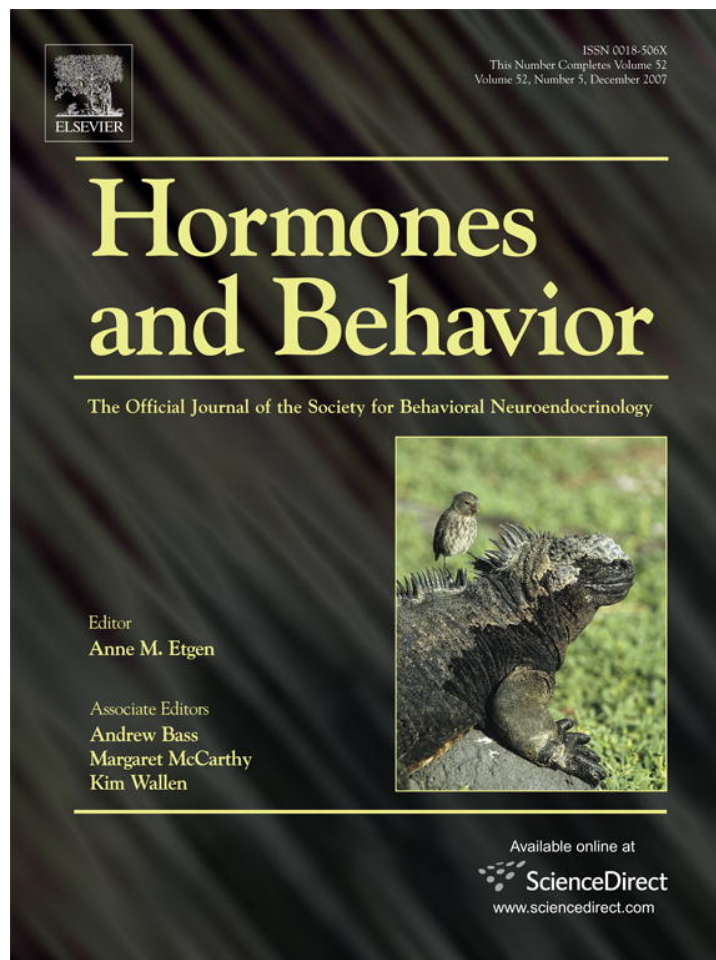


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## Does testosterone mediate the trade-off between nestling begging and growth in the canary (*Serinus canaria*)?

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### Abstract

Nestling birds solicit food from their parents with vigorous begging displays, involving posturing, jostling and calling. In some species, such as canaries, begging is especially costly because it causes a trade off against nestling growth. Fitness costs of begging like this are predicted by evolutionary theory because they function to resolve conflicts of interest within the family over the provision of parental investment. However, the mechanism that links these costs with nestling behaviour remains unclear. In the present study, we determine if the relationships between nestling androgen levels, nestling begging intensities and nestling growth rates are consistent with the hypothesis that testosterone is responsible for the trade-off between begging and growth. We test this idea with a correlational study, using fecal androgens as a non-invasive method for assaying nestling androgen levels. Our results show that fecal androgen levels are positively correlated with nestling begging intensity, and reveal marked family differences in each trait. Furthermore, changes in fecal androgen levels between 5 and 8 days after hatching are positively associated with changes in nestling begging intensity, and negatively associated with nestling growth during this time. Although these correlational results support our predictions, we suggest that that experimental manipulations are now required to test the direct or indirect role of testosterone in mediating the trade-off between begging and growth.

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**Keywords:** Signalling; Fecal androgens; Parent-offspring conflict; Sibling rivalry

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### Introduction

Evolutionary conflicts of interest within the family arise when dependent young seek resources that their siblings would prefer to have (Macnair and Parker, 1979), or that their parents would prefer to withhold for future reproduction (Trivers, 1974). The solicitation behaviours shown by many young animals are their key weapon in these conflicts of interest (Mock and Parker, 1997). By begging vigorously, for example, individual offspring can dominate the competition for food (e.g. Parker et al., 1989) or can attract a higher feeding rate from parents (e.g. Leonard and Horn, 2001).

Theoretical analyses suggest that, providing begging displays are sufficiently costly, they can bring a stable resolution to the evolutionary conflicts of interest that exist within the family (Godfray, 1995a; Godfray and Johnstone, 2000). Begging costs function to limit the extent to which offspring escalate their competition for food (Macnair and Parker, 1979; Johnstone, 1996, 1999) or to ensure the honest advertisement of offspring need (Godfray, 1991, 1995b). Whether offspring begging behaviour actually does carry associated fitness costs remains a contentious issue (e.g. Chappell and Bachman, 2002; Haskell, 2002; Leonard et al., 2003). Nevertheless, there is some indication from experimental work on birds that excessive begging can impose a growth cost on nestlings. For example, when young magpies (*Pica pica*) were forced experimentally to put greater effort into begging, they gained mass more slowly than nestlings given the same reward for a lower begging effort

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(Rodríguez-Girones et al., 2001). Similar results have also been obtained with canary (*Serinus canaria*) nestlings (Kilner, 2001). A different approach is to examine energy expenditure on growth versus begging across the whole nestling period. Measurements taken from house wren (*Troglodytes aedon*) chicks at three different ages suggest that the energy devoted to begging is negatively related to energy expenditure on growth (data from Bachman and Chappell, 1998, plotted in Kilner, 2001).

Therefore, there is some evidence to suggest that nestlings must balance the energy they spend on begging against the energy they devote to growth. In magpies, canaries and house-wrens, at least, a physiological trade-off (Stearns, 1992) constrains the exuberance of nestling begging displays. What is more, when begging functions as a signal (Godfray, 1991), its cost is what gives the signal its meaning (Grafen, 1990): a canary chick that is begging intensely is therefore signalling its need for energy to spend on growth (Kilner, 1995, 2001, 2002).

In this paper, we investigate whether our correlational data are consistent with the hypothesis that the physiological trade-off between begging and growth is mediated by androgens. Put another way, when begging functions as a signal, do androgens determine the cost of the signal and the information that is conveyed to parents? We focus on testosterone because it has been suggested to govern fitness trade-offs in other contexts (e.g. Wingfield et al., 1990; Ketterson and Nolan, 1994; Ros, 1999; Dufty et al., 2002; Ricklefs and Wikelski, 2002), including signalling, where it may impose an immunosuppressive cost on secondary sexual displays (Folstad and Karter, 1992). Altricial nestlings are known to produce their own testosterone, starting even before hatching (Ottinger and Abdelnabi, 1997), and have rapidly increasing testosterone production throughout the nestling period (Adkins-Regan et al., 1990). Elevated levels of nestling testosterone are correlated with more intense begging displays in thin-billed prions (*Pachyptila belcheri*, Quillfeldt et al., 2006) and pied flycatchers (*Ficedula hypoleuca*, Goodship and Buchanan, 2006). However, there is variation between studies, as Groothuis and Ros (2005) found higher androgen levels to cause greater aggression, but reduced begging levels, in nestling black-headed gulls (*L. ridibundus*), whilst Quillfeldt et al. (2007) recently found no association between androgen levels and begging in Cory's shearwaters (*Calonectris diomedea*). Interestingly, neither of these studies controlled for hunger levels during the begging trials and this may be crucial in terms of interpreting the effect of testosterone on begging intensity.

Testosterone may also affect growth rates. Two studies of natural variation in nestling androgen levels shows a positive association between androgens and condition. Nests of pied flycatchers which have higher testosterone levels show higher fledging success (Goodship and Buchanan, 2006), whilst Quillfeldt et al. (2006) found that higher androgen levels in thin-billed prions were associated with better body condition. However, when young chickens (*Gallus gallus domesticus*, Fennell and Scanes, 1992), turkeys (*Meleagris gallopavo*, Wise and Ranaweera, 1981), black-headed gulls (Ros, 1999; Groothuis and Ros, 2005) and Eurasian kestrels (*Falco tinnunculus*, Fargallo et al., 2007) were exposed to experimen-

tally elevated doses of testosterone after hatching, their rate of growth slowed markedly as a result. It is possible therefore that increased levels of testosterone can impede nestling growth and development.

In this preliminary, correlational study, we examined the association between nestling begging intensity, growth and androgens in young canaries. First, we biologically validated a method for sampling fecal androgens. We then quantified the begging behaviour of nestling canaries, using a standardised protocol, and assayed fecal androgens present in faeces produced immediately after this begging display as an index of the circulating levels around the time of begging. We predicted that there would be a positive correlation between extracted levels of fecal androgens and begging intensity, and that if androgen production acts to mediate a trade-off with nestling growth we should see a negative relationship between circulating androgen levels and nestling growth.

## Methods

### Assay validation

Adult canaries were held at the School of Biological Sciences, University of Bristol, in steel breeding cages (118 cm × 50 cm × 50 cm) on 12:12 photoperiod in single sex groups in spring 2001. During this time, fecal samples were gathered either immediately following production or gathered from the floor of the cage on plastic sheeting. Samples were classified as fresh when collected immediately after production or 'dried' where samples had been allowed to dry in the cage for up to 24 h before collection.

### Canary breeding

Canary chicks were bred from a separate adult population kept at the Sub-Department of Animal Behaviour, Madingley, Cambs, UK, between April and July in 2001 and 2002. The procedures we used with the canaries conformed to the high animal welfare standards enforced by the University of Cambridge. Pairs were randomly assigned to breed in separate double breeding cages (102 cm × 28 cm × 40 cm; see Kilner, 2002 for further details concerning husbandry of breeding birds). As part of a separate experiment, which will be reported elsewhere, eggs were removed from the nest, labelled, weighed and measured and either returned to the nest immediately afterwards or kept in a plastic box for 1 to 4 days, and temporarily replaced in the nest with model eggs. Eggs were always returned to the nest eventually, for incubation.

### Growth data

Canary eggs usually require at least 13 days of incubation before hatching. The day before hatching was due (i.e. from 12 days after the start of incubation), all eggs were moved to an incubator and kept individually, in labelled egg cups lined with nest material, whilst their mothers were given a clutch of plastic eggs to tend. The incubator was checked at approximate 3 h intervals from 09:00 until 22:00 for signs of hatching. As soon as they hatched, chicks were weighed with a Sartorius 300 digital balance, and a small quantity of down was plucked to give each chick in the brood a unique identity marking. Nestlings were then randomly assigned a color code and put back in the nest, and a plastic egg was removed for each chick returned. Chicks were weighed daily every evening between 17:00 and 19:00 from the day after hatching, and growth rates were calculated as the slope of a linear least squares regression relating chick mass to chick age for the first 13 days of life (for every regression,  $0.90 < R < 0.99$ ).

### Behavioural data

At 5 and 8 days after hatching, the chicks ( $N=74$  from 31 different breeding pairs) were removed from the nest and transferred to a heated test nest, supported

on a wooden platform, with one nestling per test nest. Meanwhile, their parents were left with at least one nestling to look after. Nestlings were food deprived for 40 min and then fed a standard meal of 0.5 ml Nectarblend (from Haith's of Cleethorpes, UK) mixed to a standard concentration (6.00 g sieved Nectarblend powder with 15.0 ml of tepid tap water), using a 1 ml plastic syringe. The heated test nest holding the chick was then moved into a wooden test box (described in detail in Kilner and Davies (1998) with a Sony CCD-TR680E Hi-8 video camera positioned on its lid to film the nestling begging in the test nest below. Eighty minutes after feeding, chicks were induced to beg with a tap on the nest using a plastic syringe (this stimulus was kept as standard between tests) and any begging behaviour was filmed. We tapped the nest up to three times to induce begging, but stopped tapping once begging began. Immediately after filming, chicks were fed with Nectarblend rearing mix (exactly as described above) until they reached satiation, which we defined as a failure to beg after three attempts to induce begging with the standard stimulus. We recorded the amount of food eaten before returning them to the nest. Nestlings were monitored every 10 min throughout the time they were away from their parents and any fecal samples they produced during this time were collected immediately and frozen. Only fecal samples collected immediately after a begging display were analysed for androgen levels. Video analysis of the begging trials was conducted using the randomly assigned color code to identify chicks. The observer (RMK) was therefore blind to any information about chick sex and chick hunger (i.e. the amount of food consumed at the end of the trial). Begging posture was quantified from videotapes using the scheme described in Kilner (2001). In brief, for the first 12 s of the begging bout, we paused playback of the videotape once per second and described the posture assumed by the nestling at the moment with a rank score (ranging from 0=no begging to 4=begging with great vigour). The 12 rank scores were then summed to derive an overall measure of postural begging intensity.

#### Molecular sexing

Nestlings were kept until adulthood, when one tail feather was plucked for DNA analysis ( $N=26$ ). Otherwise samples were from corpses of nestlings or adults, frozen soon after death. From these samples, a Chelex (BioRad, Richmond, CA, USA) resin-based extraction procedure (Walsh et al., 1991) was used to make DNA available for the PCR amplification of two homologous genes (CHD1-W and CHD1-Z) using primers 2550F and 2718R (following Fridolfsson and Ellegren, 1999). The PCR products were separated by electrophoresis through 6% polyacrylamide gels and visualised by silver staining (Bassam et al., 1991).

#### Hormone analysis

Fecal androgens have been quantified in a number of studies, although are often erroneously referred to as fecal testosterone despite the fact that radio-infusion studies of birds suggest that whilst testosterone does not occur in faeces, a range of androgen metabolites is present (Goymann et al., 2002). The analysis protocol was based upon previously published protocols for fecal analysis of steroids (Langmore and Cockrem, 2002), but was fully biologically validated for this species (see below).

Initial analysis of four adult pooled fresh and two adult 'dried' (collected from the cage floor within 24 h of deposition) fecal samples indicated a higher level of androgen from fresh samples (fecal androgens fresh  $4.80 \pm 1.38$  SE; dried  $1.92 \pm 0.345$  SE ng/ml extract), which is why all experimental samples were therefore taken onto ice at collection and frozen immediately. Fecal samples were stored at  $-20^\circ\text{C}$  for up to 4 months before freeze drying for analysis. The dried sample was finely ground using a mortar and pestle and 0.01 g was transferred into a conical flask. 2.5 ml of 90% ethanol was added and samples were shaken on an orbital shaker for 1 h, after which they were vortexed and decanted into a glass test tube. The samples were then centrifuged for 20 min at  $1900 \times g$ . The supernatants were removed and transferred to a second set of glass tubes and evaporated at  $37^\circ\text{C}$  under a stream of air. A further 1.25 ml of 90% ethanol was added to the first set of tubes, vortexed and centrifuged for 20 min at  $1900 \times g$ . The supernatant was removed to the second set of test tubes and evaporated. Once evaporated, a second extraction was completed using dichloromethane. Four milliliters of dichloromethane was added to the dried sample, vortexed and shaken on a rack shaker for 30 min. The samples were then

centrifuged for 20 min at  $1900 \times g$  and the supernatant was removed. This was dried at  $37^\circ\text{C}$  under a stream of air. The extracted steroids were reconstituted in 300  $\mu\text{l}$  assay buffer (0.05 M sodium phosphate buffer with NaCl 0.9%, EDTA 0.5%, BSA 0.5% and sodium azide 0.01%) and vortexed before the final assay procedure.

Total androgen concentrations were measured in the fecal extracts by direct radioimmunoassay using anti-testosterone antiserum (code 8680-6004, Biogenesis, U.K.) and [ $^{125}\text{I}$ ]-testosterone label (code 07-189126, ICN, U.K.) (Parkinson and Follett, 1995). The antiserum not only detects testosterone, but also cross-reacts with other testosterone metabolites that are present in the faeces. Therefore, for the reasons mentioned above, we refer to levels of fecal androgens, rather than testosterone, throughout. The full biochemical validation for this technique to determine the spectrum of metabolites has now been completed for four species including the canary. The results show that the radioimmunoassay protocol is appropriate for identification of the vast majority of the excreted androgen metabolites present in the extracts (Goodship et al., in preparation). In addition, in a separate study of pied flycatchers, measurements of fecal androgen levels using technique were strongly correlated with plasma testosterone sampled simultaneously from the same individual ( $R=0.653$ ,  $N=32$ ,  $P<0.001$ ; Goodship, 2006), confirming the reliability of this method to detect the principal androgens present in both faeces and blood. The fecal samples were assayed using 20  $\mu\text{l}$  aliquots of reconstituted extract. The samples were run in three assays, the inter-assay coefficient of variation (CV) was 17.6% calculated from five pooled fecal samples run in all three assays and 16.1% for six plasma pools run in all three assays. The mean 50% binding was 9–10 pg/tube, and the mean detection limit for the three assays was 0.012 ng/ml for duplicate 20  $\mu\text{l}$  aliquots of reconstituted extract. Androgen levels were quantified for 68 different nestlings in total.

#### Biological validations

A number of protocol refinements were tried and the effects on the level of androgen measured from pooled fecal samples at the start of the validation were quantified before settling on the final procedure and carrying out a full biological validation. The biological validations carried out assessed (1) the characteristics and limitations of the assay, (2) parallelism of extracts and assay standard and (3) accuracy and precision. The characteristics and limitations of the assay were determined through testing for non-specific binding and determining assay sensitivity. Non-specific binding (% non-specific binding/total binding), calculated from four validation assays run prior to data collection, was 2.95%. The sensitivity of the assay was defined by the 50% binding level and the assay detection limit (95% binding). In the four validation assays, 50% binding varied between 7.4 and 9.8 (mean 8.6) pg/tube. The detection limit for these assays varied between 0.01 and 0.067 ng/ml (mean 0.027 ng/ml) for 20  $\mu\text{l}$  samples of reconstituted extract. Parallelism was confirmed by running serial doubling dilutions of fecal extracts (Fig. 1). This demonstrates that samples dilute parallel to the standard curve, a crucial assumption of the radioimmunoassay technique. Accuracy of the assay can be defined by intra-assay and inter-assay CV. The intra-assay CV was 6.49% for one fecal sample run six times in duplicate and the inter-assay CV for six plasma pools run in the four validation assays was 7.02%. The precision of the assay was determined by assessing the mean error in recovered steroid added before extraction (31.0%) and the mean error in recovered steroid added after extraction (0.4%).

#### Statistical analysis

##### Posture measured 5 days after hatching

The statistical analyses were conducted using GENSTAT version 6 and MINITAB version 13 (Minitab Inc., State College, PA, USA). We sampled two or three nestlings from each breeding pair, and so first tested whether we could detect significant family differences in both fecal androgens and postural begging intensity measured 5 days after hatching. To control for family differences, we used a Linear Mixed Effects (LME) model, with REML (Restricted Maximum Likelihood) algorithm, to analyse the relationship between fecal androgens and begging posture measured 5 days after hatching, including brood identity as a random factor to control for repeated measures of nestlings from the same family. Fecal androgens, growth rate, chick mass, brood size and the amount eaten to satiation after the trial were included as covariates in the initial

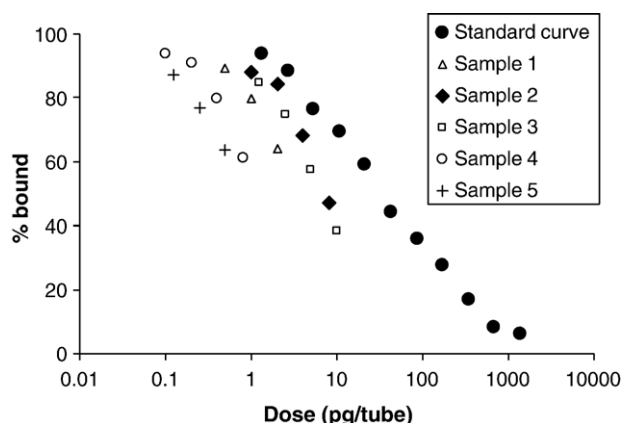


Fig. 1. Dose–response curves (inhibition of binding of labelled testosterone ligand to anti-androgen antiserum) for testosterone standard and for five fecal extracts, each run at serial doubling dilutions in the androgen radioimmunoassay. The extract dilutions are shown at arbitrary starting positions on the graph relative to the *x*-axis for clarity of representing parallelism with the standard curve.

model, with year and chick sex as factors. To investigate whether changes in fecal androgens were negatively correlated with growth rate, we ran a second LME with begging posture 5 days after hatching, brood size and fecal androgen as covariates, sex and year as factors, and brood identity as a random factor. For each model, we sequentially deleted non-significant terms to yield the minimal model. Measurements of androgens were log transformed where appropriate to fit the assumptions of the model. Colinearity diagnostics suggested that although two pairs of variables were significantly correlated, amount eaten to satiation and growth rate (Pearsons  $r=0.43$ ), and brood size and log T (Pearsons  $r=-0.24$ ), this was not sufficient to bias the results (Field, 2000).

*Posture measured eight days after hatching*

In 2001, we measured the postural begging intensity of 16 nestlings twice, once at 5 days after hatching and again 8 days after hatching. We used a General Linear Model (GLM) to investigate whether changes in fecal androgen levels during those three days were positively associated with changes in postural begging intensity during that time, and negatively correlated with chick growth.

**Results**

*Are measurements of fecal androgen levels repeatable within individuals?*

Androgen levels from fecal samples from adults ranged from 1.8 to 9.3 ng/ml reconstituted extract, whilst 5 day old chicks ( $N=68$ ) had a mean and standard error of  $0.313 \pm 0.026$  ng/ml androgen in their fecal extracts, and 8 day old chicks ( $N=19$ ) had  $0.43 \pm 0.53$  ng/ml. As there was a significant difference in fecal androgen levels from chicks in 2001 and 2002 (day 5 chicks 2001:  $0.472 \pm 0.06$  ng/ml; 2002:  $0.252 \pm 0.02$  ng/ml,  $t=3.52$ ,  $P=0.002$ ), year was retained in all further analyses, even though it was not significant. Repeated measurements of androgen levels taken on the same day from the 19 individual chicks sampled repeatedly (either day 5 or day 8 post-hatch), showed significant variation between individuals ( $F_{18,41}=2.19$ ,  $P=0.039$ ), although the repeatability value was low ( $r=0.36$ ). Measurements taken from the same individual on day 5 and then again on day 8 post-hatch were not significantly repeatable ( $F_{17,33}=1.04$ ,  $P=0.474$ ) and had an even lower repeatability value ( $r=0.21$ ) (Lessells and Boag, 1987).

*Family differences in begging and fecal androgen levels*

There were significant differences among families in the postural begging intensity of their offspring, 5 days after hatching (GLM: brood identity effect  $F_{30,73}=1.77$ ,  $P=0.043$ ; Fig. 2a). Families also differed significantly in the fecal androgen levels of their offspring 5 days after hatching (GLM: brood identity effect  $F_{30,67}=2.65$ ,  $P=0.003$ ; Fig. 2b). We therefore retained family identity as a random factor in the further analyses.

*Are fecal androgen levels positively correlated with postural begging intensity?*

As predicted, after controlling for family differences in nestling begging intensity, we found that nestlings that begged more intensely for food had correspondingly higher levels of fecal androgens at 5 days post-hatch (Table 1, Fig. 3). They were also more likely to eat a large meal at the end of the begging trial and less likely to be growing well (Table 1).

*Are changes in begging and growth during the nestling period correlated with changes in fecal androgen levels?*

We collected samples from a limited number of chicks on day 8 to test whether changes in fecal androgen levels during the

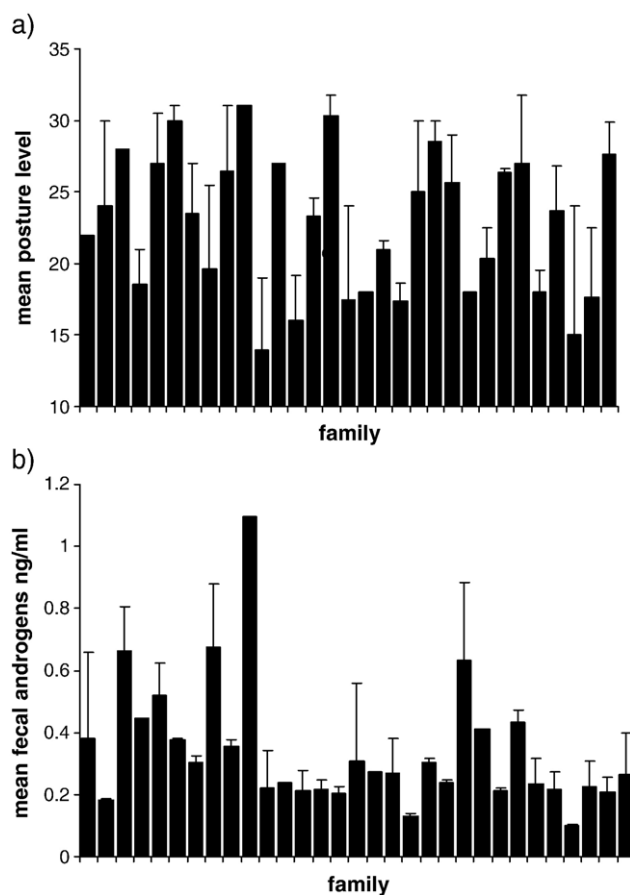


Fig. 2. Differences among families in (a) postural begging intensity and (b) fecal androgen levels. Means with standard error bars are shown.

Table 1  
A linear mixed effects model (LME) explaining variation among 5 day old nestlings in postural begging intensity

Begging posture	df	Wald	P
<i>Full model</i>			
Growth rate	1	12.69	<0.001
Amount eaten to satiation	1	12.18	<0.001
Log T	1	9.67	<0.001
Sex	1	3.69	0.06
Brood size	1	1.03	0.31
Mass	1	0.98	0.32
Year	1	0.24	0.62
Begging posture	Average effect	SE	
<i>Minimal model</i>			
Constant	23.01	1.41	
Growth rate	-11.37	3.19	
Amount eaten to satiation	8.61	2.47	
Log T	3.33	1.07	
Year 2001	0.00	0.00	
Year 2002	-0.86	1.75	

N=64 individuals at 29 nests. Nest identity was controlled for as a random factor (average effect=5.87, SE=4.67, P>0.05).

All two-way interactions were investigated and were not significant.

nestling period were positively associated with changes in nestling begging intensity. This was found to be the case. After controlling for nestling sex, the greater the increase in fecal androgen levels between day 5 and day 8 after hatching, the greater the corresponding increase in nestling begging intensity (GLM:  $F_{2,15}=4.14$ ,  $P=0.041$ ; fecal androgen effect  $F_{1,15}=5.35$ ,  $P=0.038$ ; Fig. 4a). For the same change in fecal androgen levels, there was greater change in the postural begging intensity of females than males (GLM: sex effect  $F_{1,15}=6.43$ ,  $P=0.025$ ; Fig. 4a).

Changes in fecal androgen levels during the nestling period were also negatively correlated with changes in nestling growth. There were no sex differences in this correlation, so nestling sex was dropped from the model (GLM: sex effect, marginal  $F=0.001$ ). The greater the increase in fecal androgen levels between day 5 and day 8 after hatching, the smaller the

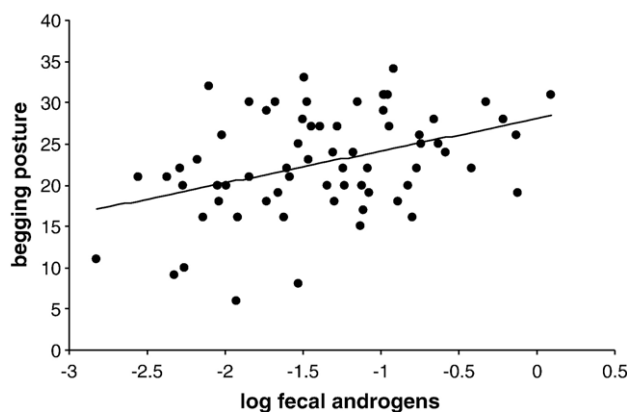


Fig. 3. The relationship between fecal androgens and postural begging intensity in 5 day old nestlings. Each datapoint corresponds to one nestling. The least squares regression line is shown,  $N=68$ ,  $r=0.34$ ,  $P=0.004$ .

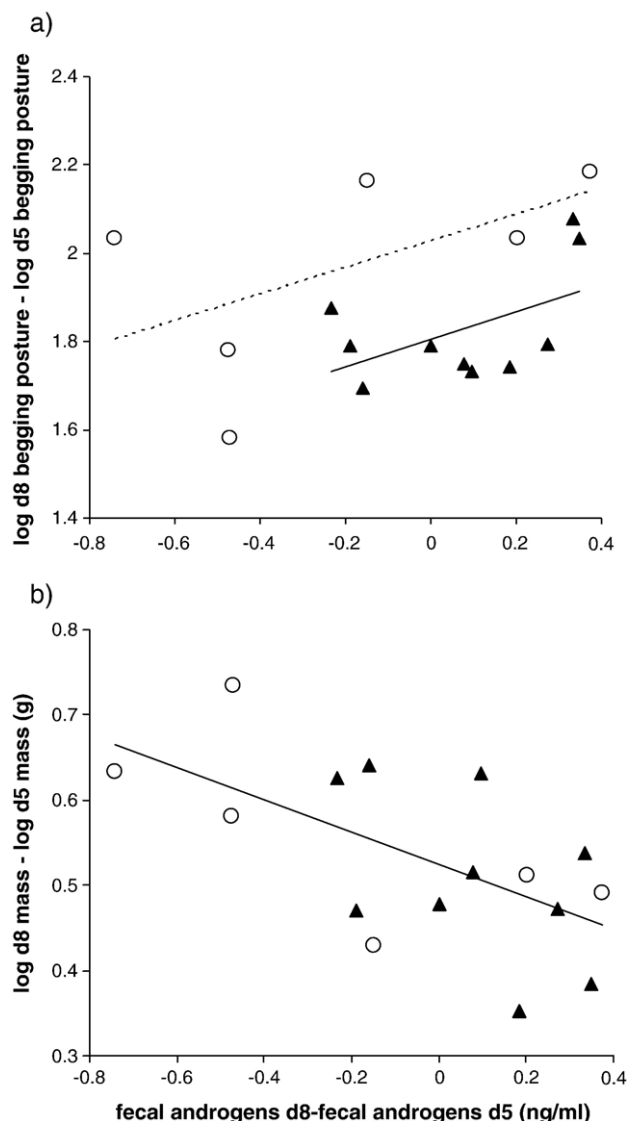


Fig. 4. The relationship between the change in fecal androgens between 5 and 8 days after hatching and the corresponding change in (a) postural begging intensity and (b) nestling mass. Female chicks are represented by open circles and male chicks by triangles. In panel a, the least squares regression lines are shown for females (dashed line) and males (solid line). In panel b, the solid line represents males and females together.  $N=16$ ,  $r=-0.61$ ,  $P=0.012$ .

associated change in nestling mass (GLM:  $F_{1,15}=8.36$ ,  $P=0.012$ ; Fig. 4b).

### Discussion

The results from our study show that begging intensity is positively related to fecal androgen levels. Although this relationship has been documented before in both passerines and non-passerines (Goodship and Buchanan, 2006; Quillfeldt et al., 2006), Groothuis and Ros (2005) found that manipulation of testosterone levels in young black headed gull chicks reduced begging. There are therefore potential differences between species in this relationship. However, these studies differ not only in species, but in timing of androgen administration and in

the method of administration. There are many possible reasons why these differences between studies could have occurred and more work is needed to clarify the role of testosterone in modulating growth and begging behaviour. Our study also shows significant interfamily differences in fecal androgens and begging intensity. Finally, this correlational study also provides some support for the hypothesis that androgens mediate the trade-off between canary nestling begging behaviour and growth. We now discuss potential explanations for the results obtained in this study.

The least conservative interpretation of our results is that they support our suggestion of a androgen-mediated trade-off between begging and nestling growth. When considering the change in nestling androgen levels during development, we found a negative relationship between fecal androgen levels and growth. These data are consistent with the interpretation that androgens increase begging intensity and therefore food intake, but at a physiological cost which impacts on growth. This could occur either by an energetic cost imposed by begging, by direct suppression of growth by testosterone or by a combination of these effects. Testosterone has several negative effects, including increased metabolic rate (Buchanan et al., 2001) and immunosuppression (Folstad and Karter, 1992), as well as the costs of associated behaviour such as aggression (Wingfield et al., 2001). Each of these could potentially offset the benefits that nestlings stand to gain from increased begging by impacting on growth and development.

Our finding that families vary both in their nestling begging intensities and in their levels of fecal androgens matches similar results from thin-billed prions (Quillfeldt et al., 2006) and pied flycatchers (Goodship and Buchanan, 2006). It is consistent with the suggestion that nestlings strategically adjust their testosterone levels, and hence their begging intensities, to match their parents' responsiveness to begging. Alternatively, this effect could be genetically determined, with some families having intrinsically higher rates of testosterone secretion than others. A combination of cross-fostering experiments and parental sampling would be needed to distinguish these two alternatives.

Derting (1989) investigated whether thyroid hormone mediates a trade-off between metabolic rate and growth in young cotton rats (*Sigmodon hispidus*). Just as predicted, she found that rats treated with thyroid hormone had an elevated metabolic rate but barely grew at all and some even lost weight. However, this trade-off was apparent only when food was restricted. When food was abundant, rats with a higher metabolic rate gained weight and grew more rapidly than control individuals (Derting, 1989). Some researchers have failed to show a link between testosterone in and condition (e.g. Tarlow et al., 2001; Fargallo et al., 2007), whereas others have found them to be positively correlated (e.g. Goodship and Buchanan, 2006; Quillfeldt et al., 2006). Furthermore, studies which do not support a positive relationship between T and begging e.g. (Groothuis and Ros, 2005; Quillfeldt et al., 2007), suggest that any T-mediated trade-off between begging and growth may be context dependent, possibly involving hunger levels.

Finally, it is possible that androgens indirectly mediate a trade-off between begging and growth and corticosterone is a plausible candidate for involvement in such a relationship. Corticosterone is produced endogenously by canary nestlings (Schwabl, 1999) and, in non-passerines at least, is known to be positively correlated with nestling begging intensity (Kataysky et al., 2001; Quillfeldt et al., 2006), yet negatively related to nestling growth (Kataysky et al., 1999; Quillfeldt et al., 2006). Alternatively, perhaps there is no direct physiological trade-off between these two traits and a more complex pathway is involved instead incorporating, say, testosterone, corticosterone and avian growth hormone (Schwabl and Lipar, 2002). In this study, we are unable to assess the importance of corticosterone as we did not blood sample the birds and as yet no validated assay procedure allows us to sample corticosterone metabolites from the faeces of this species.

A final possibility is that there is no androgen-mediated trade-off and that the negative relationship between androgen levels and growth may be due to relationships with confounding variables (e.g. nestling quality, parental aggression levels). Again, cross fostering experiments, combined with testosterone manipulations would address this issue.

In this study, we used a recently validated method for quantifying fecal androgens. This is a particularly useful technique when repeatedly sampling from small animals such as nestling birds. But any new radioimmunoassay procedure must be fully validated for the species and medium for which it is used, and this is particularly true when conducting analysis of samples obtained through 'non-invasive' procedures (Buchanan and Goldsmith, 2004; Goymann, 2005). Here we have conducted a full biological validation of this new assay technique for quantifying androgens extracted from fecal samples, including determination of the level of error and repeatability associated with this technique, as well as the assay limitations. We found that all the canary fecal samples fell within the detection limits of the assay. Furthermore, a radioinfusion study allowing a biochemical validation confirms that the principal androgen metabolites which are excreted are detected by our radioimmunoassay, as the HPLC fractions detecting androgen metabolites show concordance with radioimmunoassay levels (Goodship et al. in preparation). Finally, the correlation between plasma testosterone and fecal androgen levels suggests that the assay detects either the principal metabolites or correlates of their excretion levels.

In summary, our correlational data reveal a positive association between nestling begging intensity and fecal androgens, and highlight marked family differences in each trait. In addition, they suggest that a testosterone-mediated trade-off between begging and growth is plausible. Manipulative experiments are now needed to test whether testosterone is directly responsible for increasing begging intensity at the expense of a nestling's growth.

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