JOURNAL OF Evolutionary Biology

doi: 10.1111/j.1420-9101.2011.02242.x

Begging at high level simultaneously impairs growth and immune response in southern shrike (*Lanius meridionalis*) nestlings

G. MORENO-RUEDA* & T. REDONDO†

*Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas, Almería, Spain
†Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, Sevilla, Spain

Keywords:

begging;
growth costs;
honest signalling;
immunocompetence;
parent–offspring conflict;
sibling competition;
trade-offs.

Abstract

Theoretical models suggest that begging should be costly in order to be evolutionarily stable. However, evidence for such a cost is contradictory (e.g. for growth costs) or scant (e.g. for immunological costs). Here, we experimentally test the existence of both costs in southern shrike (*Lanius meridionalis*) nestlings. Nestlings were paired by nest of origin and similar body mass. In each pair, a nestling was forced to beg for about 30 s h^{-1}, whereas the other begged for only 2 s, both nestlings receiving the same quantity of food. At the same time, the nestling response to an antigen (phytohaemagglutinin) was measured. Nestlings forced to beg for longer showed a reduction in growth rate and in immunocompetence when compared to control chicks. The two costs occurred independently of each other and were negatively correlated to time begging. These results strongly support models of honest signalling as well as scramble competition, which predict that begging should be costly in order to be evolutionarily stable.

Introduction

Animal signals, in order to be honest, usually should be costly, either to produce or to maintain (Zahavi & Zahavi, 1997; Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005). Communication may be honest without associated costs when the interests of senders and receivers match (Maynard Smith, 1994), which typically occurs between relatives (Reeve, 1997). Offspring frequently solicit food from their parents by a set of exuberant displays called begging (e.g. Redondo & Castro, 1992). Begging could be such a type of honest signal that is relatively cheap to produce, as suggested by some models (Bergstrom & Lachmann, 1997, 1998; Lachmann *et al.*, 2001). Nevertheless, begging displays appear to be excessively complex and extravagant (wasteful) to merely accomplish an efficient transfer of food from parents to chicks. Rather, conspicuous begging behaviour may be the evolutionary outcome of a genetic conflict of interests between parents and their offspring about the amount of transferred parental resources. Within a family, the interests of offspring and parents do not completely match (Parent-Offspring Conflict; Trivers, 1974), and each offspring is selected to secure more resources from its parent than the latter is selected to give (Clutton-Brock, 1991; Mock & Parker, 1997; Kilner & Hinde, 2008). For this reason, cheap begging would be probably uninformative with respect to offspring need (Brilot & Johnstone, 2002). In contrast, begging honestly would signal offspring need if it is costly to produce (honest-signalling models; Godfray, 1991, 1995). Costly begging is also predicted by models in which begging is employed in sibling competition, not in communication (scramble competition models; Parker & Macnair, 1979; Parker *et al.*, 2002). In both scramble and honest-signalling models, an offspring that begs more receives more food, chicks in greater need beg more intensively, and both models require a cost of begging to balance the benefit of increased begging, thus ensuring the evolutionary stability of the communicative system (Royle *et al.*, 2002).

While there is convincing evidence that begging intensity varies with short-term nutritional need and that parents respond accordingly (Kilner & Johnstone, 1997; Budden & Wright, 2001), the question of whether begging signals are really costly in terms of offspring fitness still remains a troubling area of disparity between

Correspondence: G. Moreno-Rueda, Estación Experimental de Zonas Áridas (CSIC), La Cañada de San Urbano, Ctra. Sacramento s/n, 04120, Almería, Spain.
Tel.: +34 950281045 (ext. 416); fax: +34 950277100;
e-mail: gmr@eeza.csic.es

theoretical and empirical studies (Wright & Leonard, 2002; Searcy & Nowicki, 2005; Moreno-Rueda, 2007). Several costs associated with begging have been proposed. First, conspicuous calling may attract predators to the nest (Haskell, 2002). It seems that predation has played a role in the evolution of vocal communication between parent birds and their young (Redondo & Arias-de-Reyna, 1988; Briskie *et al.*, 1999; Magrath *et al.*, 2010), but there is little indisputable evidence for a predation cost of begging (Haskell, 2002; Moreno-Rueda, 2007; but see McDonald *et al.*, 2009). In any case, predation costs are not generalized in all species (Haskell, 1999). Moreover, predation costs are shared by the whole brood and may be unlikely to account for the sort of individual marginal cost required by some begging models (Godfray, 1995; Rodríguez-Gironés *et al.*, 2001). Chicks could also fall from the nest as a result of intensive begging (Bize & Roulin, 2006), or elicit punishment by parents or siblings (Leonard *et al.*, 1991), but these costs seem to be infrequent.

In most species, begging involves vigorous posturing and calling (Redondo & Castro, 1992) and considerable attentiveness (Roulin, 2001). Thus, it is often assumed that begging is physiologically costly in a way that is directly related to the intensity of the display (Roulin, 2001). The most evident physiological cost of begging should be energetic expenditure resulting from muscular and neural activity. Several studies have attempted to measure the energetic expenditure in nestling birds in a variety of species, but their results suggest that metabolic expenditure of begging is relatively low, and its evolutionary implications have proved difficult to interpret (reviews in Chappell & Bachman, 2002; Moreno-Rueda, 2007). Verhulst & Wiersma (1997) argued that, given the limited scope of growing nestlings to allocate resources to growth (13–28% of total metabolized energy), even small increases in energetic expenditure for begging may result in a disproportionate decrease in survival prospects for chicks, considering that growth rate strongly influences nestling survival (Schwagmeyer & Mock, 2008). Following this line of thought, several studies looked for a growth cost of begging in different species, but with contradictory results. In canaries (*Serinus canaria*) and magpies (*Pica pica*), chicks experimentally induced to beg at higher rates showed reduced growth rates compared to less begging controls (Kilner, 2001; Rodríguez-Gironés *et al.*, 2001). However, comparable experiments failed to detect any effect of begging on growth in three other bird species (house sparrow, *Passer domesticus*, Kedar *et al.*, 2000; Moreno-Rueda, 2010; ring dove, *Streptopelia risoria*, Rodríguez-Gironés *et al.*, 2001; tree swallow, *Tachicyneta bicolor*, Leonard *et al.*, 2003), and in a burying beetle (*Nicrophorus vespilloides*, Smiseth & Parker, 2008).

It has been suggested that these contradictory results may be the consequence of species varying in the duration of begging bouts (Kilner, 2001), differences in growth rates and peak energy demands between species (Rodríguez-Gironés *et al.*, 2001; Leonard *et al.*, 2003), or differences in begging tactics (Moreno-Rueda, 2007). Another possibility is that nestlings incur physiological begging costs other than reduced growth rate. Several authors have suggested that begging may incur a cost by reducing immunocompetence (Roulin, 2001; Goodship & Buchanan, 2006; Moreno-Rueda, 2007; Loiseau *et al.*, 2008; Ros, 2008). The immune system is costly and it has been shown that it may take resources from other costly functions (Ardia & Schat, 2008). In fact, several studies have shown a trade-off between nestling growth and the function of immune system (Soler *et al.*, 2003; Brommer, 2004; Mauck *et al.*, 2005). Moreover, less immunocompetent nestlings have a higher mortality risk (Møller & Saino, 2004; Cichoń & Dubiec, 2005; Moreno *et al.*, 2005). Therefore, offspring could be facing a three-way trade-off among investment in begging, growth and the immune system, which could explain why some species incur growth begging costs whereas others do not. Indeed, a recent study has found that house sparrow nestlings, whose growth rate is not affected by experimentally induced high begging (HB) levels, incurred a begging cost in the form of a reduced T-cell-mediated immune response (Moreno-Rueda, 2010).

In this study, we analyse whether begging incurs costs in terms of decreased immunocompetence and/or growth in southern shrike (*Lanius meridionalis*) nestlings, a medium-sized passerine. To this end, two nestlings that were still growing were forced to beg at high and low rates, respectively, while at the same time also being forced to deal with an immunological challenge. This is the second study analysing the immunological cost of begging. Our results help clarify the scarce and contradictory literature on this topic, providing a crucial test of the theoretical models: If begging is costly, nestlings begging at higher rates should show reduced growth and/or immunocompetence.

Materials and methods

The study was carried out during the spring of 2010, with a population of southern shrikes located at Lomas de Padul (SE Spain). The study area is formed by a mix of shrubland and farmland with scattered Holm oaks (*Quercus ilex*) in which most nests were located. In addition, one nest was placed on a kermes oak (*Q. coccifera*) and another in an almond tree (*Prunus dulcis*). Nest predation (sometimes associated to begging; McDonald *et al.*, 2009) in our study population was 9.4% ($n = 32$ nests), a frequency smaller than in other shrikes species and locations (e.g. Tryjanowski *et al.*, 2000). Nests were inspected regularly to determine the exact date of hatching (day 0). The experiment was performed with 36 chicks from 12 nests when nestlings were growing at the highest rate (7 days, Budden & Wright, 2000). In the afternoon on the day before the experiment, we took one or two pairs of nestlings of similar body mass from the nests, leaving two to four nestlings in the nests to avoid parental desertion. Nestlings were placed in a warm chamber and taken to a laboratory at the Animal Experimentation Unit at the University of Granada. Transportation lasted about 30 min. On that afternoon, nestlings were fed *ad libitum*. The day after the experiment, nestlings were fed *ad libitum* again and returned to their nests during the morning. On the following days, we regularly checked nests to monitor the fate of nestlings used in the experiments. All the nestlings tested in the laboratory fledged successfully, as fledgling success in the study area was unusually high that year (96%).

During the experiment, we randomly assigned one nestling of each pair of nestmates to either an HB or a low begging (LB) treatment. Each nestling was maintained isolated in a cup simulating a nest, at an ambient temperature of about 36 °C. While resting, nestlings were covered by a duster, simulating brooding by the mother. This procedure precluded nestling begging between trials. The experiment started at 8:00 (local hour) and ended at 21:00. Previously, nestlings were weighed with a digital balance (Sartorius, Frankfurt, Germany; accuracy 0.01 g). We estimated the food ingested by nestlings according to their mass during the experimental day, following the allometric relationship calculated by Weathers (1996): daily food to be consumed = $0.98 \times M^{0.814}$, where M is nestling body mass in grams. Daily food intake was divided into 14 equal portions corresponding to the 14 begging trials; any deviations from expected food intake during an hour were compensated for in subsequent trials. During 2009, we recorded parental feeding rates at 10 nests in the same study area, to calculate how frequently a nestling was fed by parents under natural conditions. At six of 10 nests, interfeeding interval per nestling was approximately 1 h (45–75 min); it was about 30 min in three nests and of 2 h in the remaining one. Consequently, we established an hourly feeding frequency for experimental trials as it was close to the feeding frequency in our study population. Food (moistened puppy chow) was of high protein content (*ca.* 50% of dry weight) and contained vitamins A, D3 and E, as well as calcium and phosphorus. Therefore, given that pairs of nestlings were matched by mass (mean difference in mass between pairs was 0.24 g), nestlings in the two treatments received similar amounts of food.

During each feeding trial, nestlings were stimulated to beg by using acoustic (a characteristic and standardized whistle) and tactile (gently touching their gapes with a forceps) stimuli. However, whereas LB nestlings were fed immediately after gaping, HB nestlings were stimulated to beg for 1 min before being fed. Begging trials were recorded with a digital camera Handycam HDR-XR155E (Sony, Tokyo, Japan). From video recordings, we measured the time each nestling spent begging by using the JWatcher 1.0 software (Blumstein & Daniel, 2007). Two categories of postural intensity were established: low-intensity begging (gaping, tarsi flexed) and high-intensity begging (gaping on extended tarsi, sometimes including wing flapping), which were assigned ranks 1 and 2, respectively, to establish a measure of chick begging intensity. Technical difficulties with eight nestlings reduced the sample size for behavioural data. The final body mass of nestlings was measured at the next day, at 8:00 h, exactly 24 h after the first measurement. Growth rate during the experimental day was estimated as final body mass minus initial body mass. Following Kilner (2001), we also computed mass lost in energy expenditure (M_{EE}) by HB and LB nestlings during the experimental period as $M_{EE} = M_I - M_G - M_F$, where M_I is mass of food ingested, M_G is mass gained (i.e. growth rate) and M_F is the mass of faeces, which were counted and weighed with the digital balance. The difference between the M_{EE} of HB minus LB nestlings has been considered a direct measure of the marginal cost of begging (Kilner, 2001).

We also measured how the experimental treatment affected immune response. Immediately before the onset of the experiment, we injected into the left patagium of each chick 0.2 mg of phytohaemagglutinin (PHA-P, L-8754; Sigma Aldrich, St. Louis, MO, USA) diluted in 0.04 mL of isotonic phosphate buffer (following Smits *et al.*, 1999). PHA-P is an innocuous protein that provokes a T-cell-mediated immune response in birds (Kennedy & Nager, 2006), although other components of the immune system are also involved in the response (Martin *et al.*, 2006; Tella *et al.*, 2008). Previously, we had measured (three times) the patagium thickness with a pressure-sensitive micrometer (Mitutoyo, Kawasaki, Japan; accuracy: 0.01 mm). At the end of the experiment (24 h later), we again measured the patagium thickness, calculating the T-cell-mediated immune response as the difference between the second and first measurements. The repeatability of measurement was 0.98 ($n = 8$; Bailey & Byrnes, 1990).

For statistical analyses, we performed general linear models (GLM) of ordinal least squares (OLS) with treatment (fixed factor) as a categorical predictor. In each model, nest of origin was introduced as a random factor to control for variance among nests, thus avoiding pseudoreplication (Hurlbert, 1984). We checked for the interaction between nest and treatment, which in all cases proved nonsignificant and was removed from final models. The lack of a significant interaction implies that the effect of treatment was independent from that of nest. In several models, other variables were introduced as covariates to examine a possible effect on the dependent variable. For every model, we checked for homoscedasticity, and we log-transformed the variable 'time begging' to fulfil homoscedasticity requirements. We also checked for normality of residuals, which never deviated from a normal distribution according to a Kolmogorov–Smirnov test (Quinn & Keough, 2002). Means are given with the standard error (SE).

Results

As expected, HB and LB nestlings did not significantly differ in initial body mass, and they consumed similar amounts of food during the experiment (Table 1). Treatment was successful at generating significant differences in total begging time between HB and LB nestlings, with HB nestlings begging for considerably longer than their LB nestmates (in average, 32.8 ± 2.3 vs. 2.0 ± 0.2 s h^{-1}, respectively; Table 1). Both HB and LB nestlings begged for a significantly shorter time than nestlings in natural nests (99.9 ± 19.0 s h^{-1}, $n = 4$ nests; Mann–Whitney *U*-test, $z = 2.97$, $P = 0.003$, in both comparisons). Nestlings in both groups begged with similar postural intensities (Table 1). During the day of the experiment, HB nestlings lost mass, whereas LB nestlings gained 0.22 g on average, differences being significant (Table 1). Growth rate was not significantly affected by the amount of food consumed ($F_{1,22} = 2.16$, $P = 0.16$), and the effect of treatment on growth rate remained significant when the amount of food consumed was included in the model ($F_{1,22} = 9.27$, $P < 0.01$). The effect of treatment on growth rate also remained significant when initial body mass was included as a covariate (effect of treatment: $F_{1,22} = 4.81$, $P < 0.05$; effect of initial body mass: $F_{1,22} = 1.23$, $P = 0.33$). The effect of treatment on growth rate was not confounded by the mass of faeces, as both groups excreted a similar mass and number of droppings (Table 1). When faeces mass was included in the model, the effect of treatment on growth rate remained significant (effect of treatment: $F_{1,22} = 3.02$, $P < 0.05$; effect of faeces mass: $F_{1,22} < 0.01$, $P = 0.97$).

The immune response also showed significant differences between HB and LB nestlings, with chicks begging for longer showing a smaller immune response (Table 1). Initial body mass did not affect immune response ($F_{1,22} = 1.71$, $P = 0.14$) and its inclusion in the model did not remove the significant effect of treatment on immune response ($F_{1,22} = 4.86$, $P < 0.05$). The amount of food consumed had no effect on immune response ($F_{1,22} = 1.64$, $P = 0.15$), but its inclusion in the model decreased the effect size of treatment on immune response ($F_{1,22} = 3.47$, $P = 0.08$).

Table 1 Mean ± SE for each variable measured in the study, and the effect of nest (random) and treatment (fixed).

	Low begging	High begging	Nest	Treatment
Initial body mass (g)	23.95 ± 0.76	24.19 ± 0.85	24.79***	0.40^{ns}
Consumed food (g)	11.50 ± 0.35	11.04 ± 0.48	7.97***	1.95^{ns}
Total time begging (s)	27.5 ± 2.7	458.8 ± 32.7	1.96^{ns}	637.4***
Begging intensity	1.52 ± 0.06	1.47 ± 0.05	0.19^{ns}	0.45^{ns}
Growth rate (g)	0.22 ± 0.14	−0.47 ± 0.26	1.14^{ns}	5.51*
Faeces mass (g)	6.49 ± 0.40	6.27 ± 0.45	6.07***	0.33^{ns}
Number of faecal sacs	9.12 ± 0.76	9.70 ± 0.68	7.57*	0.84^{ns}
Immune response (mm)	0.44 ± 0.05	0.33 ± 0.03	1.71^{ns}	4.44*
Metabolic expenditure (g)	4.80 ± 0.38	5.24 ± 0.41	3.36**	1.11^{ns}

F-values are shown. Degrees of freedom were 11 for nest, 1 for treatment and 23 for error; except for time begging, for which df were 9 for nest and 17 for error. For time begging, the analysis was performed after log-transformation, although raw data are shown. *P*-value: * for $P < 0.05$; ** for $P < 0.01$ and *** for $P < 0.001$, and ns for not significant.

HB and LB nestlings lost similar amount of mass to energy expenditure (M_{EE}; Table 1). The time spent begging explained a significant amount of variation in M_{EE} within the HB group ($r = 0.60$, $P = 0.03$; Fig. 1). Also, the magnitude of the difference in begging time within pairs was correlated with the difference in M_{EE} within pairs ($r = 0.59$, $P = 0.03$; Fig. 2), suggesting that begging significantly contributed to the energy expenditure of nestlings. Differences in M_{EE} within pairs also correlated negatively with differences in growth rate ($r = -0.62$, $P = 0.01$), but not in immune response ($r = -0.17$, $P = 0.54$), suggesting a link between metabolic expenditure and growth, but not with immunity.

In summary, we found that southern shrike nestlings begging for long periods incurred a double cost in terms of a decreased growth rate and immune response. The two costs were uncorrelated in HB as well as LB nestlings ($P > 0.40$ for both groups), suggesting that the two costs were independent of each other. In fact, the effect of treatment on growth rate held after controlling for immune response ($F_{1,22} = 5.83$; $P < 0.05$; effect of immune response on growth rate: $F_{1,22} = 0.33$; $P = 0.58$). Also, the effect of treatment on immune response after controlling for growth rate was marginally significant ($F_{1,22} = 3.91$; $P = 0.06$; effect of growth rate on immune response: $F_{1,22} = 0.33$; $P = 0.58$). Finally, considering all nestlings, total begging time was negatively correlated

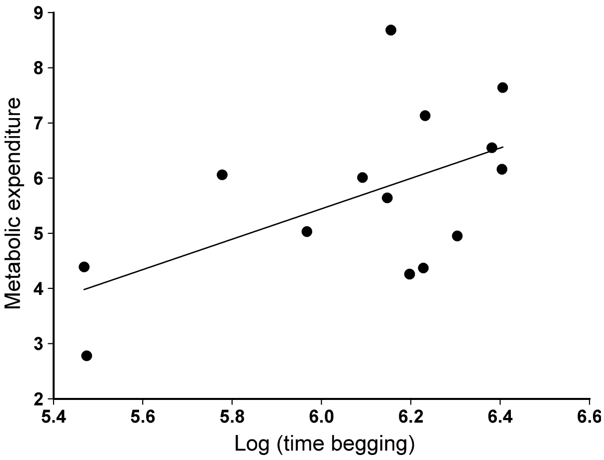

Fig. 1 Mass (g) lost to energetic expenditure (M_{EE}) as a function of time begging (in s, log-transformed) in the high begging (HB) group. The line is the regression fit.

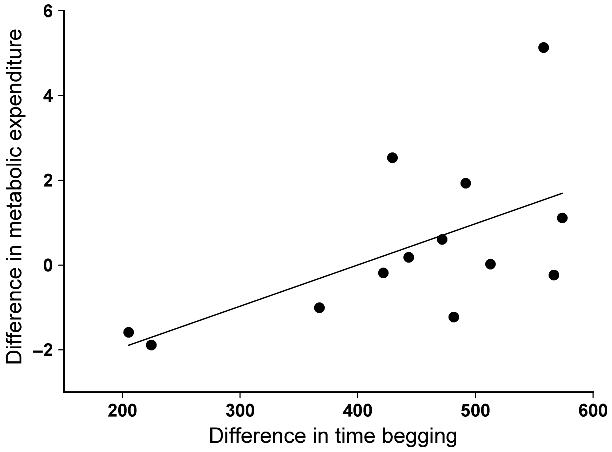

Fig. 2 Difference in metabolic expenditure (g) in relation to the difference in time begging (s), between pairs of nestlings submitted to high begging (HB) and low begging (LB) treatments (HB–LB). The line is the regression fit.

with immune response, after controlling for random variance between nests ($\beta = -0.38 \pm 0.16$; $F_{1,17} = 5.78$; $P < 0.05$), and with growth rate ($\beta = -0.39 \pm 0.18$; $F_{1,17} = 4.54$; $P < 0.05$; Fig. 3).

Discussion

Forcing chicks to beg for longer or shorter periods than they would prefer is a necessary experimental protocol to test whether begging has a marginal impact on a correlate of fitness (Kilner, 2001; Lachmann *et al.*, 2001; Számadó, 2011). By performing such an experiment, we show that southern shrike nestlings incurred costs in the form of reduced growth and reduced immune response, supporting the predictions of models of costly begging (Harper, 1986; Godfray, 1991; Parker *et al.*, 2002). Nestlings in our experimental study were fed each hour, in a routine that coincides with the natural one in the field, and they were begging, in the HB group, for an average of 32.8 ± 2.3 s h^{-1}. This begging rate was shorter than in natural nests at our population (99.9 ± 19.0 s h^{-1}) and below experimental HB rates reported in other studies (104 s h^{-1} in magpies, Rodríguez-Gironés *et al.*, 2001; 84 s h^{-1} in tree swallows, Leonard *et al.*, 2003). Therefore, we found significant costs of begging, even when nestlings were begging at relatively low rates in our experiment.

In our study, southern shrike nestlings begging for a sustained HB rate over a 14-h period showed a reduced growth rate compared to nestmates of a similar size experimentally induced to beg at much lower rates, even though both groups received the same amount of food. Nestling survival depends strongly on growth rate and body size both during the nesting phase (Ostreiher, 1999) and after leaving the nest (Schwagmeyer & Mock, 2008).

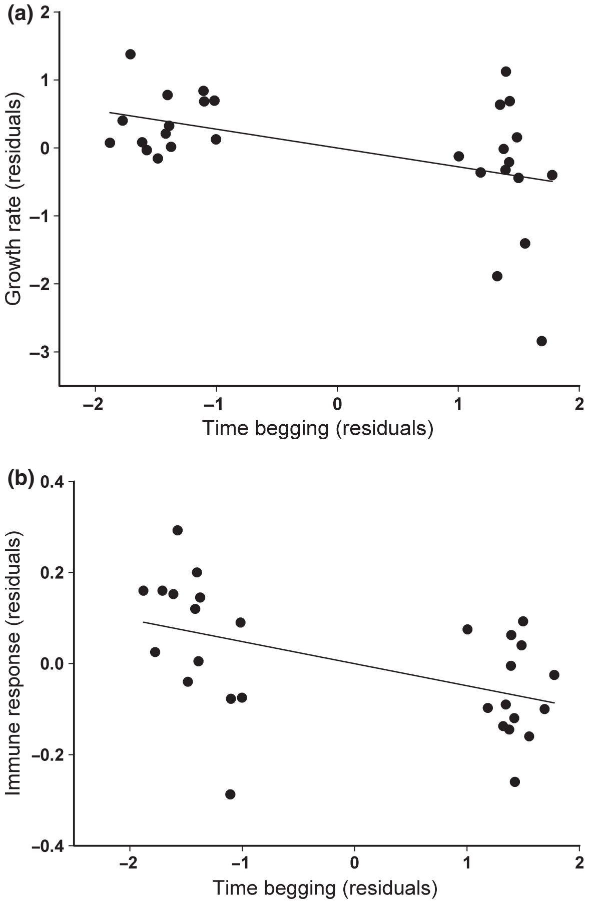

Fig. 3 Growth (a) and immune response (b) as a function of time begging. Growth is measured in g, immune response in mm and time begging in s (log-transformed), but shown are residuals after controlling for nest (random factor). The line is the regression fit.

Therefore, begging in this species may incur a marginal growth cost, and nestlings might not be able to afford spending extra effort on intensive begging unless they obtain enough food to balance the cost of begging. Evidence for a marginal growth cost of begging has also been found in canaries and magpies (Kilner, 2001; Rodríguez-Gironés *et al.*, 2001) but not in ring doves, house sparrows or tree swallows, despite considerable similarities in experimental protocols and statistical power (Kedar *et al.*, 2000; Rodríguez-Gironés *et al.*, 2001; Leonard *et al.*, 2003; Moreno-Rueda, 2010). Although the reasons for such interspecific differences remain unclear, it is likely that begging growth costs are largely influenced by behavioural, ecological and phylogenetic differences between species affecting growth energy budget (Rodríguez-Gironés *et al.*, 2001; Leonard *et al.*, 2003; Moreno-Rueda, 2007). For example, the two passerines for which this cost was not found (house sparrow and tree swallow) are cavity nesters, whereas the three species showing a cost in growth (canaries, shrikes and magpies) nest on tree branches. Nest type may affect predation rate and therefore selection on growth rates (Lack, 1968), and thus, the impact of begging on growth, this being higher in species with relatively fast growth (those nesting in branches).

Meanwhile, our estimate of energy expenditure (M_{EE}) was similar for HB and LB nestlings. At first sight, this result might indicate that begging had little impact upon a nestling's energy budget, as suggested by previous studies (review in Chappell & Bachman, 2002). However, begging time explained a substantial amount of variance in mass lost to energetic expenditure in HB nestlings and also explained differences in metabolic expenditure between nestmates. This suggests that begging did affect metabolic expenditure, but in a way difficult to detect, which might explain why it went unnoticed in previous studies.

This is the second study testing and finding that intensive begging decreases immunocompetence (see Moreno-Rueda, 2010), a trait directly linked to nestling survival (Møller & Saino, 2004; Cichoń & Dubiec, 2005; Moreno *et al.*, 2005). Parasites are one of major causes of nestling mortality (Møller *et al.*, 2009), and therefore, this cost may be particularly important in limiting the escalation of begging. In agreement with our results and those in Moreno-Rueda (2010), immune challenge weakens the expression of a begging signal, gape colour, in the barn swallow (*Hirundo rustica*, Saino *et al.*, 2003). Several mechanisms may underlie an immunological cost of intensive begging. First, begging may divert energy and resources from the immune system (Demas, 2004), as suggested by studies showing a trade-off between growth and immune response in nestlings (Soler *et al.*, 2003; Brommer, 2004). However, we found no relationship between mass expenditure in metabolism and immune response. Second, both begging activity and the immune response may increase oxidative stress (Costantini & Møller, 2009; Sorci & Faivre, 2009; Noguera *et al.*, 2010), so that immunocompetence might adaptively diminish in nestlings begging intensively to avoid oxidative damage (Råberg *et al.*, 1998; Monaghan *et al.*, 2009).

Both immunological as well as growth costs share common paths of hormonal regulation. In canaries, the growth cost is mediated by testosterone (Buchanan *et al.*, 2007). Thus, testosterone might also regulate the growth cost of begging in the southern shrike. Testosterone has also been proposed as a mediator of the immune cost of begging (Quillfeldt *et al.*, 2006; Goodship & Buchanan, 2007; Ros, 2008). Corticosterone also might mediate immunosuppression in nestlings begging for longer, as supplying this hormone to house sparrow nestlings increases begging and simultaneously it depresses immune response (Loiseau *et al.*, 2008).

Moreno-Rueda (2010) suggested that some species re-allocate resources for begging signals from growth, whereas others re-allocate resources from the immune function, and therefore, the cost of begging would be reflected either in reduced growth or reduced immunocompetence, respectively. Contrary to this idea, this study shows for the first time that both costs may occur simultaneously. In shrikes, the magnitudes of the two costs were not correlated, suggesting that the two occurred independently, i.e. begging at high rates simultaneously impaired growth and the immune response. This finding suggests that different nestlings incurred both costs to a different extent, some nestlings reduced growth rate whereas other reduced immunocompetence. The question arises why the cost of begging varied among nestlings. Moreover, that the two costs were independent also suggests that the regulatory mechanisms of each cost may be different.

In conclusion, our study, experimentally analysing the physiological cost of begging in the southern shrike, demonstrates that nestlings begging for a longer time pay a cost in terms of reduced growth and immune response. This is the third species for which a growth cost of begging is found, and the second in which an immunological cost is studied and found. Moreover, for the first time, we show that the two types of costs may occur simultaneously in the same species and, in fact, independently from each other. Begging costs of the type required by theoretical models may have gone undetected till present time largely because empirical studies have not previously considered how nestlings trade resources for growing, signalling and other less evident but costly physiological processes, such as immune function.

Acknowledgments

We are grateful to José M. Rivas (Estación Onitológica de Padul) for his assistance during nest searching. Inés Álvarez, Carlos Castillo and Antonio López contributed in the filming and analyses of natural nests in 2009. Juan M. Pleguezuelos, the staff of the Animal Experimentation Unit, and especially Jesús M. Zúñiga provided fundamental logistic support. The University of Granada allowed us to use its laboratories. G.M.-R. was economically supported by the Spanish Government (Ministerio de Ciencia y Tecnología, 'Juan de la Cierva' programme), and T.R. was supported by the Consejo Superior de Investigaciones Científicas (CSIC; Proyectos Intramurales Especiales). The entire study was performed with the permission of the Andalusian Government (Consejería de Medio Ambiente). David Nesbitt improved the English, and comments by two anonymous referees greatly improved the manuscript.

References

Ardia, D.R. & Schat, K.A. 2008. Ecoimmunology. In: *Avian Immunology* (F. Davison, B. Kaspers & K.A. Schat, eds), pp. 421–441. Academic Press, London.

Bailey, R.C. & Byrnes, J. 1990. A new, old method for assessing measurement error in both univariate and multivariate morphometric studies. *Syst. Zool.* **39**: 124–130.

Bergstrom, C.T. & Lachmann, M. 1997. Signalling among relatives. I. Is costly signalling too costly? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **352**: 609–617.

Bergstrom, C.T. & Lachmann, M. 1998. Signaling among relatives. III. Talk is cheap. *Proc. Natl Acad. Sci. USA* **95**: 5100–5105.

Bize, P. & Roulin, A. 2006. Sibling competition and the risk of falling out of the nest. *Anim. Behav.* **72**: 539–544.

Blumstein, D.T. & Daniel, J.C. 2007. *Quantifying Behavior: The JWatcher Way*. Sinauer Inc., Sunderland.

Brilot, B.O. & Johnstone, R.A. 2002. Cost, competition and information in communication between relatives. *J. Theor. Biol.* **217**: 331–340.

Briskie, J.V., Martin, P.R. & Martin, T.E. 1999. Nest predation and the evolution of nestling begging calls. *Proc. Biol. Sci.* **266**: 2153–2159.

Brommer, J.E. 2004. Immunocompetence and its costs during development: an experimental study in blue tit nestlings. *Proc. Biol. Sci.* **271**: S110–S113.

Buchanan, K.L., Goldsmith, A.R., Hinde, C.A., Griffith, S.C. & Kilner, R.M. 2007. Does testosterone mediate the trade-off between nestling begging and growth in the canary (*Serinus canaria*)? *Horm. Behav.* **52**: 664–671.

Budden, A.E. & Wright, J. 2000. Nestling diet, chick growth and breeding success in the Southern Grey Shrike (*Lanius meridionalis*). *Ring* **22**: 165–172.

Budden, A.E. & Wright, J. 2001. Begging in nestling birds. *Curr. Ornithol.* **16**: 83–118.

Chappell, M.A. & Bachman, G.C. 2002. Energetic costs of begging behaviour. In: *The Evolution of Begging* (J. Wright & M.L. Leonard, eds), pp. 143–162. Kluwer Academic Publishers, Dordrecht.

Cichoń, M. & Dubiec, A. 2005. Cell-mediated immunity predicts the probability of local recruitment in nestling blue tits. *J. Evol. Biol.* **18**: 962–966.

Clutton-Brock, T.H. 1991. *The Evolution of Parental Care*. Princeton University Press, Princeton.

Costantini, D. & Møller, A.P. 2009. Does immune response cause oxidative stress in birds? A meta-analysis. *Comp. Biochem. Physiol. A* **153**: 339–344.

Demas, G.E. 2004. The energetics of immunity: a neuroendocrine link between energy balance and immune function. *Horm. Behav.* **45**: 173–180.

Godfray, H.C.J. 1991. Signalling of need by offspring to their parents. *Nature* **352**: 328–330.

Godfray, H.C.J. 1995. Signaling of need between parents and young: parent-offspring conflict and sibling rivalry. *Am. Nat.* **146**: 1–24.

Goodship, N.M. & Buchanan, K.L. 2006. Nestling testosterone is associated with begging behaviour and fledging success in the pied flycatcher, *Ficedula hypoleuca*. *Proc. Biol. Sci.* **273**: 71–76.

Goodship, N.M. & Buchanan, K.L. 2007. Nestling testosterone controls begging behaviour in the pied flycatcher, *Ficedula hypoleuca*. *Horm. Behav.* **52**: 454–460.

Harper, A.B. 1986. The evolution of begging: sibling competition and parent-offspring conflict. *Am. Nat.* **128**: 99–114.

Haskell, D. 1999. The effect of predation on begging-call evolution in nestling wood warblers. *Anim. Behav.* **57**: 893–901.

Haskell, D. 2002. Begging behaviour and nest predation. In: *The Evolution of Begging* (J. Wright & M.L. Leonard, eds), pp. 163–172. Kluwer Academic Publishers, Dordrecht.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **54**: 187–211.

Kedar, H., Rodríguez-Gironés, M.A., Yedvab, S., Winkler, D.W. & Lotem, A. 2000. Experimental evidence for offspring learning in parent-offspring communication. *Proc. Biol. Sci.* **267**: 1723–1727.

Kennedy, M.W. & Nager, R.G. 2006. The perils and prospects of using phytohaemagglutinin in evolutionary ecology. *Trends Ecol. Evol.* **21**: 653–655.

Kilner, R. 2001. A growth cost of begging in captive canary chicks. *Proc. Natl Acad. Sci. USA* **98**: 11394–11398.

Kilner, R.M. & Hinde, C.A. 2008. Information warfare and parent-offspring conflict. *Adv. Stud. Behav.* **38**: 283–336.

Kilner, R. & Johnstone, R.A. 1997. Begging the question: are offspring solicitation behaviours signals of need? *Trends Ecol. Evol.* **12**: 11–15.

Lachmann, M., Számadó, S. & Bergstrom, C.T. 2001. Cost and conflict in animal signals and human language. *Proc. Natl Acad. Sci. USA* **98**: 13189–13194.

Lack, D. 1968. *Ecological Adaptations for Breeding in Birds*. Methuen, London.

Leonard, M.L., Horn, A.G. & Ratcliffe, L.M. 1991. Parental aggression in black-capped chickadees. *Behav. Ecol.* **2**: 228–233.

Leonard, M.L., Horn, A.G. & Porter, J. 2003. Does begging effort affect growth in nestling tree swallows, *Tachyneta bicolor*? *Behav. Ecol. Sociobiol.* **54**: 573–577.

Loiseau, C., Sorci, G., Dano, S. & Chastel, O. 2008. Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows. *Gen. Comp. Endocrinol.* **155**: 101–108.

Magrath, R.D., Haff, T.M., Horn, A.G. & Leonard, M.L. 2010. Calling in the face of danger: predation risk and acoustic communication by parent birds and their offspring. *Adv. Stud. Behav.* **41**: 187–253.

Martin, L.B. II, Han, P., Lewittes, J., Kuhlman, J.R., Klasing, K.C. & Wikelski, M. 2006. Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immunoecological technique. *Funct. Ecol.* **20**: 290–299.

Mauck, R.A., Matson, K.D., Philipsborn, J. & Ricklefs, R.E. 2005. Increase in the constitutive innate humoral immune system in Leach's Storm-Petrel (*Oceanodroma leucorhoa*) chicks is negatively correlated with growth rate. *Funct. Ecol.* **19**: 1001–1007.

Maynard Smith, J. 1994. Must reliable signals always be costly? *Anim. Behav.* **47**: 1115–1120.

Maynard Smith, J. & Harper, D. 2003. *Animal Signals*. Oxford University Press, New York.

McDonald, P.G., Wilson, D.R. & Evans, C.S. 2009. Nestling begging increases predation risk, regardless of spectral characteristics or avian mobbing. *Behav. Ecol.* **20**: 821–829.

Mock, D.W. & Parker, G.A. 1997. *The Evolution of Sibling Rivalry*. Oxford University Press, Oxford.

Møller, A.P. & Saino, N. 2004. Immune response and survival. *Oikos* **104**: 299–304.

Møller, A.P., Arriero, E., Lobato, E. & Merino, S. 2009. A meta-analysis of parasite virulence in nestling birds. *Biol. Rev.* **84**: 567–588.

Monaghan, P., Metcalfe, N.B. & Torres, R. 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol. Lett.* **12**: 75–92.

Moreno, J., Merino, S., Sanz, J.J., Arriero, E., Morales, J. & Tomás, G. 2005. Nestling cell-mediated immune response, body mass and hatching date as predictors of local recruitment in the pied flycatcher *Ficedula hypoleuca*. *J. Avian Biol.* **36**: 251–260.

Moreno-Rueda, G. 2007. Is there empirical evidence for the cost of begging? *J. Ethol.* **25**: 215–222.

Moreno-Rueda, G. 2010. An immunological cost of begging in house sparrow nestlings. *Proc. Biol. Sci.* **277**: 2083–2088.

Noguera, J.C., Morales, J., Pérez, C. & Velando, A. 2010. On the oxidative cost of begging: antioxidants enhance vocalizations in gull chicks. *Behav. Ecol.* **21**: 479–484.

Ostreiher, R. 1999. Nestling feeding-space strategy in Arabian babblers. *Auk* **116**: 651–657.

Parker, G.A. & Macnair, M.R. 1979. Models of parent-offspring conflict. IV. Suppression: evolutionary retaliation by the parent. *Anim. Behav.* **27**: 1210–1235.

Parker, G.A., Royle, N.J. & Hartley, I.R. 2002. Begging scrambles with unequal chicks: interactions between need and competitive ability. *Ecol. Lett.* **5**: 206–215.

Quillfeldt, P., Masello, J.F., Strange, I.J. & Buchanan, K.L. 2006. Begging and provisioning of thin-billed prions, *Pachyptila belcheri*, are related to testosterone and corticosterone. *Anim. Behav.* **71**: 1359–1369.

Quinn, G.P. & Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Råberg, L., Grahn, M., Hasselquist, D. & Svensson, E. 1998. On the adaptive significance of stress-induced immunosuppression. *Proc. Biol. Sci.* **265**: 1637–1641.

Redondo, T. & Arias-de-Reyna, L. 1988. Locatability of begging calls in nestling altricial birds. *Anim. Behav.* **36**: 653–661.

Redondo, T. & Castro, F. 1992. Signalling of nutritional need by magpie nestlings. *Ethology* **92**: 193–204.

Reeve, H.K. 1997. Evolutionary stable communication between kin: a general model. *Proc. Biol. Sci.* **264**: 1037–1040.

Rodríguez-Gironés, M.A., Zúñiga, J.M. & Redondo, T. 2001. Effects of begging on growth rates of nestling chicks. *Behav. Ecol.* **12**: 269–274.

Ros, A.F.H. 2008. Patterns of testosterone responsiveness and immunity in relation to competitive behavior in chicks. *Horm. Behav.* **54**: 234–237.

Roulin, A. 2001. On the cost of begging vocalization: implications of vigilance. *Behav. Ecol.* **12**: 506–510.

Royle, N.J., Hartley, I.R. & Parker, G.A. 2002. Begging for control: when are offspring solicitation behaviours honest? *Trends Ecol. Evol.* **17**: 434–440.

Saino, N., Ambrosini, R., Martinelli, R., Ninni, P. & Møller, A.P. 2003. Gape coloration reliably reflects immunocompetence of barn swallow (*Hirundo rustica*) nestlings. *Behav. Ecol.* **14**: 16–22.

Schwagmeyer, P.L. & Mock, D.W. 2008. Parental provisioning and offspring fitness: size matters. *Anim. Behav.* **75**: 291–298.

Searcy, W.A. & Nowicki, S. 2005. *The Evolution of Animal Communication*. Princeton University Press, Princeton.

Smiseth, P.T. & Parker, H.J. 2008. Is there a cost to larval begging in the burying beetle *Nicrophorus vespilloides*? *Behav. Ecol.* **19**: 1111–1115.

Smits, S.E., Bortolotti, G.R. & Tella, J.L. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol.* **13**: 567–572.

Soler, J.J., De Neve, L., Pérez-Contreras, T., Soler, M. & Sorci, G. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. *Proc. Biol. Sci.* **270**: 241–248.

Sorci, G. & Faivre, B. 2009. Inflammation and oxidative stress in vertebrate host–parasite systems. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**: 71–83.

Számadó, S. 2011. The cost of honesty and the fallacy of the handicap principle. *Anim. Behav.* **81**: 3–10.

Tella, J.L., Lemus, J.A., Carrete, M. & Blanco, G. 2008. The PHA test reflects acquired T-cell mediated immunocompetence in birds. *PLoS ONE* **3**: e3295.

Trivers, R.L. 1974. Parent-offspring conflict. *Am. Zool.* **14**: 249–264.

Tryjanowski, P., Kuźniak, S. & Diehl, B. 2000. Breeding success of the Red-backed Shrike (*Lanius collurio*) in relation to nest site. *Ornis Fennica* **77**: 137–141.

Verhulst, S. & Wiersma, P. 1997. Is begging cheap? *Auk* **114**: 134.

Weathers, W.W. 1996. Energetics of postnatal growth. In: *Avian Energetics and Nutritional Ecology* (C. Carey, ed.), pp. 461–496. Chapman & Hall, New York.

Wright, J. & Leonard, M.L. 2002. *The Evolution of Begging*. Kluwer Academic Publishers, Dordrecht.

Zahavi, A. & Zahavi, A. 1997. *The Handicap Principle: A Missing Piece of the Darwin's Puzzle*. Oxford University Press, New York.

Received 21 December 2010; revised 19 January 2011; accepted 20 January 2011