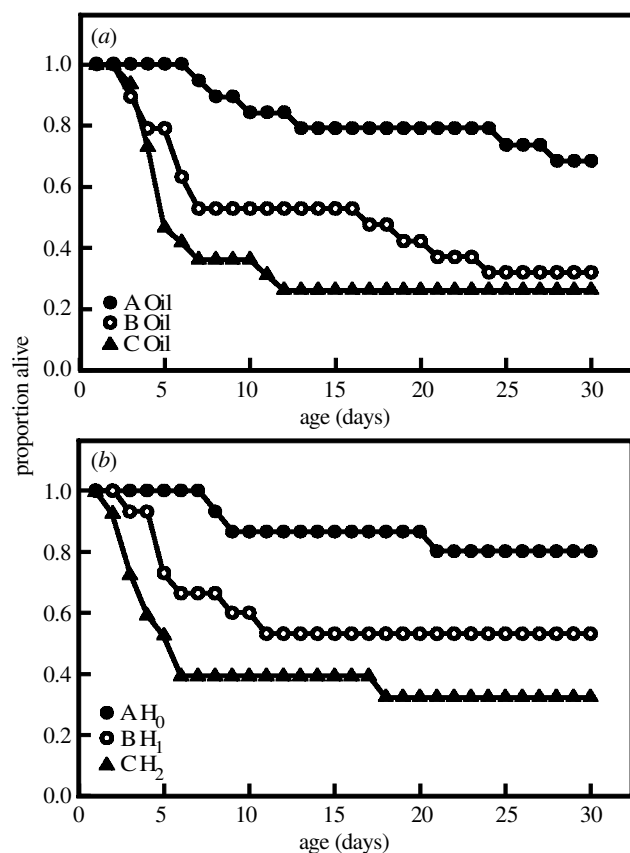


Figure 1. (a) The proportions of first-, second- and third-hatched chicks surviving in oil nests ( $n = 19$ ). (b) The proportions of first-, second- and third-hatched chicks surviving in H nests ( $n = 15$ ).

significant effect of hatching order ( $p = 0.001$ ). Furthermore, the interaction term of treatment hatch order was significant ( $p = 0.001$ ). *Post hoc* analysis showed that A chicks of H nests had significantly shorter legs than A chicks in oil nests ( $p = 0.001$ ). On the other hand, C chicks in H nests had significantly longer legs than C chicks in oil nests ( $p = 0.044$ ). The leg lengths of B chicks in H and oil nests were not different ( $p = 0.09$ ). These results suggest that the size hierarchy in nests was reduced as a result of the androgen treatment.

In summary, the above results show that, when controlling for egg quality, hatching asynchrony was the most important determinant of size hierarchy within broods. However, elevated androgen levels in later-hatching eggs appeared at least partly to compensate for the size hierarchy resulting from asynchronous hatching.

#### 4. DISCUSSION

This study shows that, in black-headed gulls, yolk androgen levels increase with laying order and can influence chick development under natural conditions. In line with the hypothesis, our experimental elevation of yolk androgen levels, while controlling for egg quality and hatching asynchrony, enhanced chick growth and thereby counteracted hatching asynchrony. It is often hypothesized that reduced growth of last-hatched chicks, as compared with growth of their earlier-hatched siblings, is a consequence of their poor ability to compete over food

with older and larger sibs (Ryden & Bengtsson 1980; Graves *et al.* 1984; Bryant & Tatner 1990; Price & Ydenberg 1995). Our data show that parents may 'help' their last-hatched offspring by favouring them with higher androgen concentrations, which seems to enable them to compete better with their sibs, as anticipated by Schwabl (1993). The only other field study, which was conducted on American kestrels (*Falco sparverius*) by Sockman & Schwabl (2000), failed to support this hypothesis. The results of that study suggested that high concentrations of androgens handicap nestlings hatching from later-laid eggs, thereby enhancing the effects of hatching asynchrony even further.

The competitive ability of gull chicks is determined by size, aggression and mobility (Royle & Hamer 1998), all of which may be influenced by androgens. We propose that differential androgen allocation to the eggs of a clutch is a mechanism that enhances reproductive output. Asynchronous incubation is a mechanism that allows economical brood reduction when food availability is low (O'Connor 1978; Hahn 1981). Parents can thus forego the high costs associated with chick feeding by rapidly eliminating the young that requires most future investment (Temme & Charnov 1987). In order to forego unwanted brood reduction when food is abundant, the size hierarchy within a brood should be diminished. There are some indications that eggs hatch more synchronously when food is abundant (Murphy 1994; Wiebe & Bortolotti 1994) or parental quality is higher (Sydesman & Emslie 1992; Wiebe & Bortolotti 1994). However, the need for the onset of incubation in order to avoid a decline in egg viability or egg predation (Magrath 1990; Stoleson & Beissinger 1995) may preclude complete hatch synchronization, thereby calling for additional mechanisms that allow the shaping of the optimal brood size.

A mechanism that may enhance reproductive output when food quantity is high is differential androgen allocation to the eggs of a clutch. Last-hatched black-headed gull chicks clearly benefited from hatching from an androgen-treated egg. At the same time, growth of the first chick was clearly suppressed by the presence of two androgen-treated sibs. Schwabl (1993) showed that maternal androgens elicit more begging behaviour from canary chicks and, in many avian species, higher begging intensity usually results in a higher rate of food provisioning by parents (Burford *et al.* 1998; Price 1998). However, parents may not be able to increase their effort in nests with three chicks. Therefore, it is likely that, in black-headed gulls, androgens enhance the competitive abilities of the chicks. This may have a pronounced effect on the distribution of food within androgen-treated nests.

Our data also show that eggs with a high level of yolk androgens hatched significantly sooner than eggs with low androgen levels, while there were no body mass differences at hatching between egg treatments. The interaction term of treatment  $\times$  hatching order effect on size was not statistically significant, indicating that the leg length of C chicks in H broods did not differ from that of A and B chicks in H broods, despite the fact that the former had hatched sooner. Moreover, there was some indication that, in oil nests, C chicks had smaller legs at hatching than A or B chicks, although all chicks hatched from eggs of the same quality and mass. This may be

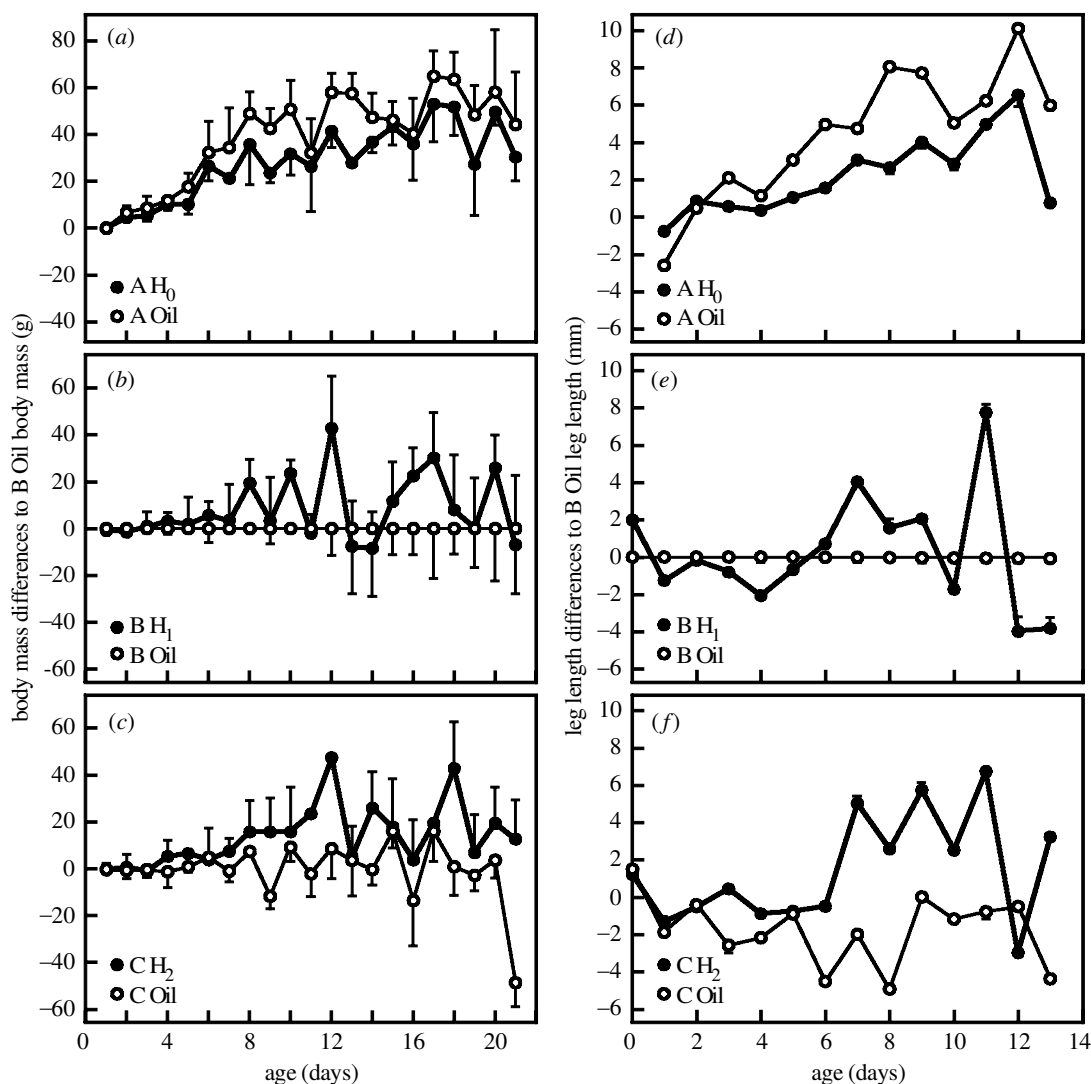


Figure 2. (a–c) Body mass changes with increasing age compared with the body mass of B chicks in oil nests for first-, second- and third-hatched chicks in all oil and H nests. Note that the mass of B chicks from control nests is 0 at all ages because we normalized the mass of the other chicks for all ages by subtracting the mean mass of the B chicks in oil nests. (d–f) Similar data for leg length. For body mass the sample size decreases over time from 22 to 5 oil nests and from 24 to 4 H nests. For leg length the sample size decreases over time from 22 to 6 oil nests and from 24 to 6 H nests. The standard errors are very small for leg length and are only missing for age 12 days in A oil chicks, age 7 and 9 days in B oil chicks, age 6, 8, 13 and 14 days in C oil chicks, age 14 days in A H<sub>0</sub> chicks, age 11 days in B H<sub>1</sub> chicks and age 12 days in C H<sub>2</sub> chicks.

explained by the fact that the incubation patterns after transfer to foster nests varied slightly with the number of hatched chicks in a nest, which influenced egg temperature and, thus, development (Evans 1990). These results indicate that, besides having a beneficial postnatal effect on competitiveness, androgens may also enhance embryonic development, as was shown by Hauser & Toran-Allerand for mice (1989). This would decrease the size hierarchy already within a brood at a very early stage. There are indeed indications that, in natural clutches, last-laid eggs, which tend to be smaller, have a tendency to hatch sooner, thereby reducing hatching asynchrony (Glutz von Blotzheim & Bauer 1982; Blair-Bollinger 1994). Although our results show that hatching asynchrony is the most important factor determining chick size in black-headed gulls, last-hatched chicks may be at least partly compensated for their handicap by faster embryonic development and enhanced post-

hatching growth via higher levels of maternal androgens in their eggs.

The effect of yolk androgen levels on chick survival was less clear. This may have been due to the small sample size. Although not statistically significant, the average number of fledglings was higher in H nests. This was predominantly caused by increased survival of B chicks. The mortality of last-hatched chicks was highest during the first week. This can be explained by the fact that last-hatched chicks were less mobile and less able to obtain food during this period. After this period the mortality of last-hatched chicks was low, indicating that they were able to fend for themselves, despite their smaller size. The lack of survival differences between oil and H nests may have been obscured by parental quality. Presumably only high-quality parents are able to raise a full brood. However, since foster parents were assigned randomly, the fractions of high- and low-quality parents

were probably equal in the oil and H nests and in both types of nests high-quality parents were probably able to supply enough food for their three chicks. However, androgen treatment seemed to change the distribution of food across the chicks. This probably resulted in the less-pronounced body mass differences at fledging in H nests as compared with oil nests. Since mass at fledging is an important determinant of first winter survival (Van der Jeugd & Larsson 1998), we suspect that the overall survival probabilities of chicks fledged from H nests might be higher.

We have shown that elevated yolk androgen levels can be beneficial during early development. High yolk androgen levels may also benefit success later in life, as demonstrated in canaries (Schwabl 1993). Canaries hatching from eggs containing higher doses of maternal androgens obtained higher social ranks later in life. This may be the long-term result of early organizing effects of androgens, which may even influence reproductive strategies. Ros (1997) showed that black-headed gulls treated early in life with testosterone have a higher sensitivity to androgens later on. This may incite birds that developed in eggs with higher androgen levels to start reproduction at a younger age and help them to acquire and defend a territory of high quality. Such consequences later in life are the focus of further studies.

We thank all the organizations and farmers in the Linthorst-Homan polder for granting us permission to work on their properties. We are much obliged to Maurine Dietz, Maarten Loonen, Ido Pen, Miguel Rodriguez-Girones and Simon Verhulst for useful comments on the data analyses. We would particularly like to thank Karen Blaakmeer, Leo Bruinzeel and Bernd Riedstra for their help in the field. Dick Visser kindly drew the figures. This project was financed by NWO (Dutch Science Foundation) grant 805.30.204 to S. Daan and T. G. G. Groothuis and approved by the animal experimentation committee under licence DEC 2158.

## REFERENCES

- Adkins-Regan, E., Ottinger, M. A. & Park, J. 1995 Maternal transfer of estradiol to egg yolks alters sexual differentiation of avian offspring. *J. Exp. Zool.* **271**, 466–470.
- Blair-Bollinger, P. 1994 Relative effects of hatching order, egg-size variation, and parental quality on chick survival in common terns. *Auk* **111**, 263–273.
- Bryant, D. M. & Tatner, P. 1990 Hatching asynchrony, sibling competition and siblicide in nestling birds: studies of swiftlets and bee-eaters. *Anim. Behav.* **39**, 657–671.
- Bryk, A. S. & Raudenbush, S. W. 1992 *Hierarchical linear models, applications and data analysis methods*. London: Sage.
- Burford, J. E., Friedrich, T. J. & Yasukawa, K. 1998 Response to playback of nestling begging in the red-winged blackbird, *Agelaius phoeniceus*. *Anim. Behav.* **56**, 555–561.
- Clark, A. B. & Wilson, D. S. 1981 Avian breeding adaptations: hatching asynchrony, brood reduction and nest failure. *Q. Rev. Biol.* **56**, 253–277.
- Conley, A. J., Elf, P., Corbin, C. J., Dubowsky, S., Fivizzani, A. & Lang, J. W. 1997 Yolk steroids decline during sexual differentiation in the alligator. *Gen. Comp. Endocrinol.* **107**, 191–200.
- Cramp, S. & Simmons, K. E. L. (ed.) 1983 *Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic. Volume III: waders to gulls*. Oxford: Oxford University Press.
- Eising, C. M., Duijs, B. & Groothuis, T. G. G. 2001 The effect of laying sequence and environmental conditions on growth and survival of black-headed gull chicks. (In preparation.)
- Evans, R. M. 1990 Embryonic fine tuning of pipped egg temperature in the American white pelican. *Anim. Behav.* **40**, 963–968.
- Feist, G., Schreck, C. B., Fitzpatrick, M. S. & Redding, J. M. 1990 Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. *Gen. Comp. Endocrinol.* **80**, 299–313.
- Forbes, L. S. 1994 The good, the bald and the ugly: Lack's brood reduction hypothesis and experimental design. *J. Avian Biol.* **25**, 338–343.
- French Jr, J. B., Nisbet, I. C. T. & Schwabl, H. 2001 Maternal steroids and contaminants in common tern eggs: a mechanism of endocrine disruption? *Comp. Biochem. Physiol.* (In the press.)
- Gil, D., Graves, J., Hazon, N. & Wells, A. 1999 Male attractiveness and differential testosterone investment in zebra finch eggs. *Science* **286**, 126–128.
- Glutz von Blotzheim, U. N. & Bauer, K. M. (ed.) 1982 *Handbuch der Voegel Mitteleuropas. Band 3*. Wiesbaden, Germany: Akademische Verlagsgesellschaft.
- Graves, J., Whitten, A. & Henzi, P. 1984 Why does the herring gull lay three eggs? *Anim. Behav.* **32**, 798–805.
- Groothuis, T. G. G. & Meeuwissen, G. 1992 The influence of testosterone on the development and fixation of the form of displays in two age classes of young black-headed gulls. *Anim. Behav.* **43**, 189–208.
- Groothuis, T. G. G. & Schwabl, H. 2001 The influence of laying sequence and habitat characteristics on maternal yolk hormone levels. (In preparation.)
- Hahn, D. C. 1981 Asynchronous hatching in the laughing gull: cutting losses and reducing rivalry. *Anim. Behav.* **29**, 421–427.
- Hauser, K. F. & Toran-Allerand, C. D. 1989 Androgen increases the number of cells in fetal mouse spinal chord cultures: implications for motorneuron survival. *Brain Res.* **485**, 157–164.
- Heaney, V., Nager, R. G. & Monaghan, P. 1998 Effect of increased egg production on egg composition in the common tern *Sterna hirundo*. *Ibis* **140**, 693–696.
- Janzen, F. J., Wilson, M. E., Tucker, J. K. & Ford, S. P. 1998 Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. *Gen. Comp. Endocrinol.* **111**, 306–317.
- Lack, D. 1947 The significance of clutch size. *Ibis* **89**, 302–352.
- Lipar, J. L., Ketterson, E. D. & Nolan, V. 1999 Intra-clutch variation in testosterone content of red-winged blackbird eggs. *Auk* **116**, 231–235.
- Loonen, M. J. J. E., Bruinzeel, L. W., Black, J. M. & Drent, R. H. 1999 The benefit of large broods in barnacle geese: a study using natural and experimental manipulations. *J. Anim. Ecol.* **68**, 753–768.
- McCormick, M. I. 1999 Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. *Oecologia* **118**, 412–422.
- Magrath, R. D. 1990 Hatching asynchrony in altricial birds. *Biol. Rev. Camb. Phil. Soc.* **65**, 587–622.
- Malickiene, D. 1999 Changes in reproductive hormones and spatial-ethological structure of the colony of black-headed gulls (*Larus ridibundus*) during the breeding period. Doctoral dissertation (summary), Institute of Ecology, Vilnius, Lithuania.
- Murphy, M. T. 1994 Breeding patterns of eastern phoebes in Kansas: adaptive strategies or physiological constraint? *Auk* **111**, 617–633.



- O'Connor, R. J. 1978 Brood reduction in birds: selection for fratricide, infanticide and suicide? *Anim. Behav.* **26**, 79–96.
- Price, K. 1998 Benefits of begging for yellow-headed blackbird nestlings. *Anim. Behav.* **56**, 571–577.
- Price, K. & Ydenberg, R. 1995 Begging and provisioning in broods of asynchronously hatched yellow-headed blackbird nestlings. *Behav. Ecol. Sociobiol.* **37**, 201–208.
- Ros, A. F. H. 1997 Testosterone and early aggression in the black-headed gull. PhD dissertation, University of Groningen, Groningen, The Netherlands.
- Royle, N. J. & Hamer, K. C. 1998 Hatching asynchrony and sibling size hierarchy in gulls: effects on parental investment decisions, brood reduction and reproductive success. *J. Avian Biol.* **29**, 266–272.
- Royle, N. J., Surai, P. F. & Hartley, I. R. 2001 Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? *Behav. Ecol.* (In the press.)
- Ryden, O. & Bengtsson, H. 1980 Differential begging and locomotory behaviour by early and late hatched nestlings affecting the distribution of food in asynchronously hatched broods of altricial birds. *Z. Tierpsychol.* **53**, 209–224.
- Schwabl, H. 1993 Yolk is a source of maternal testosterone for developing birds. *Proc. Natl Acad. Sci. USA* **90**, 11 446–11 450.
- Schwabl, H. 1996a Environment modifies the testosterone levels of a female bird and its eggs. *J. Exp. Biol.* **276**, 157–163.
- Schwabl, H. 1996b Maternal testosterone in the avian egg enhances postnatal growth. *Comp. Biochem. Physiol.* **114**, 271–276.
- Schwabl, H., Mock, D. W. & Gieg, J. A. 1997 A hormonal mechanism for parental favouritism. *Nature* **386**, 231.
- Sockman, K. W. & Schwabl, H. 2000 Yolk androgens reduce offspring survival. *Proc. R. Soc. Lond. B* **267**, 1451–1456.
- Stoleson, S. H. & Beissinger, S. R. 1995 Hatching asynchrony and the onset of incubation in birds, revisited: when is the critical period? *Curr. Ornithol.* **13**, 191–270.
- Sydeinan, W. J. & Emslie, S. D. 1992 Effects of parental age on hatching asynchrony, egg size and the 'third chick disadvantage' of Western gulls (*Larus occidentalis*) on Southeast Farallon Island, California, during the 1984–1990 breeding seasons. *Auk* **109**, 242–248.
- Temme, D. H. & Charnov, E. L. 1987 Brood size adjustment in birds: economical tracking in temporally varying environment. *J. Theor. Biol.* **126**, 137–147.
- Van der Jeugd, H. & Larsson, K. 1998 Pre-breeding survival of barnacle geese *Branta leucopsis* in relation to fledgling characteristics. *J. Anim. Ecol.* **67**, 953–966.
- Wiebe, K. L. & Bortolotti, G. R. 1994 Food supply and hatching spans of birds: energy constraints or facultative manipulation? *Ecol.-Temp.* **75**, 813–823.
- Winkler, D. W. 1993 Testosterone in egg yolks: an ornithologist's perspective. *Proc. Natl Acad. Sci. USA* **90**, 11 439–11 441.