Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study

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Black-headed gulls, Larus ridibundus, produce clutches of three eggs, which contain high levels of maternal androgens in the yolk. These levels increase with laying order and the eggs hatch asynchronously. Experiments have supported the hypothesis that this within-clutch variation in maternal androgens mitigates the disadvantage of last-hatched chicks in sibling competition, by enhancing embryonic development and early posthatching growth. We hypothesized that these effects come about by the stimulating effects of maternal androgens on begging behaviour and competitive ability. In the field, we injected first-laid eggs of a clutch (which have a low androgen level) with either an androgen solution (T eggs) or vehicle (Oil eggs). We then created pairs of chicks hatched from Oil and T eggs, matched for egg mass and hatching date. Parent–chick interactions were recorded from observation hides. Chicks from T eggs hatched almost half a day sooner than those from Oil eggs. Furthermore, chicks from T eggs were more active during the first week after hatching, were more often the first to react to the approaching parent, begged more frequently, and obtained the larger share of food. We conclude that the enhancing effect of yolk androgens on growth in this species arises at least partly through androgen-mediated effects on the chicks’ behaviour.

A certain degree of hatching asynchrony between siblings of the same brood occurs in many avian species. At least 17 hypotheses have been put forward to explain the costs and benefits of hatching asynchrony (for an overview see Clark & Wilson 1981; Magrath 1990; Stoleson & Beissinger 1995). Of these, Lack’s (1947) ‘brood reduction hypothesis’ is one of 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 a size that can be adequately fed. However, the size hierarchy and accompanying competitive asymmetry within the brood may result in unnecessary brood reduction when food is abundant, thereby reducing parental fitness (Forbes 1994).

Recently, a mechanism has been described by which mothers may compensate for such possible detrimental effects of hatching asynchrony. Schwabl (1993) was the first to show consistent within-clutch variation in androgens of maternal origin in the yolk of avian species. Since then, variation in maternal androgen levels in the yolk has been described for several avian species (Schwabl 1993, 1996a, b; Adkins-Regan et al. 1995; Schwabl et al. 1997; Gil et al. 1999; Lipar et al. 1999; Sockman & Schwabl 2000; Eising et al. 2001; Royle et al. 2001; Groothuis & Schwabl 2002; Müller et al. 2002; Whittingham & Schwabl 2002). Yolk hormone levels correlate with circulating plasma hormone levels in the female (Schwabl 1996a) and may vary adaptively with the species’ reproductive strategy and social requirements (Schwabl et al. 1997; Eising et al. 2001; Groothuis & Schwabl 2002; Müller et al. 2002; Whittingham & Schwabl 2002, but see also Sockman & Schwabl 2000). So far, effects of these maternal yolk hormones have been described primarily with respect to morphological characteristics and growth of the offspring (e.g. Lipar & Ketterson 2000; Eising et al. 2001). Schwabl (1996b) investigated the effect of maternal androgens on begging behaviour in canaries, Serinus canaria and found that testosterone treatment increased the number of begging bouts, the total time spent begging and the duration of a begging bout within the first hour after hatching. This study was done under laboratory conditions and did not assess parent–offspring interactions or the distribution of food among the brood.

We conducted an experimental field study on the begging behaviour of black-headed gull, Larus ridibundus, chicks. In this species, the three eggs of a clutch hatch asynchronously over 1–3 days. Both yolk testosterone...
and androstenedione levels increase significantly with laying order (Eising et al. 2001). Chicks hatching from eggs with experimentally elevated yolk androgen levels benefit from these higher levels: they hatch sooner and grow faster in body mass and tarsus length (Eising et al. 2001). Chicks competing with siblings from androgen-treated eggs grew more slowly than chicks that had to compete with control siblings. This suggests that maternal androgens specifically enhance the competitive ability of the chick. Therefore, we compared the behaviours of chicks that hatched from eggs with an experimentally elevated androgen content with those of matched controls. Extrapolating from Schwabl’s results, we predicted that chicks from eggs with a high androgen content would be more competitive and alert, respond faster, be more persistent in begging and more successful at obtaining food.

**METHODS**

**Study Area and Species**

We studied black-headed gull colonies (ca. 1250 and 200 breeding pairs at Westernieland, 53°24′N, 06°28′E) on the salt marshes on the north coast of The Netherlands. Black-headed gulls are monogamous colony breeders and defend small breeding territories (1–4 m²). Clutches typically contain three eggs (Cramp & Simmons 1983), laid over 3–5 days (average 3.5 days). On average the eggs are incubated for 23 days (range 22–26 days) and in our study area eggs were incubated for 28 days (Walker et al. 1998; Glutz von Blotzheim & Bauer 1992). Typically circumstances would be more competitive and alert, respond faster, be more persistent in begging and more successful at obtaining food.

**Experimental Design**

In spring 2000 we created nests containing two experimental eggs, one with an elevated androgen level and one control egg. These eggs were matched for weight and hatching date and thus chicks were more or less of equal weight and equal age at the beginning of the experiment. Since there is evidence that not only yolk androgen content, but also egg mass, protein, vitamin and water content vary with laying sequence (e.g. in common tern, Sterna hirundo: Heaney et al. 1998; lesser black-backed gull, Larus fuscus: Royle et al. 2001), we used only the first-laid eggs of a clutch (A eggs) to compose the experimental clutches. These eggs contain the lowest maternal androgen concentration and enabled us to manipulate the hormone level by injections without exceeding the levels found normally in later-laid eggs.

We used the procedures to select and inject our eggs described by Eising et al. (2001). Briefly, empty nests were marked with sticks and checked every day for egg laying. To ensure that an egg was truly a first-laid egg, we marked all single eggs with ‘1’ using a nontoxic water-resistant marker. We weighed the egg on a balance and noted the laying date. If a nest had gained an additional egg by the next visit, this egg was marked ‘2’, and the next egg ‘3’. When a nest contained three eggs, the ‘1’ egg was considered to be a 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 indicate that the nest contained eggs of more than one female and that therefore laying order was not unambiguously ascertained. When three eggs of matching size and coloration were laid within a period of 5 days we were sure we had the first-, second- and third-laid eggs of the same female and only those clutches were used for the experiment.

On the day of clutch completion the yolk of the first-laid egg was injected either with 50 μl of vehicle (sterile cold-pressed sesame oil; Oil eggs) or 50 μl of vehicle containing a mixture of 0.12 μg testosterone (T) and 10.0 μg androstenedione (A4; T eggs). Thus, hormone levels in T eggs were elevated to resemble those of natural first-laid eggs. Eising et al. (2001) showed that natural first-laid eggs contain on average 10.96 pg T/mg yolk and 607.01 pg A4/mg yolk, and levels may range up to 26.2 pg T/mg yolk and 1761.1 pg A4/mg yolk. Since the average yolk weight of first-laid eggs ± SE was 10.152 ± 0.11 g (N=90), injecting 0.12 μg testosterone and 10.0 μg androstenedione per yolk was equivalent to adding 11.82 pg T/mg yolk and 985.03 pg A4/mg yolk. Thus, the total amount of androgens after injection averaged 22.78 pg T/mg yolk and 1592.04 pg A4/mg yolk, which is well within the natural range. For the injections we used a 25-G needle near the blunt edge of the egg. The hole in the shell was then sealed with a tiny strip of flexible wound dressing (Opsite, Smith & Nephew, Hull, U.K.).

All injected eggs were relocated to foster nests at the pipping stage. All foster nests were situated within enclosures (wire mesh covered with cloth, 40–50 cm high) varying in size from 30 to 100 m². Enclosures contained from 10 to 25 nests and enabled us to follow chick development. After hatching all chicks were marked with a small numbered plastic leg ring for individual identification. In addition, chicks were randomly coloured with picric acid, rhodamin B and malachite green oxalate dissolved in water on the head, chest, belly or wings to make individual recognition on tape (see below) possible. We refer to chicks hatched from androgen-injected eggs as T chicks and chicks from oil-treated eggs as Oil chicks.

**Experimental Nests**

We injected 624 eggs (415 Oil, 209 T). Of these, 90 (58 Oil, 32 T) were predated and 118 (78 Oil, 40 T) failed to hatch. The fate of 129 eggs (84 Oil, 45 T) was not followed. Of the 287 injected eggs that hatched (195 Oil, 92 T), we created 63 nests in which one Oil chick could be matched for egg weight and hatching date with an equally old T chick. Time until hatching could be reliably determined for 255 (177 Oil, 78 T) eggs and chick growth...
was measured regularly in 41 nests. Of these, 21 were used for behavioural observations. For the analyses of the different components of begging behaviour (see below) only nests were used where this component was scored in at least six begging bouts. Since not all behaviours were observed in all nests during six begging bouts, sample sizes differ for each of the behaviours scored and are given below and in the Results. The sexes of the chicks were not known and males and females were presumed to be equally distributed over the treatments.

**Behavioural Observations**

Black-headed gull parents feed their young by regurgitating food on to the ground in the chicks’ vicinity over which the chicks then have to compete. During the first week at least one parent is usually present at the nest, but later on both are often away simultaneously to forage. During the first few days parents regularly regurgitate food spontaneously and in abundance. Thereafter, chicks develop a conspicuous begging display to stimulate the parents to regurgitate food. During the first week we scored whether the chicks were active (standing, walking, peeping versus sleeping) while a parent was present at the nest (N = 11 nests). After the first week we scored several behavioural parameters during begging bouts. A begging bout was defined to start with the first reaction of a chick to an approaching parent (N = 19 nests) and ended when the chick(s) consumed the food or did not approach or show any sign of begging behaviour for at least 5 s. Typical begging displays consisted of up and down head movements, ‘pumping’, accompanied by a call during the downward movement (N = 19 nests). Pecking at the bill of the parent often followed pumping (N = 19 nests). We scored which chick was the first to respond to the parent’s return to the nest (N = 18 nests), wake up from the nest, come out of hiding, or start vocalizing. From the second week of age onwards, we scored which chick was the first to approach the parent and to make pumping movements and which was the first to make bill-pecking movements. We also recorded the frequency of bill pecks and pumping movements (N = 19 nests). When parents regurgitated food, we scored which chick was the first to start eating the food (N = 19 nests) and if possible (e.g. if a food item could be monopolized or one chick made hardly any feeding movements), which chick obtained the larger proportion of the food (N = 13 nests). All behaviour was recorded from hides on an elevated platform next to the enclosures. Begging behaviour was recorded on videotape using Canovision EX1 cameras with a 120 mm zoom lens. We also used binoculars to observe the feeding bouts and we added comments on the video recording. Recordings of the chicks’ begging behaviour started the second week after hatching and continued until the chicks were 3 weeks old. Recordings were usually made in the morning between 0800 and 1100 hours. On average we observed a nest on 5 days during development. If possible, several nests were recorded simultaneously. However, when a lot of feeding activity was going on, the observers focused on one nest and finished commenting on the begging bout of this nest before commenting on that of another. All begging bouts were recorded on tape and these were later scored in the laboratory by an observer unaware of the chicks’ treatment.

**Survival and Growth**

We assessed survival of chicks (41 nests) by recording the presence of each chick in the enclosures at least every other day. During the first 2 weeks of age chicks were considered dead when found so (7 T, 11 Oil) or when not seen during the rest of the experiment (4 T, 2 Oil). When older than 2 weeks, missing chicks (3 T, 7 Oil) as well as their siblings were excluded from the survival analyses when they were not found dead, because they may have managed to escape from the enclosures.

We measured body mass and tarsus length of all chicks (41 nests) every other day to determine whether treatment affected growth. Measurements usually began on the day of hatching. Chick body mass was measured to the nearest 0.5 g using a Pesola spring balance. We followed chick development in the field until they were at least 30 days old. At this age the birds were considered to have fledged and were brought into the laboratory for another study to monitor development until maturity.

**Statistical Analyses**

Activity scores (1, active, 0, not active) during the first week were summed and averaged per chick over the observed feeding bouts. The differences in average scores between chicks from T and Oil eggs were tested with a paired-sample t test. The proportion of bouts in which a chick was the first to react, approach, pump, peck or eat was averaged over all begging bouts. Since the proportions are complementary for the two chicks in the nest these data were analysed with a one-sample t test to see whether the proportion deviated significantly from 50%. All proportion data were arcsine transformed before testing. The average frequency of pumps and pecks both chicks showed per feeding bout was tested with a paired-sample t test.

The average time until hatching was compared for Oil and T eggs with an independent-sample t test since eggs were matched to each other only after the pipping stage. Survival data were analysed with the life tables option in SPSS (SPSS Inc., Chicago, U.S.A.). Age at death during the first week was tested nonparametrically with a Mann–Whitney U test. Structural body size at hatching was compared with a paired-sample t test. We used hierarchical linear models (Loonen et al. 1999) to test the effect of treatment on body mass in a nested design. A chick was excluded from all analyses if its sibling was dead or missing. Statistical tests are two tailed.

**Ethical Note**

We tried to minimize disturbance of the colony as much as possible and never disturbed it for more than 45 min. We did not enter the colony during rain, cold
could observe which chick obtained the larger share of the delivered food, T chicks were significantly more often the ones to eat most of it (one-sample test: \( t_{12}=4.898, P<0.001; \) Fig. 1k).

In conclusion, in nine of 10 behavioural scores, T chicks were sooner or more persistent in begging behaviour than Oil chicks. This was significant in four of these cases, of which two remained significant after Bonferroni correction (first to react and eating most). The
significance level was almost reached in two other cases, while we found significance levels around 0.10 in the three other cases.

Survival

The survival probability until fledging was 61.0% for chicks hatching from T eggs and 53.7% for Oil chicks. This was not significantly different (Wilcoxon Gehan statistic=0.309, \( P=0.578 \)). However, because brood reduction usually occurs during the first week after hatching, we looked at how soon T and Oil chicks died during the first week of life. There were only four nests in which a T chick died and four nests in which an Oil chick died during the first week. T chicks died on average 6 ± 0.40 days whereas Oil chicks died significantly sooner at 4 ± 0.82 days (Mann–Whitney \( U \) test: \( U=0.50, N_1=N_2=4, P=0.027 \)).

Growth

To check whether T and Oil chicks had a size asymmetry at hatching that could have caused differences in their begging behaviour, we tested body mass differences at hatching for the 26 nests for which we had data. There was no body mass difference at this stage: T chicks weighed on average ± SE 29.06 ± 0.90 g and Oil chicks 29.06 ± 0.90 g (paired-sample t test: \( t_{25}=-0.155, P=0.878 \)).

An asymptotic growth curve was fitted for both groups of T and Oil chicks separately using the function: \( W=\frac{A}{(1+e^{-k(t-r)})} \), in which \( A \) is the asymptotic body mass, \( k \) is the growth constant and \( t_r \) is the point of inflection. Asymptotic body mass was 237 g for T chicks and 235 g for Oil chicks. Growth constants were 0.200 and 0.197 for T and Oil chicks, respectively, and the point of inflection of the growth curve was 9.9 days for both. Using hierarchical linear models, we tested the effect of treatment on body mass in a nested design for chicks until day 10 and after day 10. There were no significant differences in body mass between T and Oil chicks in either period (age \( \leq 10 \) days: \( P=0.80 \); age >10 days: \( P=0.93 \)). Sample sizes decreased from 41 nests at the beginning to 30 at the end since surviving chicks were also excluded from the analyses when their siblings disappeared because growth of the single chick is then likely to be enhanced.

**DISCUSSION**

We hypothesized that maternal androgens have a function in mitigating the disadvantage in sibling competition of the last-hatched chick, by enhancing begging behaviour and the competitive ability over food. By means of a field experiment in which androgen content of the eggs was manipulated in a matched design and resulting androgen levels were high, but well within the natural range, we found support for all three predictions.

Hatching and Begging Behaviour

The finding that chicks from androgen-treated eggs hatched almost half a day sooner than chicks from control nests confirms our previous result (Eising et al. 2001; but see also Sockman & Schwabl 2000 for contradictory results). The shortened time until hatching in T chicks is within the natural range recorded for this species (22–26 days, Cramp & Simmons 1983). Given the fact that the average hatching interval in natural clutches is less than a day (Glutz von Blotzheim & Bauer 1982) a reduction of half a day substantially decreases hatching asynchrony. Our finding is also consistent with the finding that androgens stimulate the development of the neck muscle (Lipar 2001, two species of songbirds), which has an important role in hatching behaviour. Since both chicks in the experimental nests were matched for hatching date and thus age, this shortened time until hatching did not affect our experimental set-up.

The stimulating effect of androgens on begging behaviour is consistent with the only other study that analysed the effect of yolk hormones on begging behaviour in an avian species (Schwabl 1996b). Canaries from testosterone-treated eggs begged more in a standard test during the first 24 h after hatching than control chicks did. We found a similar effect over a much longer time under field conditions in a semiprecocial species. We clearly showed that chicks hatching from androgen-treated eggs were more alert and faster to respond to a parent returning to the nest with food. This is in agreement with the stimulatory effect of pre-natal exposure to these steroids on locomotion activity in the domestic chicken, Gallus gallus domesticus (Wada 1982; Massa & Bottomi 1987). It is also in accordance with the early postnatal effects of androgen on attentional mechanisms described by Andrew (1975) and Clifton et al. (1988) for the domestic chicken.

The stimulating effect of androgens on begging might have come about by other effects on the brain. The hypothalamus is involved in motivational networks concerning hunger and satiation and competitive behaviour and is well known to express androgen receptors (Schwabl & Lipar 2002). Iacovides & Evans (1998) showed experimentally in ring-billed gull, Larus scopulinus, chicks that need for food affects begging rates. Indeed our prenatal androgen treatment may have increased begging rate via a direct influence on the hunger system. Alternatively, the androgens may have affected the motivation to beg more indirectly by increasing the chicks’ metabolic rate, either via the metabolic costs of increased activity or via an increase in resting metabolic rate (Buchanan et al. 2001). As yet we have no evidence that daily energy expenditure or resting metabolic rate was affected by a similar androgen treatment in our species (Eising et al. 2003).

Finally, androgens may have affected begging behaviour independently of an influence on metabolism or the hunger system. Leonard & Horn (1998) showed that begging behaviour was influenced by the behaviour of nestmates, regardless of individual hunger, in the tree swallow, Tachycineta bicolor. In Crimson rosellas,
Platycercus elegans, last-hatched chicks begged more, regardless of individual hunger levels (Krebs 2001). Some of these effects may come about via the effects of prenatal androgens on the motor system. Anabolic effects of androgens are well known, and the stimulating effect of these steroids on the neck muscle (Lipar & Ketterson 2000; Lipar 2001) may be important for the begging display in our gulls, in which the head and neck are moved up and down in long and rapid sequences. Furthermore, several motor neurons in the avian spinal cord and the hindbrain, important for begging vocalizations and other displays, are known to contain androgen receptors (Gahr 2001; Schlinger et al. 2001; Schwabl & Lipar 2002).

Our androgen treatment not only induced an earlier behavioural reaction to the approaching parent, but also increased the average number of begging pumps per bout. Such a relation between testosterone and persistency in behaviour is also known in domestic chickens (Andrew 1972; Andrew & Rogers 1972).

One could argue that the different components of begging behaviour we have presented here are not independent. However, all results point in the same direction, are consistent with earlier results and our predictions (see Introduction) and two of the significant components (first to react and eating most) remained significant even after sequential Bonferroni correction, a rather conservative statistical method.

The androgen treatment resulted not only in chicks begging more, but also in their obtaining a larger proportion of the food delivered. Although this was only estimated by eye, it was scored blindly with respect to treatment and the results are highly significant. The components of begging behaviour we scored are likely to regulate parental feeding behaviour (glaucous-winged gull, Larus glaucescens: Henderson 1975). The stimulatory effect of our steroid treatment on this begging behaviour might therefore have caused the gain in food intake. The latter could explain the growth advantages for third-hatched chicks from androgen-treated eggs in our previous experiment (Eising et al. 2001), which relates more to the natural situation.

Growth and Survival

In contrast to our expectation and previous field study (Eising et al. 2001), chicks hatching from T eggs did not show an increase in growth rate. There may be several explanations for this. First, the level of competition was probably not as high as in a normal clutch because there were only two chicks in the nest instead of three. This may also explain why overall survival probabilities were high. Second, T and Oil chicks hatched synchronously, making it difficult for one chick to outcompete the other. Third, our observations were mainly carried out during the morning, when chicks were relatively hungry after a night without food. Oil chicks may have had enough time during the rest of the day to compensate for their smaller share early in the day. Fourth, higher food intake of T chicks may have compensated only for higher energetic costs incurred in begging behaviour. However, this is unlikely since McCarthy (1996) and Bachman & Chappell (1998) showed for several avian species that the cumulative energy allocated to begging was only slight, and we did not find indications of a higher energy expenditure in T chicks relative to controls (Eising et al. 2003).

Although the stimulatory effect of the androgen treatment on behaviour did not result in increased growth rate, the treatment did increase the age at which chicks died during the first week after hatching. Since mortality in natural asynchronous nests is usually highest during the first week after hatching in gull species (Hunt & Hunt 1976; Brouwer et al. 1995; Royle & Hamer 1998), this indicates that maternal androgen deposition might have important functional consequences.

Maternal Androgens and Hatching Asynchrony

In black-headed gulls, siblings have to compete over the food that is regurgitated by the parents. Therefore, size asymmetries within the brood may strongly influence the hierarchy within a brood and the survival probabilities for individual chicks. It has long been hypothesized that by creating a competitive hierarchy within the brood, parents can quickly reduce their brood size in times of food shortages or other problems associated with rearing a full clutch (Lack 1947; O’Conner 1978; Hahn 1981; Temme & Charnov 1987; Amundsen & Stokland 1988; Pijanowski 1992; Mock & Forbes 1994; Royle & Hamer 1998). However, size asymmetry within the brood may not be beneficial for the chicks under good food conditions. Furthermore, hatching asynchrony may not be an adaptation in itself but rather a constraint. Open-ground breeders such as gulls may benefit from starting to incubate a clutch before completion to protect it from predation or environmental challenges (e.g. solar radiation, cold; Magrath 1990; Veiga 1992; Stoleson & Beissinger 1995; see also Groothuis & Schwabl 2002). Early onset of incubation may also be an epiphenomenon of the female’s hormonal mechanism (Mead & Morton 1985) and may thus induce hatching asynchrony. The resulting size asymmetry may therefore not be beneficial, but could even lead to unwanted brood reduction. By supplying the chicks of later laid eggs with a higher dose of maternal androgens, such as in the black-headed gull (Eising et al. 2001; Groothuis & Schwabl 2002), mothers may try to compensate, at least partly, for the disadvantages of later hatching.

Black-headed gull chicks hatching from androgen-treated eggs hatched sooner, were quicker to respond to their parents, were more persistent in begging behaviour, and obtained the largest proportion of the food. These differences may be crucial in circumstances where the level of sibling competition is higher than in our experiment. Especially when the eggs of a clutch hatch asynchronously and food availability is low, last-hatched chicks may benefit from an increased begging vigour. In our previous study we showed that growth of last-hatched chicks was enhanced because of elevated androgen levels in the egg, although they never weighed as much as older siblings. Furthermore, survival of T chicks...
in a period in which most of the control chicks died in unmanipulated nests was higher than that of control chicks. Therefore we conclude that the ability to provide different amounts of androgens to their offspring provides mothers with a tool to shape the competitive hierarchy within their brood and optimize their reproductive output. This clearly comes about by effects of maternal androgens on behaviour of the chicks, but the data on timing of hatching suggest other mechanisms are involved as well.

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