Nestling testosterone is associated with begging behaviour and fledging success in the pied flycatcher, 
*Ficedula hypoleuca*

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Animal signals are hypothesized to be costly in order to honestly reflect individual quality. Offspring solicitation signals given by nestling birds are thought to have evolved to advertise either need or individual quality. We tested the potential role of testosterone (T) in controlling the intensity of these signals by measuring begging behaviour as: (i) duration of the begging display and (ii) maximum height of the begging stretch, and by sampling endogenous T levels in nestling blood. We tested nestling pied flycatchers (*Ficedula hypoleuca*) using well-established experimental paradigm involving transient food deprivation to encourage begging behaviour and then blood-sampled nestlings at the end of these tests for T levels. Our results show that individual nestlings with the most intense begging displays had the highest circulating levels of T immediately after testing. In addition, we found substantial differences between broods in terms of circulating T. Finally, we found evidence that broods with higher levels of T showed increased fledging success, indicating a benefit for increased T production in nestlings. The potential trade-offs involved in T-mediated begging behaviour are discussed.

**Keywords:** offspring solicitation; begging behaviour; testosterone; signalling

1. INTRODUCTION

Models concerning the evolution and maintenance of animal signals show that costly begging displays resolve parent–offspring conflicts (Godfray 1991, 1995). In theory, conflicts of interest occur because the optimal division of food resources differs between parents and offspring, due to asymmetries in relatedness (Trivers 1974). In nestling birds, begging behaviour may resolve conflicts of interest either through: (i) scramble competition between siblings for food provided non-selectively by the parents (Parker & Macnair 1979), or by (ii) honestly advertising offspring condition so that parents choose which offspring to feed in relation to begging intensity (Godfray 1991, 1995). We sought to identify a mechanism which controls begging behaviour to improve our understanding of the evolution and maintenance of animal signals. In this study, we investigated the potential of the endocrine system and in particular testosterone (T) as a controlling mechanism of begging behaviour.

There are considerable reasons to suppose that the endocrine system regulates begging behaviour. Two types of steroid hormones: (i) endogenously produced corticosterone (Kitaysky et al. 2001b) and (ii) maternally derived androgens (Schwabl 1996; Eising & Groothuis 2003) have been shown to influence begging behaviour. Experimentally increased corticosterone levels in black-legged Kittiwake, *Rissa tridactyla* chicks have been found to increase begging effort and food provisioning rates by parents. Additionally, corticosterone-implanted chicks in broods of two begged more frequently than singletons (Kitaysky et al. 2001b). Furthermore, corticosterone levels increase with food shortage and are negatively correlated with body condition in black-legged kittiwakes (Kitaysky et al. 1999) and red-legged kittiwake, *Rissa brevirostris* chicks (Kitaysky et al. 2001a). Food restriction has also been shown to increase circulating corticosterone levels in blue-footed boobies, *Sula nebouxii* (Núñez-de La Mora et al. 1996).

Maternal T in the egg yolks of canaries, *Serinus canaria* (Schwabl 1996) and black-headed gulls, *Larus ridibundus* (Eising & Groothuis 2003) has been shown to positively correlate with post-hatching begging effort as well as nestling growth and development. In red-winged blackbird *Agelaius phoeniceus* eggs, experimentally increased T levels in the yolk result in a greater mass of the *musculus complexus*, the neck muscle used for begging (Lipar & Ketterson 2000). A logical conclusion of such studies is that maternally derived T controls offspring T production, or some other developmental control mechanism; however, no study has yet related maternal T directly with offspring endogenous T after hatching. Furthermore, while maternal T may trigger developmental pathways, it seems unlikely that maternally derived T per se controls offspring behaviour. This is because passerine nestlings are known to be able to produce their own T prior to hatching (Adkins-Regan et al. 1990; Ottinger & Abdelnabi 1997) and in addition, steroids have half-lives of hours, not days (Goymann et al. 2002). Circulating plasma T has been measured in nestling European starlings, *Sturnus vulgaris* (Williams et al. 1987), great tits, *Parus major* (Silverin & Sharp 1996), European robins, *Erithacus rubecula* (Schwabl & Lipar 2002) and zebra finches, *Taeniopygia guttata* (Naguib et al. 2004). However, these studies did not attempt to relate endogenously produced T with begging behaviour. In a recent study of thin-billed prion *Pachyptila belcherie* nestlings, both corticosterone
and T levels were positively correlated with measures of begging behaviour (Quillfeldt et al. in press). The single offspring in each nest, as is typical of petrels, showed positive covariation between body condition, begging calls and circulating levels of T. However, at present, it remains unknown what effect the T produced by passerine nestlings has on begging behaviour, within and between broods, or what consequences this may have for nestling fitness.

Pied flycatchers represent an excellent study species in which to test the relationship between begging behaviour and T, as their begging behaviour has previously been studied (Wright et al. 2002) and they have reasonably large brood sizes (3 = 7 nestlings). In adult male pied flycatchers, plasma T has been shown to affect habitat choice (Silverin 1998a) and response to predators (Silverin 1998b). The aim of this study was to test the effects of hunger on nestling begging behaviour and to correlate begging with circulating levels of endogenous T. We tested two hypotheses in pied flycatcher nestlings: (i) begging intensity correlates with circulating T levels, and (ii) individuals with higher levels of T show increased indices of fitness as a result.

### 2. MATERIAL AND METHODS

#### (a) Study subjects

We conducted manipulative experiments to determine the relationship between increasing hunger, begging behaviour and plasma T in nestling pied flycatchers during May and June 2003 and 2004. A nestbox (n = 199) population of pied flycatchers was studied at Llysdinam field centre in mid Wales, which consists of a 26.96 ha area of oak and coniferous woodland situated in steep sided valleys. Over two breeding seasons, broods from 24 randomly selected nestboxes were used in this study. Nestboxes were monitored to record: laying date; hatching date (hatching = day 0); number of eggs hatching; brood size and fledging success. Adults were caught at the nest and ringed and all nestlings were ringed at 7 days post-hatching; brood size and fledging success. Adults were caught at the nest and ringed and all nestlings were ringed at 7 days post-hatching.

#### (b) Experimental protocol

At 7 days of age, a randomly selected nestling was temporarily (for less than 5 h) removed from a nestbox (n = 24) and fed to satiation with Nectarblend rearing mix (Haith’s Seeds, bird nesting material) and covered over with a piece of dark material. In each begging trial we recorded: (i) the duration (s) of the begging display (from the start of the begging stimulus until begging behaviour ceased) and (ii) the maximum height (mm) of the begging stretch (maximum distance moved by the tip of the beak during a begging display). After 120 min food deprivation and nine begging trials, each nestling was blood sampled (100 µl) to assess circulating T concentration, weighed (accuracy 0.25 g) then returned to the original nestbox in the field. Siblings from the experimental boxes were also weighed and blood sampled at seven days post-hatching.

#### (c) Hormone assays

Blood samples (100 µl) were collected in heparinized capillary tubes (50 µl), expelled into microcentrifuge tubes (1.5 ml) after puncturing the brachial vein with a sterile syringe needle (×25 g) and stored on ice for a maximum of 3 h before centrifugation. Blood samples were centrifuged for 15 min at 13 000 rpm on a bench top centrifuge (Bocco) and plasma was transferred into a microcentrifuge tube (0.5 ml) and stored at −20 °C until assay. All samples were assayed within 10 months of collection.

Total androgen concentrations were measured in the plasma samples by direct radioimmunoassay using anti-T anti-serum (code 8680-6004, Biogenesis, UK) and [125I]-T label (code 07-189126, ICN, UK; Parkinson & Follett 1995). Although there is some cross-reaction of the antiserum with other androgens in the plasma, the cross-reactivity is low (22.2%) and, therefore, this assay presents a reliable surrogate measure of absolute T levels. It was confirmed that pied flycatcher plasma samples diluted parallel with the standard curve. The samples were run in four assays with 50% binding level at 0.34–0.56 ng per tube and a detection limit of 0.01–0.02 ng ml⁻¹ for 20 µl of sample; experimental samples were assayed in 20 µl duplicate volumes. The intra-assay coefficient of variation was 4.88% and the inter-assay variation was 10.34%.

#### (d) Statistical analysis

Statistical tests were performed in MINITAB 14.0. We used general linear models (GLM) to construct two models relating to individual begging behaviour using: (i) duration of the begging display and (ii) maximum height of the begging stretch as the dependent variables. These models asked what variables best described variation in nestling begging intensity. For both begging display duration and maximum height of begging stretch, we investigated the effects the following variables: nestling T, nestling weight, brood size, sampling date and year; the food volume used to satiate nestlings and the time of day at which nestling T was sampled were also entered to control for any variation. Mean T ± S.E. in 12 broods in 2003 was calculated to investigate inter-family disturbance throughout the experiment using a temperature-sensitive probe (Maplin, Barnsley, England) placed under the nestling.

After 30 min of food deprivation, we began recording begging trials every 10 min. In each begging trial, we stimulated the nestling to beg by removing the covering piece of dark material and making two standardized ‘tut–tut’ noises in succession while gently waving a metal spatula 3–4 cm above the head of the nestling. The spatula was continually waved until begging behaviour ceased (i.e. once more than 10 s had elapsed after head of the nestling was lowered). The begging stimulus was terminated by replacing the covering piece of material over the nestling. In each begging trial we recorded: (i) the duration (s) of the begging display (from the start of the begging stimulus until begging behaviour ceased) and (ii) the maximum height (mm) of the begging stretch (maximum distance moved by the tip of the beak during a begging display). After 120 min food deprivation and nine begging trials, each nestling was blood sampled (100 µl) to assess circulating T concentration, weighed (accuracy 0.25 g) then returned to the original nestbox in the field. Siblings from the experimental boxes were also weighed and blood sampled at seven days post-hatching.
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Subsequent analyses were, therefore, conducted using the mum begging intensity during the 120 min begging trials.
so that nestlings reached their maximum height of begging stretch by 7-day-old pied flycatchers (n=24) at 120 min food deprivation.

Table 1. Variables in a general linear model which explain individual variation in (a) duration of begging displays and (b) maximum height of begging stretch by 7-day-old pied flycatchers (n=24) at 120 min food deprivation.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) duration of begging display (s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>1</td>
<td>14.54</td>
<td>0.001</td>
</tr>
<tr>
<td>Nestling testosterone</td>
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<td>31.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(b) maximum height of begging stretch (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nestling weight</td>
<td>1</td>
<td>54.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nestling testosterone</td>
<td>1</td>
<td>21.90</td>
<td>0.001</td>
</tr>
<tr>
<td>Date</td>
<td>1</td>
<td>64.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brood size</td>
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</tr>
<tr>
<td>Year</td>
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<td>4.45</td>
<td>0.061</td>
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<tr>
<td>Nestling testosterone X Year</td>
<td>1</td>
<td>9.36</td>
<td>0.012</td>
</tr>
<tr>
<td>Nestling weight X Date</td>
<td>1</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Nestling weight X Brood size</td>
<td>1</td>
<td>28.89</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The mean duration of nestling begging displays increased from 2 s at 30 min since food satiation to 50 s at 110 min since food satiation (ANOVA $F_{9,110}=11.49$, p<0.001, n=12). The maximum height of begging stretches also increased over the same time from 7.1 to 44.4 mm (ANOVA $F_{9,110}=8.91$, p<0.001, n=12). The duration of begging displays and maximum height of begging stretches reached a plateau after 110 min since food satiation, suggesting that nestlings reached their maximum begging intensity during the 120 min begging trials. Subsequent analyses were, therefore, conducted using the data gathered at 120 min post-satiation.

(b) Nestling testosterone

The final GLM for duration of begging displays showed that only year and nestling T explained significant variation in begging duration (table 1a, figure 1). Although, the duration of begging displays were shorter in 2004 than in 2003, there was a positive correlation between begging display duration and nestling T within both years (2003: $r=0.661$, n=12, p=0.019; 2004: $r=0.731$, n=12, p=0.025). Mean nestling T (0.127 ng ml$^{-1}$±0.033) included one outlier sampled in 2003 with a substantially higher T value (0.702 ng ml$^{-1}$) compared with other nestlings in the sample. However, handling treatment and all other measured variables of the outlier including weight, brood size, date, volume of food used to satiate and sampling time were similar to the other nestlings. Removal of the outlier from the GLM made the effect of nestling T on the duration of begging displays marginally non-significant ($F_{1,17}=3.14$, p=0.094).

The final GLM for maximum height of begging stretch showed that nestling weight, nestling T, date and brood size all had a significant positive effect on maximum height of begging stretch (table 1b). The positive effect of nestling T was still significant even after the outlier was removed (ANOVA $F_{1,10}=14.06$, p=0.005). Year was found to have a marginally non-significant relationship with maximum height of begging stretch (ANOVA $F_{1,10}=4.45$, p=0.061). There were three significant interactions. The significant interaction between nestling T and year may be explained by the generally lower begging stretches observed in 2004, compared with 2003, for any given value of nestling T. The relationship between nestling weight and date changed through the season. Nestling weight at 7 days old increased with the date during the early part of the season, but later in the season, nestling weight was not affected by date. It was also found that the variance of nestling weight in larger broods was greater than that of smaller broods.

(c) Nestling family

There was significant inter-family variation on nestling T (figure 2). It was found that mean brood T level of family F, including the outlier, was significantly higher than mean brood T in all other families (except family I) in the same year (Fisher’s pairwise comparisons $F_{1,56}=5.76$, p<0.001, n=68). T levels of individual nestlings measured during the begging trials were highly correlated with mean brood T levels ($r=0.913$, n=12, p<0.001).

(d) Nestling fledging success

We tested the variables predicting significant variation in fledging success and found that mean nestling T was positively associated with fledging success across 2003 and 2004 ($F_{1,21}=6.08$, p=0.022, n=23). In the final GLM model, there was no significant effect of mean nestling weight, brood size, date or year on fledging success. Broods with high T levels at 7 days old were more likely to have a higher proportion surviving until fledging than broods with low T levels (figure 3). Although the percentage fledging success was similar between years in our sample of 24 nestboxes used for the begging trials, over the whole nestbox breeding colony at Llysdinam, fledging success was higher in 2004 (mean fledging success=87%) than in 2003 (mean fledging

Figure 1. The relationship between the duration of begging displays and circulating testosterone levels in 7-day-old pied flycatchers. Fitted data is presented for 2003 (open circles) and 2004 (filled circles).

Figure 2. The relationship between nestling testosterone levels and date. Fitted data is presented for 2003 (open circles) and 2004 (filled circles).

3. RESULTS

(a) Begging trials

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4. DISCUSSION

To our knowledge, the results of this study show for the first time, the covariation between nestling begging behaviour and circulating plasma T levels in nestling birds. We found this relationship for two different measures of begging effort: (i) duration of the begging display and (ii) maximum height of the begging stretch at a standardized time after feeding. Although this study did not address a direct causal relationship, T may affect begging behaviour by increasing metabolism and growth rate (Smith & Montgomerie 1991; Schwabl 1996), enhancing perception of cues which stimulate begging behaviour (Andrew 1975) or by increasing the mass of the hatching muscle (Lipar & Ketterson 2000). Alternatively, there may not be a causal relationship between T and begging behaviour, but T production may be a proxy for indices of condition. Further manipulative work is now needed to determine the causal links between T and begging behaviour.

A relationship between T and begging behaviour would also provide a proximate explanation as to why nestling birds vary in their level of begging effort both within and between nests (e.g. Price & Ydenberg 1995; Lotem 1998). Previous studies have suggested that maternal T may play a role in establishing brood hierarchies (Schwabl 1993; Winkler 1993), although in blue-footed booby chicks, T levels have been found to be similar between members of the same brood (Ramos-Fernández et al. 2000).

Patterns of maternal T deposited into egg yolks have been found to vary with laying order, which may either serve to counteract the competitive hierarchy due to hatching asynchrony (e.g. Eising & Groothuis 2003) or enhance it (Schwabl et al. 1997). Interestingly, circulating plasma T is known to be elevated during the first 2 days after hatching in nestling birds (Adkins-Regan et al. 1990; Silverin & Sharp 1996), but further study is required to investigate what effect individual T levels have on maintaining broods hierarchies.

Different environmental conditions change not only absolute T levels but may also change how production of T translates into begging behaviour. Our data demonstrate this effect by a year interaction, where the duration of begging displays per nanogram of T is greater in 2003 than in 2004. Studies in a range of passerine species have found that nestling begging intensity increases when food availability is low (Smith & Montgomerie 1991; Kilner 1995; Price & Ydenberg 1995; Wright et al. 2002), therefore the longer begging displays in 2003 may have been a response to lower food supplies in that year. Food availability plays a key role in fledging success (e.g. Riddington & Gosler 1995), and our data also show that fledging success for the whole of our nestbox breeding colony of pied flycatchers at Llysdinam was lower in 2003 than in 2004. In nest-bound chicks, food shortages are associated with an increase in circulating levels of corticosterone (e.g. Núñez-de La Mora et al. 1996; Kitaysky et al. 1999, 2001a). Short-term release of corticosterone in black-legged Kittiwake chicks restores energy reserves through begging behaviour and sibling competition by increasing parental provisioning (Kitaysky et al. 2001b). However, chronic elevation of corticosterone has a detrimental effect on cognitive ability (Kitaysky et al. 2003) and chicks may suppress adrenocortical activity during prolonged food shortage (Kitaysky et al. 2001a). As corticosterone and T bind with the same plasma binding globulin (Deviche et al. 2001), T production may also be affected during times of prolonged food shortage.

The results of this study also suggest that there are considerable differences in T production by nestling birds between broods, at the same age post-hatching. This may go some way to explain the apparent differences between broods of nestling birds in their rates of growth and

![Figure 2. The mean ± s.e. testosterone levels of all pied flycatcher siblings (sampled at 7 days old) in 12 individual broods (A–L) in 2003.](image1)

![Figure 3. Relationship between mean brood testosterone levels and fledging success (n=23) sampled in 2003 and 2004.](image2)

![Figure 4. Relationship between mean brood testosterone levels and fledging success (n=23) sampled in 2003 and 2004.](image3)
development (e.g. Nisbet et al. 1998; Podlesak & Blem 2001). Furthermore, our data suggest that these differences may translate into real fitness effects, as broods with higher T production at 7 days post-hatching had a greater fledging success from the nest. Even with this relatively small sample of nests, it is clear that this was not a seasonal effect, as nests fledging earlier in the season did not show increased T production. Further investigation is needed to understand the basis for these inter-family effects.

Signalling models predict that for signals to be honest indicators of need they should be costly to produce or maintain (Kilner & Johnstone 1997). Many potential costs of begging behaviour have been investigated, including predation risk (Macnair & Parker 1979; Haskell 1994; Leech & Leonard 1997 and growth costs (Kilner 2001). Endogenous T production is also thought to mediate a number of potential physiological costs including increased basal metabolic rate (Buchanan et al. 2001), immunosuppression (Folstad & Karter 1992), aggression (Johnsen 1998) and increased levels of corticosterone (Evans et al. 2000). Good quality individuals may not suffer such high costs associated with T production (Folstad & Karter 1992), or these costs may not be so detrimental to individuals most able to tolerate high T levels. If increased T levels in nestling birds produce increased physiological costs, this would infer T-mediation of the cost of nestling begging displays. Manipulative studies altering the levels of nestling T within physiological ranges are required to investigate the many potential costs involved.

In conclusion, we would suggest that there is considerable potential for T to be causally involved in controlling the level of begging effort produced by nestlings both between and within nests in this species. Such endocrine control of offspring behaviour could explain the evolution of begging as a costly signal honestly reflecting need.

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