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

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Running title: Ectoparasites reduce begging effort

Parasitised nestlings are expected to increase begging effort to obtain additional resources to compensate for those sequestered by parasites. However, begging is expected to be 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, parasitised nestlings should invest less in begging than non-parasitised ones. We tested this prediction by measuring begging in Pied Flycatcher (*Ficedula hypoleuca*) nestlings parasitised with haematophagous mites (*Dermanyssus gallinoides* and *D. gallinae*) and blowfly larvae (*Protocalliphora azurea*) and subjected to different levels of food deprivation –therefore, controlling for their short-term nutritional need. As predicted, parasitised nestlings begged for a shorter time than non-parasitised ones, especially when very hungry. Consequently, our results suggest that time begging may indicate not only the level of need, but also nestling parasitism status.

Keywords: begging, blowflies, ectoparasites, mites.

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48 Parasites, by extracting resources from their hosts and decreasing their fitness, are an
49 important ecological and evolutionary force (Schmid-Hempel 2011). Vertebrates harbour
50 several ectoparasitic arthropods, which feed mainly on blood. In birds, many haematophagous
51 ectoparasites inhabit nests, feeding on nestlings whose fitness may consequently be reduced
52 drastically (Møller *et al.* 2009). Nestlings, however, may increase resource consumption by
53 elevating begging levels (Kilner & Johnstone 1997), thus compensating –at least partially–
54 the losses due to parasitism. In fact, Great Tit (*Parus major*) and Pied Flycatcher (*Ficedula*
55 *hypoleuca*) nestlings increase begging when parasitised (Christe *et al.* 1996, Cantarero *et al.*
56 2013). These studies, however, did not control for the nutritional need of nestling and argued
57 that the increased begging in parasitised nestlings was simply due to their higher need
58 compared with unparasitised nestlings. Parasites, nonetheless, not only affect nutritional
59 condition, but also nestling physiology (e.g., reduced haematocrit [Potti *et al.* 1999] and
60 thermogenesis capacity [Simon *et al.* 2004], increased stress response [Lobato *et al.* 2008],
61 reduced antioxidant capacity [López-Arrabé *et al.* 2015]).

62 Begging behaviour is costly in different ways, including energetic expenditure (Kilner
63 2001), generation of oxidative stress (Moreno-Rueda *et al.* 2012) and reduced immune
64 response (Moreno-Rueda & Redondo 2012). Consequently, begging should be especially
65 costly for parasitised nestlings, since they are in worse physiological and nutritional condition
66 than non-parasitised ones. On this basis, we hypothesised that, for the same need of food,
67 parasitised nestlings should invest less in begging than non-parasitised ones. We tested this
68 prediction in Pied Flycatcher nestlings parasitised by the haematophagous mites *Dermanyssus*
69 *gallinoides* and *D. gallinae*, and by larvae of the blowfly *Protocalliphora azurea*. Parasitism
70 by these arthropods negatively impacts nestling fitness by reducing their growth and survival
71 (Merino & Potti 1995), the latter even affecting reproduction when they reach adulthood

(Potti 2008). We experimentally examined the begging behaviour (typified 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

The study was carried out in 2011 and 2013 in an intensively studied Pied Flycatcher population breeding in nest-boxes near La Hiruela (Central Spain; more details in Camacho *et al.* 2013). Nest-boxes were regularly inspected to determine hatching date (day 1). When nestlings were 8-days old, we took 36 nestlings of intermediate size from 18 nests (one pair of nestlings with similar body mass per nest) in the afternoon, always leaving in the nests at least three nestlings to prevent parental desertion. Nestlings were placed in a warm chamber and taken to a nearby laboratory, which took about 20 min. Nestmates were 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. Given that parasites analysed in this study are nest-dwelling, nestlings were free of parasites in the lab. Heat was provided by bulb lamps and temperature inside nest cups was monitored with a probe digital thermometer and kept at ca. 36°C. Upon arrival to the lab, 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 made sure that all nestlings were conditioned to the stimulus and begged similarly to how they behave in their nests (based on nest video recordings, see Redondo *et al.* 2016).

On the following morning, nestlings were stimulated to beg with the parental feeding call at 7:30, 8:00 and 8:30 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 need of food. 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 thrice in each trial. Nestling behaviour was recorded with a digital camera Handycam HDR-XR155E. By using the JWatcher 0.9 software (Blumstein & Daniel 2007), we measured from video recordings: (1) Latency to beg, as the time taken for nestlings to open the gape to beg since the onset of the playback stimulus. (2) Time begging, as the time each nestling spent begging. Once nestlings started to beg, they were allowed to beg without interference until they voluntarily ceased begging. And (3) begging intensity, by establishing five categories of postural intensity (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). The analysis of begging behaviour was blind regarding parasitism status.

When the experiment ended (at midday), nestlings were fed *ad libitum* again, individually marked with non-toxic felt pens, and took back to their nests. In total, nestlings were in the lab for less than 24 hours. On the following days, we regularly checked nests to monitor the fate of nestlings used in the experiments, re-marking them when necessary. All experimental nestlings fledged successfully, except three (one from unparasitised and two from parasitised nests), which died from natural causes. Therefore, no nestling was damaged or died as a consequence of our experiment. When nestlings were 13 days-old, they were

ringed, weighed and measured (tarsus length). Moreover, we scored nests as parasitised or not by haematophagous mites following Merino & Potti (1995): nestlings were placed in a white cotton bag during 5 min, and then 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 (Merino & Potti 1995). This fine analysis of parasites estimation showed however that “absence of mites” did not necessarily imply their complete absence, but rather a low parasitic load, at levels for which mites have no detectable effects on nestling fitness (Merino & Potti 1995). In any case, nests in which mites were categorized as “present” should be interpreted as having considerably higher (with detrimental effects for nestlings) parasitic load than nests in which mites were categorized as “absent” (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. We are confident that such estimations reflect parasite load at the time begging was measured, although we estimated parasite load 5-15 days (5 days for mites, 10-15 for blowflies) after, considering: 1) the time these parasites need to complete their cycles (10-36 days, Gold & Dalhsten 1989, Bruneau *et al.* 2001), and 2) in infected nests, blowflies and mites are usually detected at an early stage of the nestling period (3-5 days) and at a parasitism level similar to that found at the end the nestling period (personal observations).

Statistical analyses were performed with Linear Mixed Effects Models of Restricted Maximum Likelihood (REML-LMM; Zuur *et al.* 2009), by 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 parasitised by mites, blowflies or both (ANOVA, $F_{2,27} < 1.40$, $P > 0.25$ for all begging variables and levels of food deprivation; Fig. S1), we repeated the analyses with two levels for “Parasitism status”: uninfected versus 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, p. 243).

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 legislations on the protection of animals used for scientific purposes. 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 ± 6.26 mites (range: 0-71) and 5.45 ± 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 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 a trend for nestlings in non-

parasitized nests to weigh more at day 13 than those in parasitized nests ($F_{3, 29} = 2.89$, $P = 0.052$), suggesting that ectoparasites had a detrimental effect on nestling growth.

Irrespective of parasitism status, time begging and begging intensity increased with hunger (Table 1; Fig. 1). As expected, the time spent begging was significantly shorter in nestlings reared in parasitised than in uninfected nests (Table 1; Fig. 1a). Differences in begging time were especially marked at the highest level of food deprivation, as indicated by the significant interaction deprivation*parasitism (see Table 1). That is, when nestlings were very hungry (90 min), nestlings from uninfected nests begged for longer than those from infected nests (Fig. 1a). In fact, after 90 min of food deprivation, differences in begging time among nestlings differing in parasitism status were highly significant (REML-LMM, $\chi^2 = 10.85$, $P < 0.001$), while differences were not significant for the trials at 30 and 60 min since the last feeding ($P > 0.65$), and only were closed to significance at 15 min ($\chi^2 = 3.42$, $P = 0.064$). In contrast to begging time, parasitism status had no effect on begging postural intensity or latency (Table 1; Figs. 1b and 1c). Findings were qualitatively similar when we repeated the statistical models with 4 levels of parasitisation (uninfected, only mites, only blowflies, and both; Table S2; Fig. S1).

DISCUSSION

Our study shows that nestlings from parasitised nests begged for shorter than non-parasitised nestlings in a similar state of nutritional need, especially at the highest level of food deprivation. 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 it 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 correlational 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.

At first glance, our findings contrast with those of Cantarero *et al.* (2013) in a nearby population of Pied Flycatchers, in which nestlings in parasitised nests (with mites, blowflies, and fleas) begged more intensely than non-parasitised ones; similar findings were found in Great Tits (Christe *et al.* 1996). In these studies, increased begging effort of parasitised nestlings could be explained by their presumably higher nutritional need, as long as parasitised nestlings were in more need than unparasitised nestlings. Therefore, to ascertain the effect of parasites on begging, it is necessary to control for short-term needs. Our findings are novel because we controlled for short-term nutritional need, and by doing so, we found that parasitised nestlings begged less than non-parasitised nestlings for the same level of food deprivation. Similarly, O'Connor *et al.* (2014) found that Darwin's Finch (*Geospiza fuliginosa*) nestlings parasitised with larvae of the fly *Philornis downsi* begged less than non-parasitised nestlings. That is, nestlings in bad 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), but this is probably not possible when physical condition is much deteriorated (O'Connor *et al.* 2014). Along this line, we found that the largest differences between parasitised and non-parasitised nestlings in begging time occurred when nestlings' need was highest.

The underlying mechanisms of reduced begging effort in parasitised nestlings are still unclear. An energetic cause may be invoked (Moreno-Rueda & Redondo 2011), 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 since, for example, corticosterone levels have been reported to increase with parasitism (Lobato *et al.* 2008). However, corticosterone also increases begging effort (Loiseau *et al.* 2008), so their potential effects would markedly contrast with our findings. A third possibility is that the reduction in begging effort is mediated by oxidative stress. One of the main responses against ectoparasites is inflammation, which is associated with the release of several pro-oxidant substances, such as nitric oxide (NO[•]) or superoxide anion (O₂^{•-}) (reviewed in 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, parasitised nestlings may pay a cost in the way of increased oxidative stress. Therefore, parasitised nestlings would benefit from reducing begging effort in order to keeping their oxidative balance within safe limits. Finally, another possibility is that the reduction in begging is due to a trade-off with the immune response. Birds frequently show an immune response against ectoparasites (review in Owen *et al.* 2010), and begging impairs immune response in our population (Redondo *et al.* 2016). Consequently, it is possible that parasitised nestlings reduced begging in order 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. Parasitised 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 parasitised broods (Saino *et al.* 2000). Health

signalling by nestlings is usually associated to 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 begging may also inform about parasitism status. Therefore, time begging should no longer be viewed as only an indicator of need, as it may also indicate aspects of individual quality (see Mock *et al.* 2011).

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Table 1. Results of Linear Mixed Effects Models of Restricted Maximum Likelihood (REML-LMM) analysing the effect of “Deprivation” (4 levels: 15, 30, 60, and 90 min since the last feeding), “Parasitism status” (2 levels: uninfected vs. infected) and its interaction on “Postural Intensity”, “Time 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.

	χ^2	d.f.	P
a) Time 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

Figure 1. 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 in the nest (black circles: uninfected; white squares: infected). Sample size: 6 uninfected nestlings, 30 infected nestlings.

a)

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386 b)

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389 c)

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