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*Published in:*  
Hormones and Behavior

*DOI:*  
[10.1016/j.yhbeh.2013.08.004](https://doi.org/10.1016/j.yhbeh.2013.08.004)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2013

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

*Citation for published version (APA):*

Boncoraglio, G., & Groothuis, T. G. G. (2013). Effect of rapid modulation of circulating plasma testosterone concentration on begging, aggressive behavior and competition for food in black-headed gull (*Larus ridibundus*) chicks. *Hormones and Behavior*, 64(3), 487-493. <https://doi.org/10.1016/j.yhbeh.2013.08.004>

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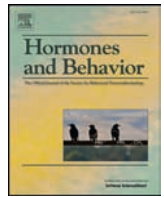
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# Effect of rapid modulation of circulating plasma testosterone concentration on begging, aggressive behavior and competition for food in black-headed gull (*Larus ridibundus*) chicks



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## ARTICLE INFO

### Article history:

Received 17 January 2013

Revised 6 July 2013

Accepted 9 August 2013

Available online 17 August 2013

### Keywords:

Aggressive behavior

Androgens

Begging behavior

Sibling competition

Testosterone

## ABSTRACT

Sibling competition mediated by begging behavior is extremely common in avian species and recent studies have highlighted the role of endogenous testosterone in regulating such phenomenon. However, current literature depicts an inconsistent pattern in altricial vs. semi-precocial species, with stimulating versus inhibitory effects of the hormone respectively. This is possibly due to a difference in the methodology of hormone treatment (short-term moderate dose versus a long-term stronger elevation, respectively) between the studies performed so far. In this study, we induced short-term moderate peaks in plasma testosterone levels, as applied in altricial bird species, and assessed the effects of our manipulation on begging, competitive and aggressive behavior in black-headed gull (*Larus ridibundus*) chicks, a semi-precocial species. Our results suggest that, unlike in altricial songbirds, temporary increase of plasma testosterone concentration suppresses begging and enhances aggressiveness towards intruders. However, it also increases aggression and the chances of getting priority while scrambling with nest mates to gain access to food. Thus, the inconsistencies in the hormonal control of begging behavior observed between altricial vs. semi-precocial birds seem real and perhaps related to species differences in complexity of the display and the nature of competition. These may be elucidated by future comparative studies.

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## Introduction

The offspring period represents a critical phase of individual life history in vertebrate species. While prenatal ontogeny is spent in a relatively stable developmental environment, after birth offspring have to face a much more unpredictable environment, being potentially exposed to novel antigens and harsh competition for the access to resources (Roff, 1993; Stearns, 1992). During post natal development, offspring often experience intense competition for food or other critical resources by siblings and adult conspecifics, whenever no diversification in the trophic or ecological niche between different age classes occurs (Roff, 1993; Stearns, 1992). Moreover, in species performing parental care, conflict among siblings over the allocation of care occurs. Parental fitness is positively affected by the increase of the number of offspring reaching sexual maturity and because offspring viability and food availability at reproduction are not always easily predictable, parents can be selected to overproduce marginal offspring in the beginning of the breeding bout (Mock and Forbes, 1995; Stearns, 1992). However,

the resources an adult can devote to parenting during a single reproduction attempt are limited, because life history trade-offs impose costs for future survival and fecundity (Clutton-Brock, 1991; Stearns, 1992). As a consequence, the overall demand by the progeny typically exceeds the total availability of parental resources, leading to the evolution of behaviors by which offspring try to solicit care from their parents and compete among each other (Mock and Parker, 1997).

In birds, sibling competition is extremely common and often lethal to part of the progeny, either because of physical aggressions among the offspring ultimately leading to siblicide (e.g. Drummond, 2006), or because of starvation of young, weak offspring, whose access to parental resources can be restrained by stronger siblings via non-aggressive ways, such as for example scrambling mediated by begging behavior (sensu Kilner and Johnstone, 1997). In addition, chicks of colonial species often have to face harsh competition by adult and young conspecifics from other families aiming at seizing the resources brought to the nest by the parents, and actively participate in the defense of the nesting territory by means of threatening and aggressive displays (e.g. Groothuis, 1989; Groothuis and Ros, 2005; Ros et al., 2002).

Recent studies have highlighted the role of androgen hormones in regulating the expression of competitive behavior in birds (for a review see Boncoraglio et al., 2006; Ros, 2008; Smiseth et al., 2011). For example, the allocation of testosterone in the yolk by the mothers is known to

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affect key traits for both early and long-term competitiveness of the offspring, such as prenatal development rate, postnatal growth, begging behavior, immune function, as well as the future social and sexual status (reviewed in Groothuis et al., 2005; Gil, 2008; von Engelhardt and Groothuis, 2011). Moreover, endogenous testosterone, which is secreted by chicks very early during ontogeny (Adkins-Regan et al., 1990; Ottinger and Abdelnabi, 1997; Ros et al., 2002) has been found to positively covary (Buchanan et al., 2007; Goodship and Buchanan, 2006) with soliciting behavior for parental food (begging) in passerine species, which was confirmed by an experimental study (Goodship and Buchanan, 2007), with clear benefits for chick survival until fledging (Goodship and Buchanan, 2006).

In sharp contrast, postnatal exposure to testosterone suppresses begging in black-headed gull (*Larus ridibundus*) chicks (Groothuis and Meeuwissen, 1992; Groothuis and Ros, 2005). In this species testosterone facilitates chick aggression, like in booby chicks (Ferree et al., 2004). Both species are colonial, non-altricial species in which the chicks perform aggression either towards siblings or intruders already early in life.

The aim of this paper is to investigate the discrepancy between the effects of testosterone on begging behavior in the altricial songbirds and the semi-precocial colonial species highlighted by current literature. One possibility to explain this discrepancy is the difference in experimental treatment between the studies. In the songbird study, testosterone was applied by oral dosing inducing a short peak of T elevation up to 0.32 ng/ml (Goodship and Buchanan, 2007). In the black-headed gull study, testosterone treatment consisted of an implantation treatment, inducing persistent high plasma concentrations over a much longer time period (after 3 weeks average plasma T levels amounted 1.58 ng/ml). Although the latter condition is not supraphysiological for gull chicks involved in aggressive interactions (Ros et al., 2002), these levels might be too high, and especially too high over too long a period for inducing begging behavior and might have shifted the chicks prematurely into an adult-like reproductive life history phase. Hence, evidence from studies assessing the effect on begging and aggressive behavior by a short-term moderate increase of circulating plasma testosterone levels in the gull chicks is required.

In the present study, we investigated the effect of inducing short-term moderate peaks in plasma testosterone concentration on the begging, competitive and aggressive behavior of black-headed gull chicks. Black-headed gull chicks are known to produce endogenous testosterone from an early age onwards with basal levels around 0.1 ng/ml and challenged levels on average 0.75 ng/ml (Ros et al., 2002). Chicks were hand-reared under standard laboratory conditions and subjected to experimental trials during the third week after hatching to assess their begging output, aggressiveness towards intruders and ability in outcompeting brood mates for accessing to food in response to three experimental treatments. We administered hormone treatments via intraperitoneal injections and tested testosterone-treated chicks alongside oil-injected and not injected, unmanipulated chicks as control groups. We employed these two different control groups based on the fact that oil injection can induce an increase in the circulating plasma levels of corticosterone (Pinson et al., 2008) which has been found to enhance begging performance in kittiwake chicks (Kitaysky et al., 2001).

## Materials and methods

### *Study species and general housing conditions*

Black-headed gulls breed in large colonies of up to thousands of pairs. Modal clutch size is three. Chicks are semi-altricial and depend on parental provisioning during the first five weeks after hatching. Begging behavior consists of vocal and postural displays, such as calling, gentle pecking of the parent's bill to solicit regurgitation of food and, when chicks are older, head pumping (Cramp, 1994; Groothuis, 1989).

Families defend a small territory surrounding their nest, employed by the parents to deliver the food for their offspring. Chick's territorial behavior is addressed to both young and adult intruders and consists of threatening postures accompanied by loud, harsh calls, up to pecking and fighting, while aggression towards siblings only very seldom occurs (Groothuis, 1989).

Thirty-six *Larus ridibundus* chicks of 2–3 days hatched from first laid eggs were collected in June 2009 from a large breeding colony in the North of the Netherlands and brought to the laboratory about 30 km away from the field. Chicks were matched for weight, marked individually on their head or belly feathers with non-toxic colours and housed in groups of three peers in metal cages of 85 × 75 × 85 cm. A group size of three matches the most frequent brood size naturally occurring for this species and is therefore considered ideal for hormone studies on captive chicks (Groothuis and Meeuwissen, 1992). Groups from adjacent cages could hear but not see each other. Cages were supplied with straw on the floor and, for the first two weeks, a thermal lamp providing a temperature of ca. 37 °C in the middle of the floor. Additional tube lighting was provided on a 16 L:8D schedule, the temperature of the room being kept at ca. 23 °C. Food and water were daily refreshed and available ad libitum, except when specified. Food consisted for the first two weeks of a moistened mixture of pellets used in trout farming (Trouvit, Trouw, Gent) supplemented daily with smelt (*Osmerus eperlanus*). Thereafter the diet gradually shifted to dry trout pellets, with smelt provided every third day. Chicks were hand-reared by offering food by means of tweezers especially during the first two weeks, after which time they became gradually autonomous. Body mass was measured at day 3, 12 and 20 and tarsus length was measured at day 12 and 20. Two chicks each from different cages died before day 14 and were not included in the datasets; all the other chicks successfully completed our experiments. This study was performed according to Dutch laws on animal research (Institutional Animal Care and Use Committee authorization number: 5343A).

### *Hormone manipulation*

At the days when behavioral testing was conducted (days 14, 17 and 20) we manipulated circulating plasma hormone levels of the chicks. Chicks were food deprived since the evening before the tests by removing food from the cages. Then, on the morning of testing, 30 min after the lights turned on (8:30 AM) and 45 min before being subjected to the experimental trials, chicks were assigned to one of the following hormone treatments: testosterone injection (T treatment hereafter), oil injection (O treatment hereafter), or control (C treatment hereafter). In the T treatment, chicks were intraperitoneally injected with 50 µl of a sterile sesame oil solution containing T in a concentration of 100 µg/ml (i.e. 5000 ng T per chick), while only 50 µl of sterile sesame oil was injected in the O treatment. Chicks from the C treatment were not injected nor handled to avoid any change in endogenous steroid production. Individual assignment of hormone treatments preceding the experimental test at day 14 was random for each group of 3 and the same treatment was subsequently maintained on the following days. Hormone treatment administration was quickly accomplished for all birds (ca. 15 min in total) before every test. *Ad libitum* food was provided again directly after the end of the tests. All three hormone treatments were represented in each three-chick group while only the T and O treatments were administered in the remaining two-chick groups, giving final sample sizes of 12, 12 and 10 chicks for T, O and C treatment, respectively. Chicks from different treatments did not differ in body mass or tarsus length at any stage (see above) of the study (one-way ANOVA, always  $F_{2, 31} < 1.810$ ,  $P > 0.180$ ; average body mass, day 3:  $28.12 \pm 5.79$  SD g; day 12:  $124.88 \pm 26.88$  SD g; day 20:  $230.98 \pm 24.07$  SD g). During all trials, observers were blind with respect to chick treatment. In total, 12 T chicks, 12 O chicks and 10 C chicks were employed; additional information about the sex of the chicks was considered unnecessary since we never found sex differences or specific

effects of T in this monomorphic species in which both sexes defend the nest and show the same displays (Groothuis and Meeuwissen, 1992; Ros et al., 1997), even in adult birds in early spring; also, sample sizes were too small to test reliably these differences.

The timing and dosages of the injection protocol were chosen according to the results of two pilot studies on gull chicks about 20 days post-hatching that were performed in 2008. In Pilot 1, we injected intraperitoneally 5 chicks with 0, 10, 100, 5000 or 100000 ng T dissolved in 50  $\mu$ l sterile sesame oil and measured their circulating plasma T levels 15, 45, 180 and 360 min after injection by radioimmunoassay (see below). A dosage of 100 ng T or lower did not produce any appreciable changes over time with respect to oil injection, while the 100000 ng treatment strongly increased the T concentration over the three hours following the administration (Fig. 1a). Conversely, the 5000 ng treatment resulted in a peak of T in the plasma 45 min after injection, such effect being quickly dissipated in the next hours (Fig. 1a). Therefore, in Pilot 2 ( $N = 7$  18–20 days old chicks and 18 data points in total; average body mass:  $235.50 \pm 31.03$  SD g) we focussed only on the 5000 ng dosage and compared its effect over time to the 0 ng T injection (i.e. injection with only 50  $\mu$ l sesame oil alias O treatment; no uninjected, C chicks were employed for this pilot) as control treatment. Plasma T and corticosterone (Cort hereafter) levels of the chicks were sampled before the administration of treatments to assess the baseline levels, and 40 and 100 min after injection. Blood (250  $\mu$ l, 150 for T and 100 for Cort determination) was sampled from the brachial vein within 3 min after taking the bird from the cage and finished for each cage within 10 min after taking out the first bird. Blood was extracted with diethyl/petroleum benzene 70:30 (vol/vol) and subsequently 70% methanol. For the RIA we used commercially available kits (for T: Active Testosterone Coated-Tube RIA DSL-400, Diagnostic Systems Laboratory, with a sensitivity of 0.08 ng/ml; for Cort: RS49011, MP Biomedicals, Ohio, USA DSL Benelux Office). We checked and confirmed parallelism by using known pools for controls. For further details see Goerlich et al. (2009).

The chosen dosage for the T treatment was shown to significantly increase circulating plasma T levels with respect to baseline levels 40 min after injection (Mann-Whitney U-test,  $Z = -2.393$ ,  $P = 0.017$ ) while no difference emerged after 100 min ( $Z = -1.464$ ,  $P = 0.222$ ). Conversely, O treatment was shown not to significantly increase circulating plasma levels of T, either 40 or 100 min after injection (always  $P > 0.111$ , Fig. 1b), but tended to increase circulating Cort levels 40 min after injection ( $Z = -1.937$ ,  $P = 0.067$ ,  $N = 13$  data points in total; Fig. 1c). As intended, the elevation by T injection was well within the physiological range of this species (Ros et al., 2002), much lower than the level induced by T-implantation in the earlier study on this species (Groothuis and Ros, 2005; Ros et al., 2002) and restrained within a 100 min interval.

#### Begging test

Chicks were food deprived since the evening of day 13. At day 14, 45 min after the hormone treatment administration, following the same order in which the hormone treatment was administered across cages, we opened the door of each cage and presented in random order with respect to the treatment of the chicks each chick with empty tweezers to solicit begging pecks towards the tweezers while its cage mates were temporarily kept aside, as during normal hand-feeding events. Begging pecks, being the most obvious and frequently performed expression of begging in the tests, can be easily distinguished as gentle tapping on the parent's bill (or tweezers, in our case) by the chick's beak, often accompanied by soft high pitch peeping calls (Groothuis, 1989). The total number of pecks given by the chicks in the first 10 section of the individual test was recorded. The order of testing of the chicks within the cage did not differ among treatment groups (Kruskal–Wallis ANOVA,  $\chi^2_2 = 0.639$ ,  $P = 0.726$ ). Repeatability

of baseline (= without hormone manipulation) begging pecks measured was high in a dataset collected at day 12 ( $F_{33, 34} = 9.148$ ,  $P < 0.001$ ,  $R^2 = 0.899$ ,  $N = 2$  replicas per chick collected over two sessions at 30 min interval).

#### Aggression test

In the morning of day 17, we used a stuffed adult black-headed gull in threatening posture to simulate a territorial aggression inside the experimental cages. Forty-five min after the administration of the hormone treatment, applied according to the same protocol as in the begging test, we placed the stuffed model at the entrance of the cages. Then, every 6 s over the first 30 s and in random order but almost simultaneously for all the chicks in the cage, we ranked the intensity of aggression towards the intruder by each chick on the basis of a simplified version of the classification of aggression and threat displays as proposed by Groothuis (1989); see also Groothuis and Ros, 2005). Aggressive pecking was set at the top (level 5) of the aggression ranking, while freezing/not reacting and begging for food towards the intruder were set at the bottom (levels 2 and 1, respectively). Two threat displays, described by Groothuis (1989) as “oblique” (erect postures accompanied by loud threatening calls addressed to the intruder, often followed by attack or a charge) and “choking” (non-erect postures

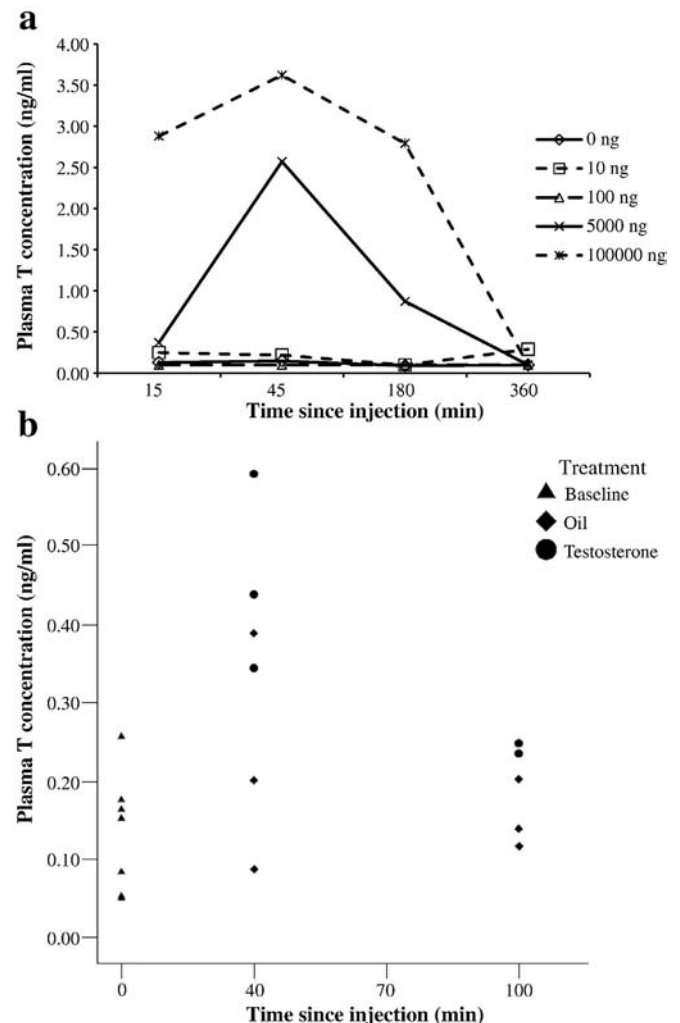


Fig. 1. Circulating plasma testosterone concentration of chicks sampled during the pilot study 1 (a) and 2 (b). See Materials and Methods for details.

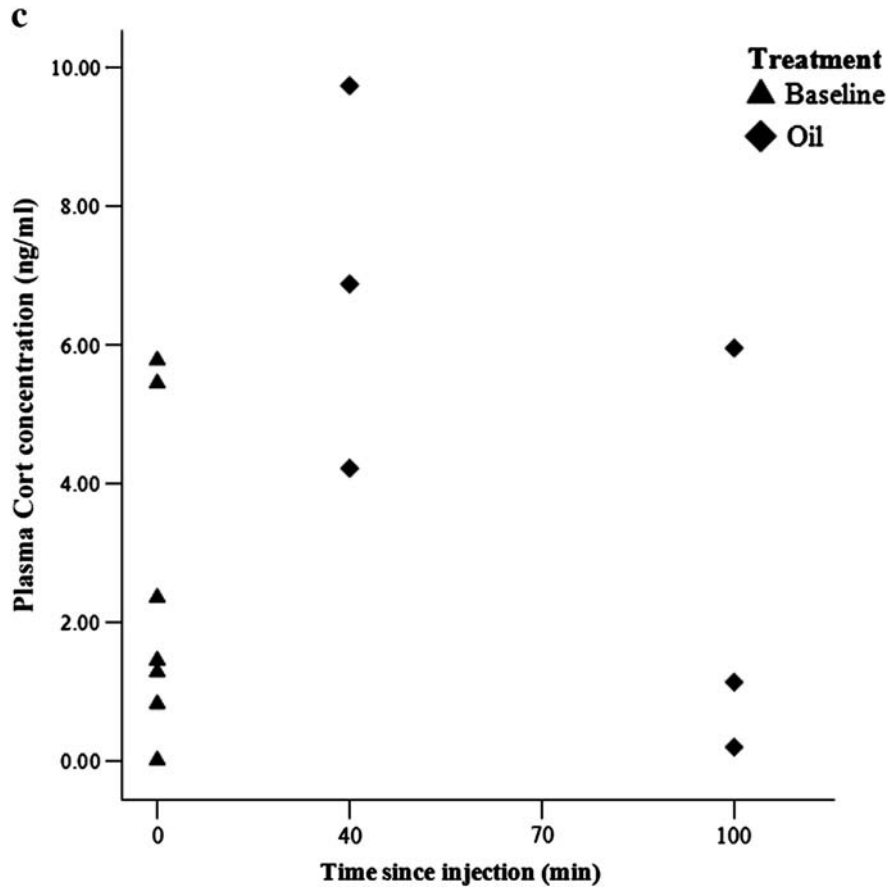


Fig. 1 (continued).

accompanied by repetitive, shorter calls while the beak is pointing downwards), corresponded to levels 4 and 3, respectively. Cages were tested following the same order as for the administration of hormone treatment and a second session of testing was performed ca. 20 min after the first. Two observers blind to the treatment scored independently the postures assumed by the chicks during the trials and consulted each other in the end of the tests for each given cage: and no disagreement was recorded. The order of scoring the aggression intensity of the chicks within the cage did not differ among treatment groups (Kruskal–Wallis ANOVA,  $\chi^2_2 = 2.732$ ,  $P = 0.255$ ). Repeatability of the aggression score within individual was high ( $F_{33, 306} = 20.836$ ,  $P < 0.001$ ,  $R^2 = 0.692$ ,  $N = 10$  replicas per chick over the two sessions).

*Food competition test*

Chicks were food deprived since the evening of day 19. At day 20, 45 min after the hormone treatment administration, following the same order in which the hormone treatment was administrated across cages, we performed a food competition test conducted as follows. After opening the cage door, we presented the chicks with a small food item (0.4–0.8 g piece of smelt) that was easy to monopolize and swallow, held with tweezers and kept close to the floor at equal distance from each chick in the cage. Chicks headed for food straight away, and the identity of the chick that swallowed the item was noted. This was repeated over ten trials, at the end of which we estimated the number

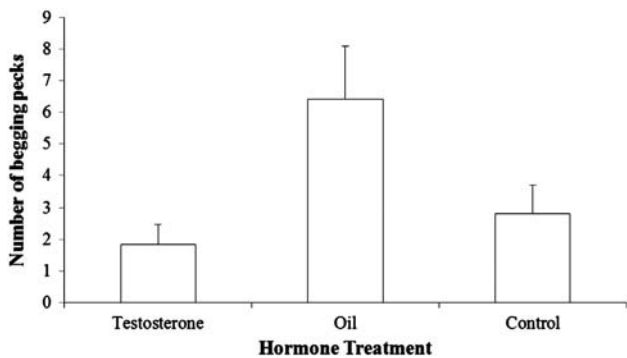


Fig. 2. Mean (+ SE) number of begging pecks over 10 s recorded during the begging test at day 14 with respect to hormone treatment.

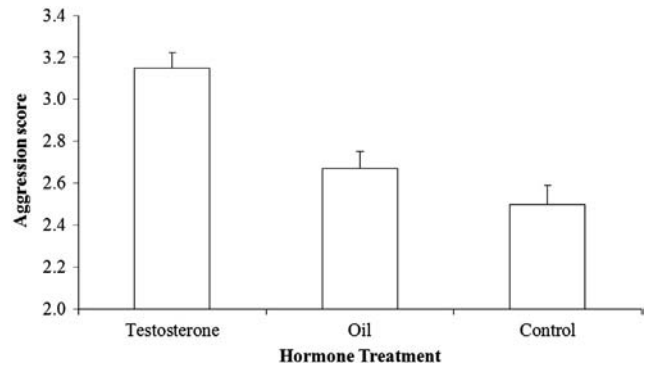
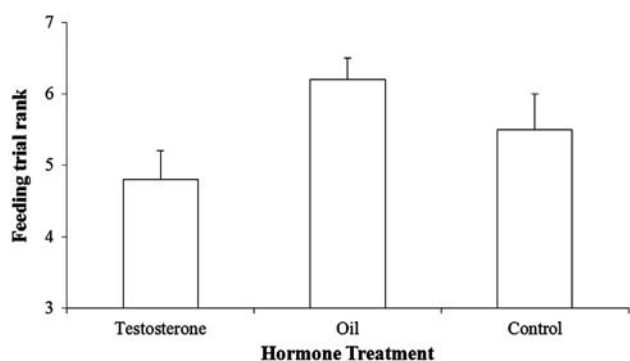


Fig. 3. Mean (+ SE) postural score recorded during the aggression test at day 17 with respect to hormone treatment. Mean values for each treatment are shown for easier appreciation of the variation patterns, although the analyses relied on the whole dataset (see Materials and Methods).





**Fig. 4.** Mean (+ SE) rank of trials where the chicks obtained food during the food competition test at day 20 with respect to hormone treatment.

of food items eaten by each chick. In addition, we assigned to each trial a ranking number from 1 to 10, number 1 being the first and number 10 being the last trial of the test, and computed for each chick the average ranking of trials in which food was obtained. Thus, the total number of swallowed food items being equal, a lower average ranking identifies a more competitive chick, feeding itself earlier than its cage peers. All chicks obtained at least one food item during the test (range 1–8) and were included in the analyses.

#### Statistical analyses

All analyses were performed with Sas 9.1.3. Begging data were analysed with a general linear mixed model (proc MIXED) including cage as a random factor and hormone treatment (three levels: 1 for T, 2 for O and 3 for C) as a fixed factor. Post-hoc comparisons were performed adopting Sidak correction for multiple testing. Chick body mass at day 12 was added as a covariate in a subsequent analysis to test its effect on begging intensity as a low body mass may indicate a higher hunger state facilitating begging behavior.

Aggression scores collected over the two sessions of the test performed at day 17 ( $N = 340$  data points in total) were analysed with a generalized mixed model assuming a multinomial distribution of the response variable and a cumulative logit link function (proc GLIMMIX), different levels of the response scale being ranked from minimum to maximum. Hormone treatment was included as a fixed effect, while cage and chick identity nested within cage were included as random effect factors.

Data of the proportion of swallowed food items (arcsin-square root transformed values of number of eaten items divided by 10) and average feeding rank during the food competition test were first analysed with Kruskal–Wallis non-parametric ANOVAs including hormone treatment (see above) as the grouping variable. Post-hoc comparisons were performed with separate Mann–Whitney U-tests. We also used an alternative approach to be able to include the covariate body mass. This was based on logistic mixed model analyses including information from each trial for all the chicks and assuming a binomial distribution of the response variable (fed vs. not fed, one response value per feeding trial per chick,  $N = 340$  data points in total; proc GLIMMIX) and a logit link function was also applied. These analyses included cage and chick identity nested within cage as random factors, hormone treatment as a fixed factor, trial number and day 20 body mass as covariates. The trial number \* hormone treatment and trial number \* day 20 body mass interaction were then needed to test for specific effects of hormone treatment and chick body mass on taking priority over cage mates in accessing to food. Post-hoc comparisons of the trial number \* hormone treatment interaction effect were performed on separate models including only the data points of the pairs of treatment groups to be compared. Since Kruskal–Wallis and logistic mixed model

analysis gave qualitatively identical results, we presented full statistics only for the latter for brevity.

## Results

### Begging test

Hormone treatment significantly affected chick begging behavior at day 14 ( $F_{2, 21.1} = 4.74$ ,  $P = 0.020$ ; Fig. 2). Post-hoc comparisons showed that T treatment suppressed begging pecks with respect to O treatment, as T chicks gave significantly fewer pecks than O chicks ( $P = 0.023$ ), while no difference emerged between T and C ( $P = 0.917$ ) or O and C ( $P = 0.111$ ) chicks. The number of begging pecks did not vary across cages ( $z = 0.35$ ,  $P = 0.362$ ). A subsequent analysis including also body mass at day 12 among the predictors confirmed the effect of hormone treatment ( $F_{2, 20.8} = 6.26$ ,  $P = 0.007$ ; post-hoc comparisons, T vs. O:  $P = 0.012$ ; T vs. C:  $P = 0.994$ ; O vs. C:  $P = 0.033$ ) and showed that begging intensity significantly declined with increasing body mass of the chicks ( $F_{1, 29.5} = 4.94$ ,  $P = 0.034$ , slope:  $-0.055 \pm 0.025$  SE). Thus, smaller and needier chicks begged more than their peers at day 14.

### Aggression test

Chick behavior during the aggression test at day 17 significantly varied with hormone treatment ( $F_{2, 18.3} = 3.88$ ,  $P = 0.039$ ; Fig. 3). Post-hoc comparisons showed that T chicks tended to assume more aggressive postures towards the intruder than C and O chicks ( $P = 0.021$  and  $P = 0.094$ , respectively), while no difference was found between C and O chicks ( $P = 0.430$ ). Based on the latter we tested T against C + O yielding values of  $F_{1, 19.5} = 7.33$ ,  $P = 0.014$ . Aggression behavior significantly varied with chick identity ( $z = 2.66$ ,  $P = 0.008$ ), while no difference emerged among cages ( $z = 1.25$ ,  $P = 0.210$ ).

### Food competition test

The chance for the chicks to acquire the food item was not affected by hormone treatment when only this variable was entered as predictor ( $F_{2, 31.33} = 1.55$ ,  $P = 0.228$ ; T:  $4.00 \pm 0.56$  SE items; O:  $3.75 \pm 0.55$  SE items; C:  $2.70 \pm 0.45$  SE items). However, when correcting for body mass at day 20, trial number and its interactions with hormone treatment and body mass, testing the rank order of food items obtained, we found a significant effect of the interaction between trial number and hormone treatment ( $F_{2, 27.0} = 4.09$ ,  $P = 0.028$ ; Fig. 4), in line with the non-parametrical testing (see Methods). This showed T chicks being more successful to obtain food during early compared with late trials when compared with O and C birds. Although all groups had negative slopes, the slope of the T group was the strongest (T-slope:  $-1.21 \pm 0.46$  SE;  $t_{27} = -2.64$ ,  $P = 0.014$ ; O-slope:  $-0.96 \pm 0.44$  SE;  $t_{27} = -2.19$ ,  $P = 0.038$ ; C-slope:  $-0.95 \pm 0.42$  SE;  $t_{27} = -2.24$ ,  $P = 0.034$ ). Post-hoc comparisons confirmed that T chicks had priority during early trials over O chicks (trial number \* hormone treatment:  $F_{1, 27.0} = 6.50$ ,  $P = 0.017$ ) and over C chicks ( $F_{1, 27.0} = 5.31$ ,  $P = 0.029$ ), while no difference between O and C chicks was found ( $F_{1, 27.0} = 0.01$ ,  $P = 0.944$ ). In addition, the odd success rate of obtaining food during the trials tended to increase with decreasing body mass of the chicks ( $F_{1, 27.0} = 2.80$ ,  $P = 0.106$ ; slope:  $-0.020 \pm 0.012$  SE), while the combined effect of the covariation between trial number and day 20 body mass was significant and positive (trial number \* day 20 body mass:  $F_{1, 27.0} = 5.79$ ,  $P = 0.023$ ; covariation coefficient:  $0.005 \pm 0.002$  SE). Thus, smaller, needier chicks had higher chance to obtain food during early trials than large chicks. Finally, no significant effects for the random factors emerged (cage and chick identity:  $z > 1.33$ ,  $P > 0.183$  in both cases).

## Discussion

In this study, we aimed at providing further insight in the hormonal control of begging behavior, sibling competition and social aggressiveness in the young of avian species, as the current literature depicts a complex and inconsistent pattern. We investigated to what extent the discrepancy between the role of testosterone in enhancing begging behavior in altricial songbirds and inhibiting begging while temporarily promoting more aggressive, adult-like behaviors in semi-precocial birds may be due to a difference in the methodology of hormone treatment between the studies performed so far (short-term moderate dose versus a long-term stronger elevation, respectively). Since the experimental evidence for songbirds (Goodship and Buchanan, 2007) is backed-up by several correlative studies (see Introduction; Smiseth et al., 2011) and the findings of the semi-precocial black-headed gulls have been replicated, although always through manipulations that persistently increased testosterone levels in the long-term (Groothuis and Meeuwissen, 1992; Groothuis and Ros, 2005), this discrepancy seems real. Therefore, we felt that a short-term manipulation of testosterone levels in gull chicks, being much more suitable to a proper comparison with Goodship and Buchanan (2007) findings than the former implantation studies, was required. Furthermore, our injection protocol simulates probably much better natural variation in endogenous T production than an implantation protocol would do and might therefore reveal more realistic results. Moreover, we studied the T effects for the first time under such experimental conditions on direct competition among chicks in a food shortage context, testing its functional relevance. In addition, since previous implantation studies on gulls not only showed a suppressive effect on begging but also a strong facilitating effect on aggression, we assessed the effect of a short term moderate elevation of T on aggression.

In the previous study on the same species, T treatment was applied by silicone implants filled with T and delivering for at least 10 days elevated circulating T concentrations of around 1.58 ng/ml, which was more than 20 times higher compared with the average T concentration measured in the control group (Groothuis and Ros, 2005). In our study, the injection treatment elevated T levels only for about 100 min and to a level of less than 0.05. Nevertheless, as in the previous implantation studies, T treated birds performed less begging than oil injected birds. This indicates that the discrepancy between the experimental study on songbirds (oral dosing with T in flycatcher chicks) and our study is very unlikely to be caused by a difference in the experimental treatment and rather suggests the existence of species-specific differences in the role of T in controlling begging behavior. Indeed, in the related semi-precocial kittiwake it has been shown that corticosterone, elevated during phases of poor body condition, stimulates begging (Kitaysky et al., 2001), in contrast to the case of songbirds (for a review see Smiseth et al., 2011). One possibility to explain this species-specific difference is that, especially during the first stages of the rearing period, begging in songbirds mainly consists of an elaborate postural display in which the chicks stretch their necks extensively, gape widely and perform loud begging calls. This complex motor pattern may require anabolic effects of androgens on the muscles of neck and syrinx that are indeed sensitive for T already early in life (Groothuis and Meeuwissen, 1992; Lipar and Ketterson, 2000). The begging display of kittiwakes and the begging pecks of the black headed gull are much less elaborate and may therefore not require peripheral elevation of T. However, when gull chicks get older, they beg also with elaborate pumping movements of the neck and head (Groothuis, 1989) and these were nevertheless suppressed by T implantations in the earlier studies (Groothuis and Meeuwissen, 1992; Groothuis and Ros, 2005). As in the field this display is performed immediately preceding begging pecks, it seems therefore unlikely that head pumping is facilitated by lower and temporary elevation of T, also since this was hardly performed in our experiment even in the T group. Further studies with T manipulation in gulls and kittiwakes (Muller and Groothuis, in prep.) are needed.

Although T lowered the number of begging pecks in T chicks relative to the O group, T birds did not differ from unmanipulated control birds, while the latter showed a tendency to beg less than O chicks. This can be explained in two ways. Either the injection lowered T production, releasing the inhibition on begging, or it induced elevation of corticosterone in the O birds relative to the controls as corticosterone is known to stimulate begging in the closely related kittiwake (Kitaysky et al., 2001). Suppression of T in the O birds is unlikely as the latter performed a similar level of T dependent aggression compared with controls. Conversely, the second hypothesis is likely, as such an elevation of plasma corticosterone levels by sham injection has been previously demonstrated in poultry literature (Pinson et al., 2008). This is also suggested by our preliminary findings on variation in plasma corticosterone levels following oil injection during Pilot 2 (Fig. 1c). Overall, our study further warrants testing the effect of corticosterone manipulations on begging in our species.

Aggression was still elevated with our more subtle T treatment. This is to be expected since in this species T implantation leading to an elevation up to only 0.5 ng/ml also elevated aggression, whereas social challenges, known to temporarily elevate T levels, do this too (Ros et al., 2002). Nevertheless, our results confirm that T facilitates aggression outside a sexual context and already early in ontogeny even with a much more moderate treatment than previously shown. This has clear benefits for chicks reared in large breeding colonies, where competition by intruders for accessing to food resources stored in the nesting territory by parents can be very strong since very early life, like in many seabirds. Further evidence in favour of a positive relationship between aggressive behavior and endogenous T levels in the chicks is provided by studies of other semi-precocial species not related to Charadriiformes (Ferree et al., 2004; Sasvari et al., 1999).

Interestingly, our T treatment facilitated food competition behavior, although it suppressed begging pecks. This would fit the explanation above that T may be required for motor activity in a competitive situation, such as in this case running towards the food item, that can happen over substantial distances in the field. Thus, endogenous testosterone regulation may have a crucial role in determining food intake and ultimately chick survival during a life stage where harsh competition by siblings can strongly increase the risk of starvation depending upon food availability. In birds, prenatal exposure to testosterone of maternal origin is can affect the density of androgen receptors in the hypothalamic region and the amount of endogenous T production in the first week after hatching (Pfannkuche et al., 2011). Thus, our study possibly also shed light on one of the mechanisms by which systematic variation over the laying sequence in the allocation of androgens of maternal origin can differentially affect the fitness of single brood mates (e.g. Boncoraglio et al., 2011; Eising et al., 2001). Also, as expected, a lower body condition enhanced both chick begging and the scrambling competition over food among cage mates.

In conclusion, the role of testosterone and corticosterone in facilitating begging and sibling competition may differ between bird species depending either on their mode of development (altricial vs. semi-precocial) or, perhaps in relation to this, the nature of the competition and begging displays. Further comparative studies on the role of these steroids in chicks of species of different developmental modes and with a different elaboration of begging display and level of competition may further elucidate the causes behind the complexity of these phenomena in avian species.

## Acknowledgments

We thank Linda van Zomeren for precious help with data collection, Albert Ros for sharing his data for Pilot 1, and Bonnie de Vries and Maarten Lasthuizen for help with the hormone assays. GB was funded by a post-doc grant from the Ministero Italiano dell'Università e Ricerca.

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