



## Short communication

# Nest-dwelling ectoparasites reduce begging effort in Pied Flycatcher *Ficedula hypoleuca* nestlings

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Parasitized nestlings might be expected to increase begging effort to obtain additional resources to compensate for those sequestered by their parasites. However, begging is costly and chicks harbouring parasites may find it more difficult to attain high begging levels. Consequently, we predicted that, for the same level of nutritional need, nestlings that are parasitized will invest less in begging than those that are not parasitized. We tested this prediction by measuring begging in Pied Flycatcher *Ficedula hypoleuca* nestlings parasitized with haematophagous mites *Dermanyssus gallinoides* and *Dermanyssus gallinae* and blowfly larvae *Protocalliphora azurea*, and subjected to different levels of food deprivation in order to control for short-term nutritional need. Nestlings from nests with ectoparasites spent less time begging than those from nests without parasites, especially when very hungry, although there was no association with latency to beg or begging intensity. Our results suggest that time invested in begging may indicate not only the level of need, but also nestling parasitism status.

**Keywords:** begging, blowflies, ectoparasites, mites.

By extracting resources from their hosts and decreasing their fitness, parasites are an important ecological and evolutionary force (Schmid-Hempel 2011). Vertebrates harbour several ectoparasitic arthropods, which feed mainly on blood. In birds, many haematophagous ectoparasites inhabit nests, feeding on nestlings whose

fitness may consequently be severely reduced (Møller *et al.* 2009). Nestlings, however, may increase resource consumption by elevating begging levels (Kilner & Johnstone 1997), thus compensating at least partially for losses due to parasitism. Great Tit *Parus major* and Pied Flycatcher *Ficedula hypoleuca* nestlings increase begging when parasitized (Christe *et al.* 1996, Cantarero *et al.* 2013). These studies, however, did not control for the nutritional need of the nestling and argued that the increased begging in parasitized nestlings was simply due to their higher need compared with non-parasitized nestlings. Parasites, nonetheless, not only affect nutritional condition but also aspects of nestling physiology such as reduced haematocrit (Potti *et al.* 1999), thermogenesis capacity (Simon *et al.* 2004), increased stress response (Lobato *et al.* 2008) and reduced antioxidant capacity (López-Arrabé *et al.* 2015).

Begging behaviour is costly in different ways, including energetic expenditure (Kilner 2001), generation of oxidative stress (Moreno-Rueda *et al.* 2012) and reduced immune response (Moreno-Rueda & Redondo 2012). Consequently, begging should be especially costly for parasitized nestlings, as they are in worse physiological and nutritional condition than those that remain unparasitized. On this basis, we predicted that, for the same nutritional need, parasitized nestlings will invest less in begging than will unparasitized nestlings. We tested this prediction in Pied Flycatcher nestlings parasitized by the haematophagous mites *Dermanyssus gallinoides* and *Dermanyssus gallinae*, and by larvae of the blowfly *Protocalliphora azurea*. Parasitism by these arthropods reduces nestling fitness through effects on growth and survival (Merino & Potti 1995), the latter even affecting reproduction during adulthood (Potti 2008). We experimentally examined the begging behaviour (measured as latency to beg, postural intensity and time spent begging) of nestlings from ectoparasite-infested and non-infested nests in response to a standard stimulus (playback of a parent feeding call), and at different but standardized levels of food deprivation.

## METHODS

### Study design and behavioural data collection

The study was carried out in 2011 and 2013 in an intensively studied Pied Flycatcher population breeding in nestboxes near La Hiruela (central Spain; more details in Camacho *et al.* 2013). Nestboxes were inspected regularly to determine hatching date (day 1). When nestlings were 8 days old, we took 36 nestlings of intermediate size from 18 nests (two nestlings with similar body mass per nest) in the afternoon, always leaving at least three nestlings in each nest to prevent parental desertion. Nestlings were placed in a warm chamber and taken to a nearby laboratory, which took about 20 min. Nest-mates were

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kept together in artificial nests lined with cotton fabric, covered by a duster in a quiet room to avoid begging in response to visual or acoustic stimulus unrelated to the experiment. Because the parasites considered in this study are nest-dwelling, nestlings were free of these parasites in the laboratory. Heat was provided by bulb lamps and the temperature inside nest cups was monitored with a probe digital thermometer and kept at c. 36 °C. Upon arrival in the laboratory, nestlings were conditioned to a begging stimulus and fed *ad libitum* with commercial dipteran larvae just after playing a parental feeding call recorded from the same population. We ensured that all nestlings were conditioned to the stimulus and begged similarly to how they behave in their nests (based on nest video recordings; Redondo *et al.* 2016). On the following morning, nestlings were stimulated to beg with the parental feeding call at 07:30, 08:00 and 08:30 h while being fed *ad libitum* in order to eliminate differences in nutritional need among nestlings. Our rationale for this is that, for measuring begging behaviour, it is important that all nestlings are completely satiated at the start of the experiment, so that any difference found is not due to initial differences in nutritional need. To quantify begging behaviour, we established four begging trials at 15-, 30-, 60- and 90-min intervals since the last meal. Begging trials consisted of stimulating begging in a standardized way, by playing a recording of an adult Pied Flycatcher feeding call. Given that sleeping sometimes prevented responses to the playback feeding call, this was played three times in each trial. Nestling behaviour was recorded with a digital camera Handycam HDR-XR155E. Using JWATCHER 0.9 software (Blumstein & Daniel 2007), the following behavioural measures were recorded:

- 1 *latency to beg* – the time taken for nestlings to open the gape to beg since the start of the playback stimulus,
- 2 *time spent begging* – once nestlings started to beg, they were allowed to beg without interference until they voluntarily ceased begging,
- 3 *begging intensity* – five categories of postural intensity were established (following Redondo & Castro 1992): 0 (no response), 1 (gaping, tarsi flexed), 2 (gaping, neck extended, tarsi flexed), 3 (gaping, neck extended, body up) and 4 (gaping on fully stretched feet and tarsi, sometimes including wing flapping). Note that these categories represent a gradient of increased energy invested in begging, which correlates well with need levels and the probability of being fed (Gottlander 1987).

When the experiment ended (at midday), nestlings were fed *ad libitum* again, individually marked with non-toxic felt pens and returned to their nests. In total, nestlings were in the laboratory for < 24 h. On the following days, we regularly checked nests to monitor the fate of nestlings used in the experiments, re-marking them

when necessary. Thirty-three of 36 experimental nestlings fledged successfully and three died from natural causes that we believe to be unrelated to the experiment. When nestlings were 13 days old, they were ringed, weighed and measured (tarsus length).

### Recording parasitism status of nests

We scored nests as parasitized or not by haematophagous mites following Merino and Potti (1995). Nestlings were placed in a white cotton bag for 5 min, after which we recorded whether any mite was left by nestlings in the cotton bag. This estimation of mite occurrence is tightly correlated with the number of mites in the nest, as revealed by fine examination of nesting material with Berlese funnels, so that absence of mites in the cotton bag (i.e. 'absence' for the purposes of this study) reflects a sufficiently low parasitic load to have no detectable effects on nestling fitness (Merino & Potti 1995).

After fledglings left the nest, the complete material of the nest was examined and the presence of blowfly larvae and pupae was recorded. Although we estimated parasite load for 5–15 days (5 days for mites, 10–15 days for blowflies) after behavioural observations, we are confident that such estimations reflect parasite load at the time begging was measured because the parasites need 10–36 days to complete their life cycles (Gold & Dahlsten 1989, Bruneau *et al.* 2001) and because, in infected nests, blowflies and mites are usually detected at an early stage of the nestling period (3–5 days) at a parasitism level similar to that found at the end of the nestling period (C. Camacho, D. Canal & J. Potti, unpubl. data).

Because the parasitism status of nests was only recorded after the behavioural data were collected, this means that all trials were blind with respect to the nest parasite status of the nest of origin.

### Data analysis

Statistical analyses were performed with linear mixed effects models fitted with Restricted Maximum Likelihood (REML-LMM; Zuur *et al.* 2009), using the package 'nlme' (Pinheiro *et al.* 2012) in R (R Development Core Team 2012). In each model, 'Nestling identity' nested in 'Nest of origin', at the same time nested in 'year' (Gelman & Hill 2007), was introduced as a structured random factor to control for variance among years, nests and repeated measures of nestlings. As fixed predictors, we used 'Deprivation' (four levels: 15, 30, 60 and 90 min since the last feeding), 'Parasitism status' (four levels: uninfected, only mites, only blowflies, and both mites and blowflies), and their interaction. However, given that there were no differences in the dependent variables among nests parasitized by mites,

blowflies or both (ANOVA,  $F_{2,27} < 1.40$ ,  $P > 0.25$  for all begging variables and levels of food deprivation; Supporting Information Fig. S1), we repeated the analyses with two levels for 'Parasitism status': uninfected vs. infected. Dependent variables were 'Latency', 'Intensity' and 'Time begging'. For every model, we checked for homoscedasticity and normality of residuals. Means are given with one standard error (se). Main effects were tested with a type III model, which is recommended for unbalanced designs (Quinn & Keough 2002). The complete dataset is available in Table S1.

## RESULTS

Eight nestlings came from nests infected with mites only, 10 from nests infected with blowflies only, 12 from nests simultaneously infected by the two ectoparasites, and six from nests that remained uninfected (total sample size,  $n = 18$  nests). Nests harboured a mean of  $9.18 \pm 6.26$  mites (range: 0–71) and  $5.45 \pm 2.57$  blowflies (range: 0–29). There were no differences according to parasitism status in either hatching date ( $F_{3,14} = 0.49$ ,  $P = 0.70$ ), brood size ( $F_{3,14} = 0.70$ ,  $P = 0.57$ ) or body mass at 8 days of the nestlings used in the study ( $F_{3,14} = 0.64$ ,  $P = 0.60$ ). For the nestlings that survived until fledging (33 of 36 nestlings), there were no differences in tarsus length at day 13 ( $F_{3,29} = 1.24$ ,  $P = 0.31$ ), according to the presence of mites, blowflies or both. However, there was weak evidence that nestlings in unparasitized nests were heavier at day 13 than those in parasitized nests ( $F_{3,29} = 2.89$ ,  $P = 0.052$ ), suggesting that ectoparasites may have had a detrimental effect on nestling growth.

Contrary to our predictions, latency to beg and begging intensity increased with hunger irrespective of parasitism status (Table 1, Fig. 1). However, the third measure of begging behaviour, time spent begging, was significantly lower in nestlings reared in parasitized nests (Table 1, Fig. 1a). Differences in time spent begging were especially marked at the highest level of food deprivation, as indicated by the significant interaction deprivation\*parasitism (Table 1, Fig. 1a). After 90 min of food deprivation, differences in begging time among nestlings differing in parasitism status were highly significant (REML-LMM,  $\chi^2 = 10.85$ ,  $P < 0.001$ ), but they were not significant for the trials at 30 and 60 min ( $P > 0.65$ ), and only were close to significance after 15 min ( $\chi^2 = 3.42$ ,  $P = 0.064$ ). Findings were qualitatively similar when we repeated the statistical models with four levels of parasitism (uninfected, only mites, only blowflies and both; Table S2, Fig. S1).

## DISCUSSION

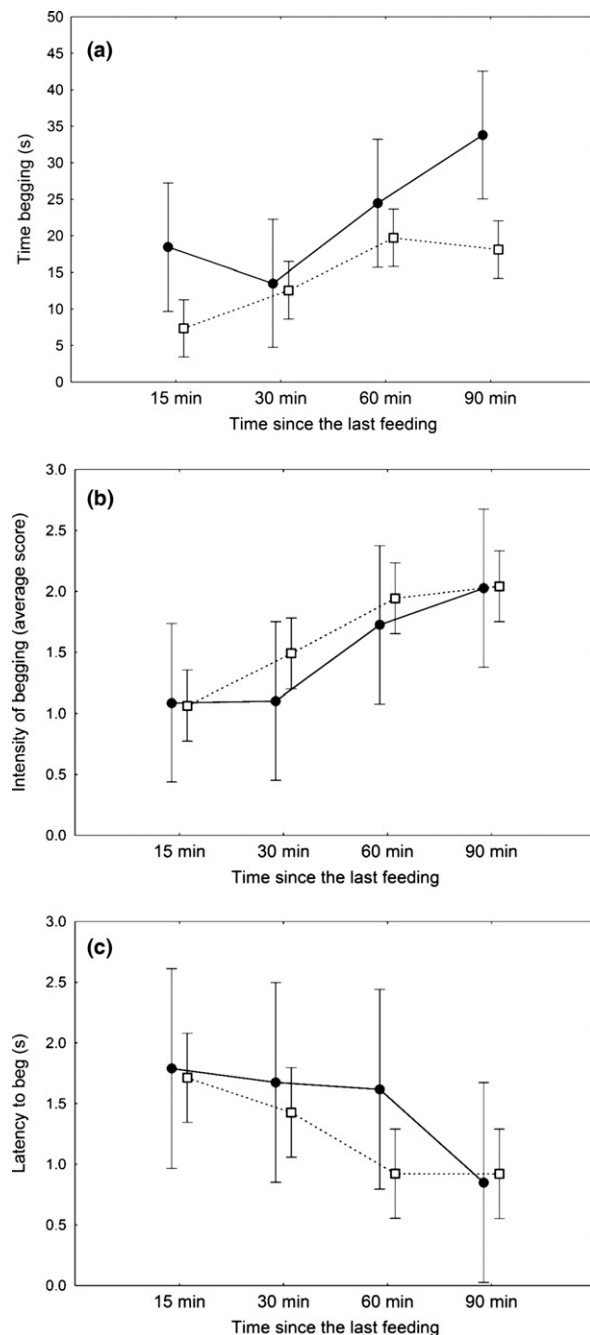
Our study shows that nestlings from parasitized nests begged for less time than nestlings in a similar state of

**Table 1.** Results of linear mixed effects models of restricted maximum likelihood (REML-LMM) analysing the effect of deprivation (four levels: 15, 30, 60 and 90 min since the last feeding), parasitism status (two levels: uninfected vs. infected) and its interaction on postural intensity, time spent begging, and latency to beg. In each model, nestling identity ( $n = 36$ ) is nested within nest of origin ( $n = 18$ ), and nested within year, and introduced as a structured random factor to control for variance among years and nests and the repeated measured of nestlings.

	$\chi^2$	df	P
a) Time spent begging			
Deprivation	35.82	3	< 0.001
Parasitism status	3.91	1	0.048
Deprivation*Parasitism status	16.83	3	< 0.001
b) Postural intensity			
Deprivation	9.67	3	0.022
Parasitism status	0.02	1	0.88
Deprivation*Parasitism status	1.34	3	0.72
c) Latency to beg			
Deprivation	5.59	3	0.13
Parasitism status	0.50	1	0.48
Deprivation*Parasitism status	2.80	3	0.42

nutritional need from unparasitized nests, especially at the highest level of food deprivation (90 min). This suggests that the effect of parasitism on begging may be exacerbated at higher levels of begging effort. Parasitism, on the other hand, had no detectable effect on the postural intensity or latency of begging. Therefore, it seems that parasitism has no detectable effect on the neural and muscular capacity of nestlings, but limits the time they may invest in begging. Begging time, in fact, seems to be the variable most related to begging effort and begging costs (Moreno-Rueda & Redondo 2011). Our study, however, is correlative and sample size is limited and thus we cannot rule out the possibility that an unknown factor (e.g. nest-site quality or microclimate) is simultaneously affecting parasite prevalence and begging behaviour, resulting in the relationship found here.

Our findings seem in contrast to those of Cantarero *et al.* (2013) in a nearby population of Pied Flycatchers, in which nestlings in parasitized nests (with mites, blowflies and fleas) begged more intensely than those in unparasitized nests, as well as similar findings from a study of Great Tits (Christe *et al.* 1996). In these studies, however, increased begging effort of parasitized nestlings may be explained by higher nutritional need. To ascertain the effect of parasites on begging, it is necessary to control for short-term needs. Our findings are novel because we do this, and we found that parasitized nestlings begged less than unparasitized nestlings for the same level of food deprivation. Similarly, O'Connor *et al.* (2014) found that Small Ground Finch *Geospiza fuliginosa* nestlings parasitized with larvae of the fly



**Figure 1.** Average values, with 95% confidence intervals, for Time spent begging (a), Begging postural intensity (b) and Latency (c), according to time since the last feeding (hunger) and prevalence of parasites in the nest (black circles: uninfected; white squares: infected). Sample size: six uninfected nestlings, 30 infected nestlings.

*Philornis downsi* begged less than unparasitized nestlings. That is, although nestlings in poor condition may increase begging effort to some degree to increase food

consumption and compensate for their mass loss due to parasitism (Christe *et al.* 1996, Cantarero *et al.* 2013), this is probably not possible when physical condition has deteriorated (O'Connor *et al.* 2014). Accordingly, in this study we found that the largest differences in begging time between nestlings from parasitized and unparasitized nests occurred when nestlings' nutritional need was highest.

The underlying mechanisms of reduced begging effort in parasitized nestlings are still unclear. An energetic cause (Moreno-Rueda & Redondo 2011) is consistent with a higher effect of parasitism on begging at higher levels of begging effort. Nonetheless, nestlings did not differ in body mass at the time the experiment was performed, which suggests that they had similar energy reserves. Alternatively, the reduction in begging might be mediated by hormones, for example because corticosterone levels have been reported to increase with parasitism (Lobato *et al.* 2008). However, corticosterone also increases begging effort (Loiseau *et al.* 2008), so its potential effects would be likely to contrast with our findings. A third possibility is that the reduction in begging effort is mediated by oxidative stress. One of the main responses to ectoparasites is inflammation, which is associated with the release of several pro-oxidant substances, such as nitric oxide ( $\text{NO}^{\cdot}$ ) or superoxide anion ( $\text{O}_2^{\cdot-}$ ) (Sorci & Faivre 2009). Indeed, parasitism depletes antioxidant defences in Pied Flycatcher nestlings (López-Arrabé *et al.* 2015). Begging behaviour implies considerable neural and muscular activity, which is also associated with the release of pro-oxidant molecules (Costantini 2014), and some studies have found evidence of oxidative stress associated with begging (Noguera *et al.* 2010, Moreno-Rueda *et al.* 2012). In such a situation, parasitized nestlings may pay a cost of increased oxidative stress, and parasitized nestlings might benefit from reducing begging effort in order to keep oxidative balance within safe limits. Finally, another possibility is that reduction in begging reflects a trade-off with immune response. Birds frequently show an immune response to ectoparasites (Owen *et al.* 2010), and begging is known to impair immune response in our study population (Redondo *et al.* 2016). Consequently, it is possible that parasitized nestlings reduced begging to avoid negative consequences of impaired immune response.

An important implication of our findings is that time employed in postural begging may indicate not only nutritional need, but also nestling parasitism status. Parasitized nestlings are of less reproductive value for parents (Forbes 1993), and thus parents may gain useful information on nestling quality from begging signals (Mock *et al.* 2011), and respond accordingly by feeding preferentially less parasitized broods (Saino *et al.* 2000). Health signalling by nestlings is usually associated with mouth colour (e.g. Saino *et al.* 2003) and it actually indicates parasitism by haematophagous mites



*Pellonyssus reedi* in House Sparrow *Passer domesticus* nestlings (Dugas & Doumas 2014). Our study, nevertheless, shows that time spent begging may also carry information about parasitism status. Time spent begging should therefore not only be viewed simply as an indicator of nutritional need, but also as an indicator of aspects of individual quality (Mock *et al.* 2011).

The study was licensed by the Madrid government. Experimental procedures were approved by the CSIC Ethical Committee (ref. CGL2011-29694) and the Andalusian Committee of Animal Experimentation (ref. 2011\_03Potti) to comply with Spanish and European legislation on the protection of animals used for scientific purposes. This study was funded by the Spanish government (Ministerio de Ciencia e Innovación; projects CGL2011-29694 and CGL2014-55969-P). María Cuenca, Gerardo Giménez, Guadalupe Macías, Carlos Marfil-Daza and Alba Ruiz helped us during fieldwork. C.C. received financial support from the Spanish Ministry of Economy and Competitiveness, through the Severo Ochoa Programme for Centres of Excellence in R&D&I (SVP-2013-067686). Comments by Sarah Burthe and three anonymous referees improved the manuscript.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Average values, with 95% confidence intervals, for Time begging (a), Begging postural intensity (b) and Latency (c), according to time since the last feeding (hunger) and prevalence of parasites (black circles: absence; white squares: only mites; white rhombuses: only blowflies; white triangles: both parasites).

**Table S1.** Rawdata: year, nest code, ring of fledglings, test of hunger (T15: 15 min since the last feeding, T30: 30 min, T60: 60 min and T90: 90 min), values for time begging (seconds), begging intensity (average category of postural begging, see Methods) and latency to begging (in seconds), and prevalence of mites, blowflies or any parasite (Y = yes, N = no).

**Table S2.** Results of Linear Mixed Effects Models of Restricted Maximum Likelihood (REML-LMM) analysing the effect of 'Deprivation' (four levels: 15, 30, 60 and 90 min since the last feeding), 'Parasitism status' (four levels: uninfected ( $n = 6$ ), only mites ( $n = 8$ ), only blowflies ( $n = 10$ ) and both parasites ( $n = 12$ )) and its interaction on 'Postural Intensity', 'Time begging' and 'Latency to beg'.