Open access • Journal Article • DOI:10.1111/J.1600-048X.2013.00134.X

Behavioural responses to ectoparasites in pied flycatchers Ficedula hypoleuca: An experimental study — Source link

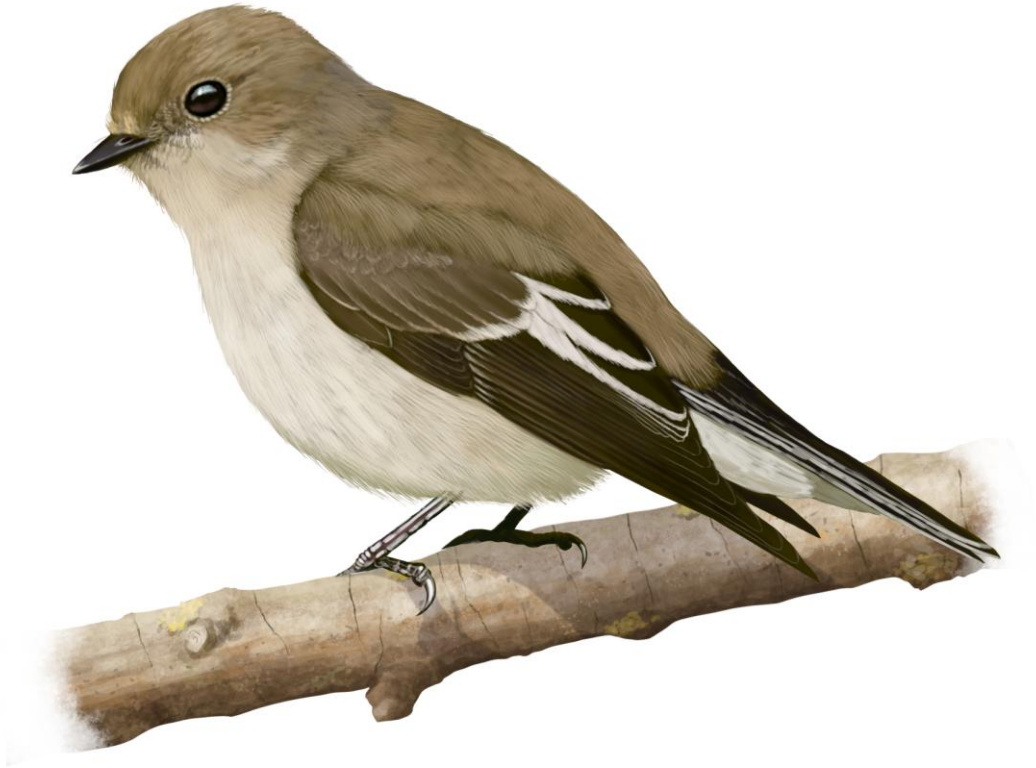
Alejandro Cantarero, Jimena López-Arrabé, Alberto Redondo, Juan Moreno

Institutions: Spanish National Research Council

Published on: 01 Nov 2013 - Journal of Avian Biology (Blackwell Publishing Ltd)

Topics: Ficedula

CHAPTER IV



This chapter reproduces entirely the manuscript:

Cantarero A., López-Arrabé J., Redondo A. J. & Moreno J. 2013. Behavioural responses to ectoparasites in Pied Flycatchers *Ficedula hypoleuca*: an experimental study. Journal of Avian Biology 44: 591-599.

CHAPTER IV

Behavioural responses to ectoparasites in Pied Flycatchers *Ficedula hypoleuca*: an experimental study

Alejandro Cantarero[1], Jimena López-Arrabé[1], Alberto J. Redondo[2] and Juan Moreno[1]

[1] Museo Nacional de Ciencias Naturales - CSIC. Dpto Ecología Evolutiva. C/ José Gutiérrez Abascal 2, 28006 Madrid.

[2] Departamento de Zoología, Universidad de Córdoba, Córdoba, Spain

ABSTRACT. Nests of cavity-nesting birds usually harbor some species of haematophagous ectoparasites that feed on the incubating adults and nestlings. Given the negative impact of ectoparasites on nestlings there will be selection on hosts to reduce parasite infestations through behavioural means. We have experimentally reduced the abundance of all ectoparasites in nests of pied flycatchers *Ficedula hypoleuca* to explore both whether there are changes in the frequency and duration of putative anti-parasite behaviours by tending adults, as well as whether such anti-parasite behaviours are able to compensate for the deleterious effects that parasites may have on nestlings. Heat treatment of nests substantially decreased the density of ectoparasites, and thereby positively affected nestling growth. The frequency and intensity of female grooming and nest sanitation behaviours during the incubation and nestling periods decreased as a consequence of the experimental reduction of ectoparasite infestation. Although nestlings begged more intensely in infested nests, the experiment had no significant effect on parental provisioning effort. Reduction of parasites resulted in larger nestlings shortly before fl edging and increased fledging success. This study shows a clear effect of a complete natural nest ectoparasite fauna on parental behaviour at the nest and nestling growth in a cavity-nesting bird. Although ectoparasites induce anti-parasite behaviours in females, these behaviours are not able to fully remove parasite's deleterious effects on nestling growth and survival.

INTRODUCTION

Parasites have been proposed as an important ecological and evolutionary force affecting avian life histories and behaviour (Atkinson and van Riper 1991, Møller 1997). Cavity-nesting birds have been traditionally associated with selective pressures arising from the thermal environment and the impact of nest predation (Hansell 2000). However, the microclimatically stable environment of cavity nests and the presence of an abundant food supply may offer excellent breeding conditions for ectoparasites, and it is thought that many nest ectoparasitic arthropods have evolved specifically in such nesting environments (Waage 1979, Marshall 1981). Thus, ectoparasites in the nest may be an additional important evolutionary factor modulating adaptations of cavity-nesting birds (Heeb et al. 1999, Tripet et al. 2002a).

Ectoparasites cause removal of nutritional and energy resources from hosts that could otherwise be used for growth, maintenance, or reproduction (Møller 1993). They may also induce costly immune and inflammatory responses (Møller et al. 2005, Owen et al. 2009). Conversely the immature immune systems of altricial nestlings result in stronger direct impacts from ectoparasitism faced by nestlings with the need to assign sufficient nutritional resources to growth (Saino et al. 1998, Szep and Møller 1999). Given the negative impact of ectoparasites on host fitness there will be selection on hosts to avoid parasite infestations through behavioural, physiological and immunological responses (Møller and Erritzoe 1996, Hart 1997, Heeb et al. 1998, Cantarero et al. 2013). All these responses are complementary and may be induced in adults, nestlings or both (Hart 1992, Keymer and Read 1991, Simon et al. 2005). Nesting adults may avoid nest sites with high ectoparasite loads (Moore 2002), due to the association between old nest material and higher abundance of certain types of ectoparasites (Mazgajski 2007, Tomás et al. 2007, López-Arrabé et al. 2012) and bacteria (González-Braojos et al. 2012). Adults may also take measures to indirectly minimize the effects of nest parasites through incorporation of fresh plant material containing compounds that either directly affect the development of parasites (Malan et al. 2002, Clark and Mason 1988, Lafuma et al. 2001, Tomás et al. 2012) or stimulate elements of the immune system of chicks that help them to cope better with the harmful

activities of ectoparasites (Mennerat et al. 2008). Nevertheless, adult cavity-nesting birds are faced with the presence of nest ectoparasites, and likely have a suite of behaviours directed a minimizing the impacts of parasites (Loye and Zuk 1991, Keymer and Read 1991, Hart 1992, Mazgajski 2009).

The main behavioural defenses against ectoparasites are grooming and nest sanitation (Christe et al. 1996). Grooming behaviour may be operationally defined as manipulation of the plumage with the bill (Nelson et al. 1977, Murray 1990). One of its functions may be to dislodge ectoparasites hiding or residing among feathers (Cotgreave and Clayton 1994, Waite et al. 2012). Thus both adults and nestlings may groom themselves in the presence of ectoparasites (O'Connor et al. 2010). Nest sanitation (Welty 1982) refers to behaviours by parents in altricial species tending to remove ectoparasites on nestlings or nest material (Hurtrez-Boussès et al. 2000), removing from the nest both these as well as eggshells (Montevecchi 1974), fecal material (Blair 1941) or dead nestlings (Skutch 1976). Parents are expected to allocate time to nest sanitation in order to control the load of harmful ectoparasites in the nest materials and on the nestlings. Such anti-parasite behaviours may be time-consuming (Cotgreave and Clayton 1994) and therefore may reduce the time that a parent bird can devote to foraging and provisioning offspring.

Nest ectoparasites are a community of species, and the entire community must be considered when examining the influences of these ectoparasites on host behaviour and fitness. For example the nests of Pied Flycatchers (*Ficedula hypoleuca*) in Iberian populations usually harbour three species of haematophagous ectoparasites, namely mites (*Dermanyssus gallinoides*), blowflies larvae (*Protocalliphora azurea*) and hen fleas (*Ceratophyllys gallinae*) (Merino and Potti 1995, 1996). Nests may also contain haematophagous flying insects such as blackflies and biting midges (Martínez-de la Puente et al. 2009). In mites, adult and nymphal stages are haematophagous, while their short generation times allow the build-up of very large populations with detrimental effects on host reproductive success (Merino and Potti 1995, 1996, Moreno et al. 2009). Mites may be present in nest materials even before nestlings hatch and may feed on incubating females (Pacejka et al. 1996). They have been shown to be the most virulent ectoparasite of Iberian pied flycatcher populations (Merino and Potti 1995, Moreno et al. 2009).

Blowfly larvae live in bird nests and feed intermittently on nestling blood (Bennett and Whitworth 1991, Remeš and Krist 2005). Larvae of fleas are not haematophagous, but adult fleas need blood to produce eggs (Tripet and Richner 1997). Therefore, the number of flea larvae in nests indicates the fecundity of adult fleas (Eeva et al. 1994). Fleas may be present in nest materials already during incubation (Harper et al. 1992).

To explore behavioural anti-parasite strategies it is necessary to conduct field experiments where the levels of infestation are strictly controlled in all treatments (Christe et al. 1996, Heeb et al. 1998, Tripet et al. 2002b, Fitze et al. 2004) or experiments in which nests with a reduced ectoparasite loads are compared with natural controls (Allander 1998, Martínez-de la Puente et al. 2010). Each approach has its advantages and disadvantages. Controlled levels of infestation are useful when dealing with a single parasite and reduce environmentally induced variation. On the other hand, natural controls versus experimental reductions allow manipulations of complete ectoparasite faunas while retaining natural levels of infestation as controls, and are especially useful when the effects of whole ectoparasite faunas with their natural interactions are of interest. Moreover, natural controls reflect the effects of whole ectoparasite faunas on nestlings in the wild.

Our goal is to examine the impacts of an entire nest ectoparasite community on reproductive behaviour of their hosts. In our study of Pied Flycatchers in central Spain, we have reduced the abundance of all ectoparasites by a heat treatment of nestboxes. We have assumed that experimental reduction would have a negative impact on ectoparasite abundance and a positive impact on nestling growth and survival. We have then compared control and experimental host behaviour within the nest-box using data from video films (see Hurtrez-Boussès et al. 2000 for a similar approach). Video-recordings inside the nest-box were made during the incubation and at two stages of the nestling period (nestlings of 3 and 9 days of age). Our objectives were to explore changes in the frequency and duration of parental grooming and nest sanitation behaviours as a consequence of the abundance of ectoparasites, and to examine the impacts of these behaviours of adult birds. We have hypothesized that:

(1) Behavioural responses to ectoparasites should be more frequent in control nests than in experimental nests. This pattern should occur during both the incubation and nestling periods;

(2) There should be a trade-off between brooding nestlings and nest sanitation behaviours at the early nestling stage;

(3) Nestlings should beg more intensely in control nests due to the increased food demand induced by ectoparasites;

(4) Parents should respond to higher begging levels in control nests by increasing provisioning rates only if time consumed by anti-parasite behaviours does not compromise that available for foraging.

MATERIAL AND METHODS

General field methods

The study was conducted during the spring of 2012 in a montane forest of Pyrenean oak, *Quercus pyrenaica*, at 1200 m.a.s.l. in Valsaín, central Spain (40˚ 54' N, 4˚ 01' W) where pied flycatchers breeding in nest-boxes have been studied since 1991 (see Sanz et al. 2003 for general description). Of 552 nest-boxes, 91 were occupied by pied flycatchers and the rest by other species, mainly great tits, nuthatches and blue tits (see Lambrechts et al. 2010 for dimensions, structure and placement of nest-boxes).

Egg laying in the Pied Flycatcher population under study typically begins in late May, and modal clutch size is six. The female incubates and broods alone and receives part of her food from her mate (Moreno et al. 2011). No brooding is observed after nestlings attain 7 days of age (Sanz and Moreno 1995). Breeding activities are followed routinely every year and laying and hatching dates and brood sizes at hatching and fledging are determined.

On day 3 (hatching day = day 1), we weighed all nestlings in each brood together with a digital scale to the nearest 0.1 g to give an average nestling mass when divided by brood size. On day 13 (hatching day = day 1), we ringed nestlings and measured their tarsus lengths with a digital callipers to the nearest 0.01 mm and their wing lengths with a stopped ruler to the nearest mm. Nestlings were also

weighed with a Pesola spring balance to the nearest 0.25 g. Nestlings from one nest flew before being measured on day 13.

Experimental reduction of ectoparasites protocol

Of the 91 nest boxes occupied by pied flycatchers we selected those whose laying date was between dates 45 and 51 (April 1=day 1). We assigned 56 nests randomly to two groups, to minimize any possible confounding effects such as variation in microclimate among nest-boxes. The first group was left unmanipulated (control group, N=37). In the second experimental group (N=19), we reduced the number of ectoparasites by a heat-treatment for 30 s at 750 W using a microwave oven. For the time that the original nests were treated (around 30 minutes), a fresh substitute nest was introduced into the nest-box (these nests had been collected in previous seasons after being abandoned prior to laying and kept frozen at -20 ºC until use). This treatment ensured that experimental nests did not contain live arthropods when placed in the nest-box (Rendell and Verbeek 1996), although some arthropods may colonize the nest material after the treatment. To avoid the loss of water during the heat-treatment, the nests were placed into a hermetic plastic container. To prevent recurrence of ectoparasite colonization a total of three heat-treatments were made in the experimental group: (1) 7 days after clutch completion, (2) when nestlings were 2 days old (hatching day=day 1) and (3) when nestlings were 8 days old. Furthermore, before returning the nest the flame from a butane jet torch lighter (Microtorch GT-3000) was passed across the walls of the nest-box to kill ectoparasites that might remain there. Nests in the control group were visited on the same days and handled in a similar way to experimental ones.

Ectoparasite abundance estimation

One or two days after nestlings fledged (17 days after hatching), all nests were removed in sealed plastic bags and taken to the laboratory, where they were subjected to arthropod removal in Berlese funnels for 48 h until nests were thoroughly dried and no arthropods were moving in the nest material. Ectoparasite identification was made with the aid of a stereoscopic microscope (Olympus SZX7). We assume that all mites are hematophagous given their red

color as a consequence of the ingested blood (for arthropods collection and abundance estimations see Moreno et al. 2009).

Video recordings

Seven days after clutch completion (day 7 of incubation), we recorded nest activity inside nest-boxes for about 90 min (91.45±SE 24.63 min, n=58) with a cold white light (LED 5 mm) powered by a 3 V battery and a camera (GoPro HD Hero1) mounted on the roof inside the nest-box. Video recordings were made one day after experimental treatment and nest handling. Nest-boxes were again recorded two days after the day of hatching of the young (88.63±13.01 min, n=57) and 8 days after hatching of the young (85.04±20.01 min, n= 55). In two nests all chicks died after day 3 but we have included records taken during incubation and day 3. All films were recorded between 08:00-17:00 h, and no differences between experimental groups with respect to time of filming were found (incubation period: U=297.0, P=0.346; nestling period day 3: U=314.5, P=0.522; nestling period day 9: U=272.5, P=0.277). We excluded the time until the first nest visit by parents (14.31±11.55 min, n=164). No evidence of stress or unnatural behaviour like extremely long absence periods from the nest or trying to peck at the camera system were observed after the first visit.

Because of technical problems, we failed to record the behaviour at two different nests in the control group, one from young nestlings and another from older nestlings.

Behavioural data analysis

From recordings taken during incubation we estimated the proportion of time spent by the female inside the nest-box or "egg attendance" which includes the time allocated to incubating and turning the eggs, and the mean duration of incubation sessions and recesses. Furthermore, we monitored two specific types of female behaviour: "grooming" and "nest sanitation". "Grooming" is the combined time in which female spends preening or scratching herself (Cotgreave and Clayton 1994) while "nest sanitation" is a period of active search with the head buried, sometimes deeply, into the nest material (Cotgreave and Clayton 1994). In our study, we define nest sanitation as burying the bill in the nest material or carrying

out nest materials. As scratching by females resting on the nest could not be observed accurately, grooming refers mostly to preening with the bill. We assume that these behaviours in our study population have the functions implied by the terms derived from the literature, although our experiment intends precisely to confirm these functional interpretations. We obtained the proportion and the mean duration of these behaviours over the time that the female was inside the box. In addition, we also counted the number of incubation feedings by males.

From recordings during the early nestling phase we obtained hourly provisioning rates by males and females and the amount of time spent by females in "nest attendance", "brooding", "grooming" or "nest sanitation". "Nestlings attendance" includes the proportion of time spent by the female inside the nest-box. "Brooding" activity is defined as the proportion of time spent by the female inside the nest-box covering young nestlings in relation to the total time spent inside the nest-box. "Nest sanitation" and "grooming" were calculated in the same way as during the incubation stage. Pied flycatcher females do not exhibit "sleeping" behaviours during the incubation or nestling periods like in other species (Tripet et al. 2002b).

From recordings during the late nestling phase we obtained hourly provisioning rates by males and females and nest sanitation behaviour. Nest sanitation at this stage only considers removal of nest material from nest-boxes as the chicks do not need brooding and female visits to the nest-box are just for feeding. We also recorded the posture during begging of nestlings. Nestling postures were assigned based on a modification of the scale used by Leonard et al. (2003) following a scale of increasing intensity: 0 = head down, no gaping; 1 = head down, gaping, sitting on tarsi; 2 = head up, gaping, sitting on tarsi; 3 = same as 2, plus neck stretched upward; and 4 = same as 3, but body lifted off tarsi. On each visit of an adult to the nest with food, we scored the maximum postural begging intensity of each nestling. We then estimated the average value of the maximum begging intensity at each visit for the whole brood.

Statistical analyses

Many response variables were normally distributed or successfully normalized through logarithmic transformation (Kolmogorov-Smirnov, $P>0.20$) and were therefore analyzed with GLM models (STATISTICA package) assuming a normal error with treatment as explanatory factor. Hatching success (proportion eggs that hatched) was not normally distributed even when transformed but its residuals were, so it was analyzed with a GLM with treatment as explanatory factor and laying date and clutch size as continuous predictors. Clutch size and brood size were analyzed with GLM models assuming a Poisson distribution with treatment as explanatory factor. Grooming and nest sanitation variables could not be normalized and were analyzed with non-parametric tests (Mann–Whitney U test**)**. Nest sanitation (nestling day 9) and fledging success (proportion hatched chicks that fledged) were analyzed as frequencies (Yes-1/No-0 observation of sanitation in the nest and Yes-1/No-0 cases of all chicks hatched becoming fledglings) with Chi-squared contingency tables.

All parametric behavioural variables were analyzed with treatment as explanatory factor and hatching date, brood size, date and time of filming as continuous predictors. Nonsignificant predictors were sequentially removed until only significant effects remained in the final model. Only the effects of treatment are presented in all cases, even when non-significant.

RESULTS

The two treatments did not differ with respect to laying date, hatching date, clutch size or brood size (Table 1). In order to assess the efficiency of our manipulation, we compared the ectoparasite abundances of the two treatments. The experiment was successful because the experimental nests differed from controls in the abundances of ectoparasites sampled in the predicted direction (Table 1).

	Control	Experimental	Statistic	p
Breeding data				
Laying date	48.162 ± 1.642(37)	47.386 ± 1.012(19)	F_1 = 3.70	0.060
Hatching date	66.351 ± 1.230(37)	66.947 ± 1.311(19)	F_1 = 1.30	0.260
Clutch size	5.622 ± 0.594(37)	5.84 ± 0.501(19)	*Wald* =0.107	0.743
Brood size 13	4.722 ± 1.446(36)	5.263 ± 0.733(19)	*Wald* =0.741	0.389
Ectoparasites				
Blowflies	6.162±8.748(37)	0.684±1.887(19)	U_1 = 172.0	<0.005
Mites	3347.57±4543.55(37	274.053±906.913(19)	F_1= 17.76	<0.001
Fleas	24.946±88.329(37)	0.000±0.000(19)	U_1 = 247.0	<0.01

Table 1. Differences in breeding variables and ectoparasite abundances (means ± SE, n in parenthesis) and results of GLM analyses and Mann-Whitney U-tests.

The control (3.36±0.49) and the experimental group (3.76±0.61) differed in the mean nestling mass (g) on day 3 (F=6.87, P=0.011). We then tested for the effects of treatment on brood-averaged nestling morphometric measurements and mass near fledging (tarsus length, wing length and body mass at day 13), controlling for hatching date and brood size. Nestlings in experimental nests were significantly larger with respect to wing length and tarsus length (Table 2), while there were no differences between treatments in body mass (Table 2). Tarsus length was negatively related to hatching date (adjusted R^2=0.25). The control (90.09±14.13) and the experimental (90.30±11.61) groups did not differ in hatching success (F=0.003, P>0.90) when controlling for laying date and clutch size (both P>0.20), while fledging success was marginally lower (χ^2=2.82, P=0.093) in control nests (0.89±0.32) than in experimental nests (1.00±0.00).

	Control	Experimental	Treatment	Hatching Date	Brood size
Nestling day 13					
Tarsus length (mm)	17.42±0.47(34)	17.79±0.38(19)	F=6.615*	F=9.027**	F=0.133
Body mass (g)	13.98±0.94(34)	14.17±0.92(19)	F=0.510	F=0.231	F=0.478
Wing length (mm)	46.85±2.40(34)	48.57±2.32(19)	F=7.126*	F=1.050	F=0.072

Table 2. Differences in brood-averaged nestling morphology and mass (means ± SE, number of broods in parenthesis) and results of GLM models on nestling condition parameters with treatment as explanatory factor and hatching date and brood size as continuous predictors (**P<0.01, * P<0.05).

Treatment did not significantly affect incubation attendance (% incubation time) or the mean of recess and incubation session durations of females (Table 3). Female grooming behaviour was less frequent and the mean duration of grooming sessions were significantly shorter in the experimental group than in the control group (Table 3). Nest sanitation behaviour time was also more intensive in the control group (Table 3).

	Control	Experimental	Statistic	P
Incubation				
Grooming (%)	0.400±0.586(37)	0.122±0.240(19)	U = 182	**0.012**
Mean grooming (s)	3.00±3.00(37)	2.00±3.00(19)	U =185	**0.015**
Egg attendance (%)	63.844±11.74(37	60.750±12.645(19	F_1= 0.81	0.372
Mean session (min)	10.516±4.433(37	9.317±6. 167(19)	F_1 = 0.68	0.412
Mean recess (min)	5.950±2.000(37)	4.217±2.217(19)	F_1 = 1.55	0.218
Nest sanitation (%)	1.905±1.596(37)	1.041±1.289(19)	F_1= 5.50	**0.029**
Nestling day 3				
Nestling attendance (%)	53.274±14.59(36	48.358±13.106(19	F_1= 1.34	0.254
Brooding (%)	93.045±3.127(36	95.997±3.981(19)	F_1= 7.60	**0.008**
Mean grooming duration	2.00±2.00(36)	1.00±2.00(19)	U=172	**0.048**
Grooming (%)	0.108±0.143(36)	0.047±0.121(19)	U = 168	**0.039**
Nest sanitation (NS) (%)	5.367±2.562(36)	2.879±3.659(19)	F_1 = 7.80	**0.007**
Mean NS duration (s)	5.00±3.00(36)	3.00±2.00(19)	U = 121	**<0.005**
Male provisioning (h^{-1})	9.298±5.102(36)	11.108±4.566(19)	F_1 = 1.62	0.209
Female provisioning (h^{-1})	5.950±3.076(36)	5.791±2.557(19)	F_1 = 0.03	0.853
Nestling day 9				
Nest sanitation (yes/no)	0.176±0.387(34)	0.000±0.000(19)	χ^2=3.78	0.052
Male provisioning (h^{-1})	11.934±5.685(34	11.290±5.268(19)	F_1 = 0.16	0.688
Female provisioning (h^{-1})	11.384±5.541(34	10.345±5.074(19)	F_1 = 0.45	0.503
Begging intensity score	1.149±0.636(34)	0.741±0.376(19)	F_1 = 6.16	**0.016**

Table 3. Differences (means ± SE, n in parenthesis) in behavioural variables between the two treatments and results of GLM analyses (significant p-values in bold), Mann-Whitney U-tests and Chi-squared contingency tables (sanitation present or absent).

In relation to the second hypothesis, the proportion of brooding time on day 3 was significantly higher in the experimental group than in the control one as predicted (Table 3). Grooming variables showed the same pattern between treatments as during incubation (Table 3). Nest sanitation time was again higher in the control group (Table 3). There was no experimental effect on provisioning

rates of males and females (all $P>0.2$). Control nests where nest sanitation behaviours occurred showed higher mite infestations than control nests where these behaviours did not occur (Fig. 1). This relationship was not found for blowfly larvae or fleas. There were marginally more nest sanitation events in control nests than in experimental nests (Table 3).

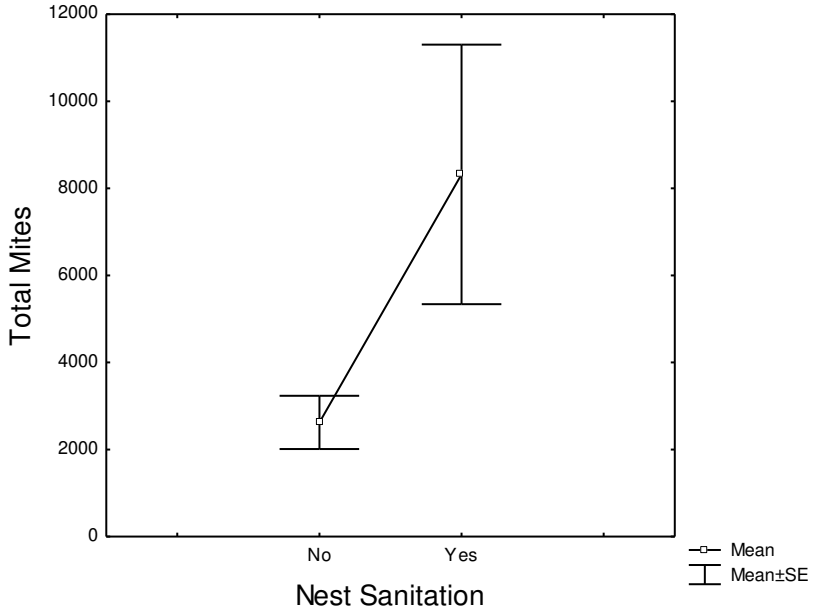


Figure 1. Total mites (±SE) in relation to the presence (Yes) or absence (No) of nest sanitation behaviour in the control group ($P=0.0045$) in the late nestling phase.

In accordance with our third hypothesis, begging intensity of nestlings was higher in control nests (Table 3) and was positively associated with parental provisioning rates (Fig. 2).

Contrary to our fourth hypothesis and despite the association with begging intensity male and female provisioning rates on day 9 were not related to treatment (Table 3).

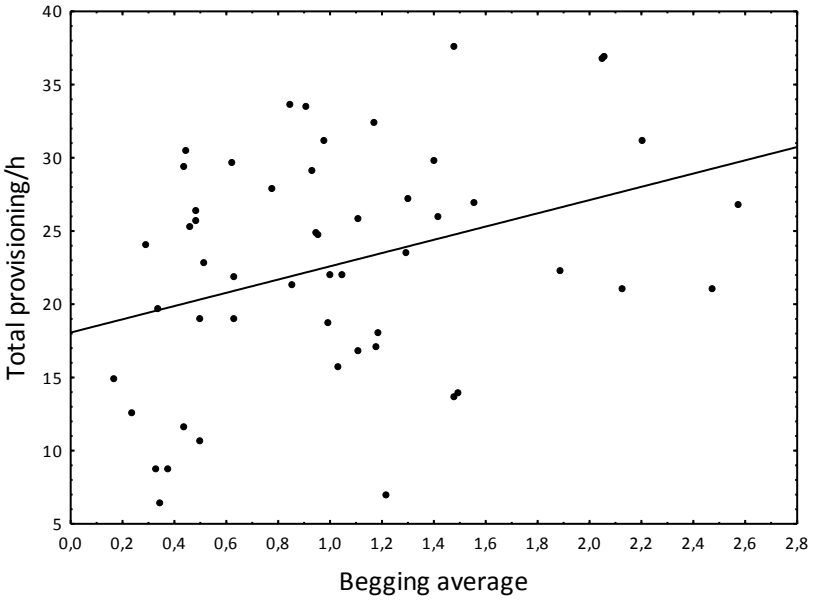


Figure 2. Association between hourly provisioning rates (male and female) and begging average intensity in the late nestling phase (Spearman correlation: r=0.48, $P<0.005$).

DISCUSSION

This study shows that the behaviour of pied flycatcher females inside the nest-box was clearly affected by ectoparasite abundance. The heat treatment of the nests decreased strongly the nest density of blowflies, mites and fleas, and thereby positively affected nestling growth. Experimental nests resulted in larger nestlings shortly before fledging. The frequency and intensity of female grooming and nest sanitation behaviours during the incubation and nestling periods decreased as a consequence of the experimental reduction of ectoparasite infestation. The experimental treatment did not affect incubation attendance and there were no effects on male or female provisioning rates to the chicks at any stage.

There is mixed evidence concerning the impact of ectoparasites on reproductive success in altricial cavity-nesting birds. While some experimental studies have found strong deleterious effects on nestling growth and survival (Heeb et al. 1998, Richner et al. 1993), others have only found weak effects or none at all (Tripet et al. 2002b, O'Brien and Dawson 2008, Bouslama et al. 2002). These differences among host species and populations may depend on the absolute levels of infestation found in different regions or habitats (Hurtrez-Boussès et al. 1997,

Eeva et al. 1994, Fitze et al. 2004). Strong effects are thus mostly found in areas where climate is favourable for arthropod survival and dispersal during and between avian breeding seasons (Merino and Potti 1996, Dufva and Allander 1996). Reproductive success in Iberian pied flycatcher populations has been previously shown to suffer the impacts of nest-dwelling ectoparasites (Merino and Potti 1995, 1996, Merino et al. 1998, Merino and Potti 1998, Moreno et al. 2009).

In agreement with several previous experimental studies we found marked effects of ectoparasites on nestling growth (Heeb et al. 2000, Tomás et al. 2008, Brommer et al. 2011). Tarsus and wing length of nestlings were negatively affected by ectoparasite abundance although we did not find an effect on body mass. Tarsus length of pied flycatcher nestlings has been related to their recruitment probability from fledging until breeding (Alatalo and Lundberg 1986), so the effects of ectoparasites may affect the future fitness of nestlings. For the observation that we found no effect of treatment on nestling body mass, there are two non-mutually exclusive alternative interpretations of our results. On the one hand, nestling growth improves under favourable conditions for breeding (Sanz 1995). Conditions during the year of study (2012) must have been especially favourable as nestlings attained their largest masses since the inception of the study (1991), which may explain why we found no effect of treatment on body mass. Additionally, control nestlings could reduce ectoparasite effects on body mass by increasing self preening or standing on top of one another (O'Connor et al. 2010).

Control nestlings showed as expected an increase in begging intensity, which is positively associated with parental provisioning rates, as was found in the Great Tit *Parus major* (Christe et al. 1996). Older nestlings suffering from higher ectoparasite loads begged more intensely as a response to their higher nutritional needs. Parental provisioning frequency depends on begging intensity as found in other studies on begging intensity (Kedar et al. 2000, Kölliker et al. 2000, Wright et al. 2002). Like Fitze et al. (2004) we noticed no effect of ectoparasite reduction on parental provisioning rates at any nestling age despite the strong correlation between begging intensity and provisioning rates. However, we found no effects of the experiment on parental provisioning frequency which could explain the lack of difference in the body mass of nestlings between treatments and it could have

been caused by factors for which we did not control such as prey quality, ectoparasite virulence, nestling resistance or environmental constraints (e.g. Møller 1994, Lehmann 1993). Roger et al. (1991) also showed no effect in parental provisioning frequencies in response to ectoparasites (see also Tripet et al. 2002b). There is also evidence that parental effort in pied flycatchers is energetically tightly constrained thereby precluding responses to variation in brood demand (Moreno et al. 1997, Moreno et al. 1999). This lack of parental response may explain why their food provisioning was incapable of compensating for ectoparasite effects leading to smaller size at fledging in control nests. It is also possible that increased dedication to nest sanitation in control nests contributed to reduce the capacity of parents to augment their provisioning rates sufficiently to be detectable. That fledging success was marginally higher in experimental nests supports the existence of ectoparasite effects on nestling survival (Lundberg and Alatalo 1992, Moreno et al. 1999) although other fitness costs such as the probability of recruitment could be expressed after fledging (Thomas et al. 2007).

It is assumed that as a consequence of the negative impact of ectoparasites on nestlings, hosts have evolved behavioural responses (Cantarero et al. 2013). Ectoparasites present during incubation in pied flycatcher nests are mites and fleas. Females groom themselves more in control nests which may imply a direct response to the attachment of these ectoparasites on their skin and plumage. Nevertheless, grooming activity may not occupy sufficient time to constrain incubation attendance in females (Hurtrez-Boussès et al. 2000). However, our results on grooming behaviour indicate that tending females may suffer some costs induced by nest ectoparasites through attachment and possibly blood-sucking even before nestlings hatch (see also Tomás et al. 2008). When the nestlings hatch, the blowflies lay their eggs on their skin, and emerging larvae then begin feeding on nestling blood. The blood-sucking larvae of blowflies feed intermittently on the blood of nestling birds (Rognes 1991), although they may try to attach also to brooding females (Bennett and Whitworth 1991) given that their belly skin is naked at this stage. The combined effect of nest ectoparasites induced a lower body mass of chicks in control nests compared with treated nests already two days after hatching. This indicates that ectoparasites impair the growth of nestlings from hatching, a cost for which parents are apparently not able to compensate. If

variation in parasite abundance is obvious to attending parents, we should expect that females in the control group compared to those in the treated group should allocate more time to anti-parasite behaviours and restrict the time spent on brooding chicks, sleeping (Tripet et al. 2002b) or foraging and provisioning nestlings (Christe et al. 1996). We found that control females reduced their proportion of time spent in the nest-box brooding compared to experimental females, but not with respect to total nestling attendance. The fact that females from control nests increased anti-parasite behaviours but maintained similar brooding attendances and provisioning rates as at experimental nests suggests that the time costs of these behaviours are not sufficiently important to reduce time available for foraging at this early stage of nestling development.

The function of nest sanitation behaviour by introducing the bill in the nest material has been debated (Haftorn 1994). One possibility is that birds actually destroy and even consume ectoparasitic arthropods (Rothschild and Clay 1952). This behaviour may also occur in pied flycatchers because we observed females swallowing some collected items on video-recordings of control nests. Nest sanitation could also be used to chase blowfly larvae or adult fleas away from their own body or that of their nestlings, thereby preventing them from biting the incubating female or the nestlings. We also observed one female attacking an adult blowfly that entered the nest-box while she was brooding which could prevent oviposition in the nest. The difference in the time invested in behavioural defences indicates that females may be able to choose to increase the amount of time allocated to control of nest ectoparasites.

Changes in the frequency and duration of grooming or nest sanitation may be interpreted as responses to ectoparasites. Our results are consistent with several previous studies in Great Tits (Richner et al. 1993) and Blue Tits (Christe et al. 1996, Tripet et al. 2002b, Hurtrez-Boussès et al. 2000) that showed that females spent more time on nest sanitation when the nest was infested with fleas. The number and duration of grooming sessions also increased in control nests. This suggests that this behaviour may have evolved in response to ectoparasites and that females could thereby minimize the fitness costs associated with ectoparasite infestations (Richner et al. 1993).

This is the first study showing a clear effect of a complete natural ectoparasite fauna on parental behaviour and nestling growth in a cavity-nesting bird. Our test of effects of ectoparasites is conservative as we were not able to completely remove all ectoparasites and as the study was performed under especially good conditions for breeding. Ectoparasites induce significant changes in female grooming and nest sanitation behaviours which are not able to fully remove their natural deleterious effects on nestling growth and survival.

ACKNOWLEDGMENTS

This study was financed by project CGL2010-19233-C03-02 to JM from Spanish MICINN. JLA and AC were supported by FPI and FPU grants from MICINN and MECD respectively. We were legally authorized to ring and measure nestlings by Consejería de Medio Ambiente de Castilla y León and by J. Donés, director of "Centro Montes de Valsaín" to work in the study area. We thank S. González-Braojos, E. Jiménez-Vaquero, S. Merino and E. Pérez-Badás for collaboration in the field and S. Redondo for technical support. This study is a contribution to the research developed at 'El Ventorrillo' field station.

REFERENCES

Alatalo RV, Lundberg A (1986) Heritability and selection on tarsus length in the pied flycatcher (*Ficedula-hypoleuca*). Evolution 40:574-583

Allander K (1998) The effects of an ectoparasite on reproductive success in the Great Tit: a 3-year experimental study. Canadian Journal of Zoology-Revue Canadienne De Zoologie 76:19-25

Atkinson CT, van Riper C (1991) Pathogenicity and epizootiology of avian haematozoa: *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*. In: Loye JE, Zuk M (eds) Bird-parasite interactions. Ecology, Evolution and Behaviour Oxford University Press, New York, pp 19-48

Bennett GF, Whitworth TL (1991) Studies on the life-history of some species of *Protocalliphora* (Diptera, *Calliphoridae*). Canadian Journal of Zoology-Revue Canadienne De Zoologie 69:2048-2058

Blair RH (1941) Nest-sanitation with additions from published sources by B. W. Tucker. British Birds 34:206-215, 226-235, 250-255

Brommer JE, Pitala N, Siitari H, Kluen E, Gustafsson L (2011) Body size and immune defense of nestling blue tits (*Cyanistes caeruleus*) in response to manipulation of ectoparasites and food supply. Auk 128:556-563

Cantarero A et al. (2013) Factors affecting the presence and abundance of generalist ectoparasites in nests of three sympatric hole-nesting bird species. Acta Ornithologica 48:39-54

Clark L, Mason JR (1988) Effect of biologically-active plants used as nest material and the derived benefit to starling nestlings. Oecologia 77:174-180

Cotgreave P, Clayton DH (1994) Comparative-analysis of the time spent grooming by birds in relation to parasite load. Behaviour 131:171-187

Christe P, Richner H, Oppliger A (1996) Of great tits and fleas: Sleep baby sleep. Animal Behaviour 52:1087-1092

Dufva R, Allander K (1996) Variable effects of the Hen Flea *Ceratophyllus gallinae* on the breeding success of the Great Tit *Parus major* in relation to weather conditions. Ibis 138:772-777

Eeva T, Lehikoinen E, Nurmi J (1994) Effects of ectoparasites on breeding success of great tits (*Parus-major*) and pied flycatchers (*Ficedula-hypoleuca*) in an air-pollution gradient. Canadian Journal of Zoology-Revue Canadienne De Zoologie 72:624-635

Fitze PS, Tschirren B, Richner H (2004) Life history and fitness consequences of ectoparasites. Journal of Animal Ecology 73:216-226

González-Braojos S, Vela AI, Ruiz-de-Castañeda R, Briones V, Cantarero A, Moreno J (2012) Is nestling growth affected by nest reuse and skin bacteria in Pied Flycatchers *Ficedula hypoleuca*? Acta Ornithologica 47:119-127

Haftorn S (1994) The act of tremble-thrusting in tit nests, performance and possible function. Fauna Norvegica Ser. C, Cinclus 17:55-74

Hansell M (2000) Bird Nests and Construction Behaviour. Cambridge: Cambridge University Press

Harper GH, Marchant A, Boddington DG (1992) The ecology of the hen flea *Ceratophyllus-gallinae* and the moorhen flea *Dasypsyllus-gallinulae* in nestboxes. Journal of Animal Ecology 61:317-327

Hart BL (1992) Behavioral adaptations to parasites - an ethological approach. Journal of Parasitology 78:256-265

Hart BL (1997) Behavioural defence. In: Clayton DH, Moore J (eds) Host-parasite evolution. General principles and avian models. Oxford University Press, Oxford

Heeb P, Kölliker M, Richner H (2000) Bird-ectoparasite interactions, nest humidity and ectoparasite community structure. Ecology 81:958-968

Heeb P, Werner I, Kölliker M, Richner H (1998) Benefits of induced host responses against an ectoparasite. Proceedings of the Royal Society B-Biological Sciences 265:51-56

Heeb P et al. (1999) Ectoparasite infestation and sex-biased local recruitment of hosts. Nature 400:63-65

Hurtrez-Boussès S, Renaud F, Blondel J, Perret P, Galan MJ (2000) Effects of ectoparasites of young on parents' behaviour in a Mediterranean population of Blue Tits. Journal of Avian Biology 31:266-269

Kedar H, Rodriguez-Gironés MA, Yedvab S, Winkler DW, Lotem A (2000) Experimental evidence for offspring learning in parent-offspring

communication. Proceedings of the Royal Society B-Biological Sciences 267:1723-1727

Keymer AE, Read AF (1991) Behavioural ecology: the impact of parasitism. In: Toft CA, Aeschli mann A, Bolis L (eds) Parasite-host association: coexistence or conflict? Oxford University Press, Oxford, pp 37-61

Kölliker M, Brinkhof MWG, Heeb P, Fitze PS, Richner H (2000) The quantitative genetic basis of offspring solicitation and parental response in a passerine bird with biparental care. Proceedings of the Royal Society B-Biological Sciences 267:2127-2132

Lafuma L, Lambrechts MM, Raymond M (2001) Aromatic plants in bird nests as a protection against blood-sucking flying insects? Behavioural Processes 56:113-120

Lambrechts M et al. (2010) The design of artificial nestboxes for the study of secondary hole-nesting birds: a review of methodological inconsistencies and potential biases. Acta Ornithologica 45:1-26

Lehmann T (1993) Ectoparasites - Direct impact on host fitness. Parasitology Today 9:8-13

Leonard ML, Horn AG, Parks E (2003) The role of posturing and calling in the begging display of nestling birds. Behavioral Ecology and Sociobiology 54:188-193

López-Arrabé J, Cantarero A, González-Braojos S, Ruiz-de-Castañeda R, Moreno J (2012) Only some ectoparasite populations are affected by nest re-use: an experiment on pied flycatchers. Ardeola 59:253-266

Loye JE, Zuk M (1991) Bird-parasite interactions. Ecology, Evolution and Behaviour. Oxford Ornithology Series:i-xv, 1-406

Lundberg A, Alatalo RV (1992) The pied flycatcher. Poyser, London

Malan G, Parasram WA, Marshall DJ (2002) Putative function of green lining in black sparrowhawk nests: Mite-repellent role? South African Journal of Science 98:358-360

Marshall AG (1981) The ecology of ectoparasitic insects. Academic Press, London

Martínez-de la Puente J et al. (2009) Does weather affect biting fly abundance in avian nests? Journal of Avian Biology 40:653-657

Martínez-de la Puente J et al. (2010) Nest ectoparasites increase physiological stress in breeding birds: an experiment. Naturwissenschaften 98:99-106

Mazgajski TD (2007) Effect of old nest material on nest site selection and breeding parameters in secondary hole nesters - a review. Acta Ornithologica 42:1-14

Mazgajski TD (2009) Breeding success of hole-nesting birds - effects of nest sites characteristics and predator avoidance strategies. Wiadomosci Ekologiczne 55:159-183

Mennerat A, Perret P, Caro SP, Heeb P, Lambrechts MM (2008) Aromatic plants in blue tit *Cyanistes caeruleus* nests: no negative effect on blood-sucking *Protocalliphora* blow fly larvae. Journal of Avian Biology 39:127-132

Merino S, Moreno J, Potti J, de León A, Rodríguez R (1998) Nest ectoparasites and maternal effort in pied flycatchers. Biologia e Conservazione della Fauna 102:200-205

Merino S, Potti J (1995) Mites and blowflies decrease growth and survival in nestling pied flycatchers. Oikos 73:95-103

Merino S, Potti J (1996) Weather dependent effects of nest ectoparasites on their bird hosts. Ecography 19:107-113

Merino S, Potti J (1998) Growth, nutrition, and blow fly parasitism in nestling Pied Flycatchers. Canadian Journal of Zoology-Revue Canadienne De Zoologie 76:936-941

Møller AP (1993) Ectoparasites increase the cost of reproduction in their hosts. Journal of Animal Ecology 62:309-322

Møller AP (1994) Parasite infestation and parental care in the barn swallow *Hirundo-rustica* - a test of the resource-provisioning model of parasite-mediated sexual selection. Ethology 97:215-225

Møller AP (1997) Parasitism and the evolution of host life history. In: Clayton DH, Moore J (eds) Host-parasite evolution: general principles and avian models. Oxford University Press, Oxford, pp 105-127

Møller AP, Christe P, Garamszegi LZ (2005) Coevolutionary arms races: increased host immune defense promotes specialization by avian fleas. Journal of Evolutionary Biology 18:46-59

Møller AP, Erritzoe J (1996) Parasite virulence and host immune defense: Host immune response is related to nest reuse in birds. Evolution 50:2066-2072

Montevecchi (1974) Eggshell removal and nest sanitation in ring doves. Wilson Bulletin 86:136-143

Moore J (2002) Parasites and the Behaviour of Animals. Oxford University Press, Oxford

Moreno J et al. (2009) Nest-dwelling ectoparasites of two sympatric hole-nesting passerines in relation to nest composition: An experimental study. Ecoscience 16:418-427

Moreno J, Merino S, Potti J, de León A, Rodríguez R (1999) Maternal energy expenditure does not change with flight costs or food availability in the pied flycatcher (*Ficedula hypoleuca*): costs and benefits for nestlings. Behavioral Ecology and Sociobiology 46:244-251

Moreno J, Potti J, Merino S (1997) Parental energy expenditure and offspring size in the pied flycatcher *Ficedula hypoleuca*. Oikos 79:559-567

Moreno J, Redondo AJ, Cantarero A, Ruiz-de-Castañeda R, González-Braojos S (2011) Handicapped females receive more feedings during incubation from their mates: support for the female nutrition hypothesis. Acta Ethologica 14:85-89

Murray MD (1990) Influence of host behaviour on some ectoparasites of birds and mammals. In: Parasitism and host behaviour, pp 290-315

Nelson WA, Bell JF, Clifford CM, Keirans JE (1977) Interaction of ectoparasites and their hosts. Journal of Medical Entomology 13:389-428

O'Connor JA, Robertson J, Kleindorfer S (2010) Video analysis of host-parasite interactions in nests of Darwin's finches. Oryx 44:588-594

Owen JP, Delany ME, Cardona CJ, Bickford AA, Mullens BA (2009) Host inflammatory response governs fitness in an avian ectoparasite, the northern fowl mite (*Ornithonyssus sylviarum*). International Journal for Parasitology 39:789-799

Pacejka AJ, Santana E, Harper RG, Thompson CF (1996) House Wrens *Troglodytes aedo*n and nest-dwelling ectoparasites: Mite population growth and feeding patterns. Journal of Avian Biology 27:273-278

Remeš V, Krist M (2005) Nest design and the abundance of parasitic *Protocalliphora* blow flies in two hole-nesting passerines. Ecoscience 12:549-553

Rendell WB, Verbeek NAM (1996) Are avian ectoparasites more numerous in nest boxes with old nest material? Canadian Journal of Zoology-Revue Canadienne De Zoologie 74:1819-1825

Richner H, Oppliger A, Christe P (1993) Effect of an ectoparasite on reproduction in great tits. Journal of Animal Ecology 62:703-710

Rogers CA, Robertson RJ, Stutchbury BJ (1991) Patterns and effects of parasitism by *Protocalliphora sialia* on Tree Swallow nestlings. In: Bird-parasite interactions. Ecology, Evolution and Behaviour. Oxford University Press, New York, pp 123-139

Rognes K (1991) Revision of the bird-parasitic blowfly genus *Trypocalliphora-peus* 1960 (Diptera, *Calliphoridae*). Entomologica Scandinavica 15:371-382

Rothschild M, Clay T (1952) Fleas, flukes and cuckoos. Collins, London

Saino N, Calza S, Møller AP (1998) Effects of a dipteran ectoparasite on immune response and growth trade-offs in barn swallow, *Hirundo rustica*, nestlings. Oikos 81:217-228

Sanz JJ (1995) Environmental restrictions on reproduction in the Pied Flycatcher *Ficedula hypoleuca*. Ardea 83:421-430

Sanz JJ, Moreno J (1995) Mass loss in brooding female Pied Flycatchers *Ficedula hypoleuca*: No evidence for reproductive stress. Journal of Avian Biology 26:313-320

Sanz JJ, Potti J, Moreno J, Frías O (2003) Climate change and fitness components of a migratory bird breeding in the Mediterranean region. Global Change Biology 9:461-472

Simon A, Thomas DW, Speakman JR, Blondel J, Perrer P, Lambrechts MM (2005) Impact of ectoparasitic blowfly larvae (*Protocalliphora spp.*) on the behavior and energetics of nestling Blue Tits. Journal of Field Ornithology 76:402-410

Skutch AF (1976) Parent Birds and Their Young. University of Texas Press

Szep T, Møller AP (1999) Cost of parasitism and host immune defence in the sand martin *Riparia riparia*: a role for parent-offspring conflict? Oecologia 119:9-15

Thomas DW, Shipley B, Blondel J, Perret P, Simon A, Lambrechts M (2007) Common paths link food abundance and ectoparasite loads to physiological performance and recruitment in nestling blue tits. Functional Ecology 21:947-955

Tomás G et al. (2012) Interacting effects of aromatic plants and female age on nest-dwelling ectoparasites and blood-sucking flies in avian nests. Behavioural Processes 90:246-253

Tomás G, Merino S, Martínez-de la Puente JM, Moreno J, Morales J, Lobato E (2008) Determinants of abundance and effects of blood-sucking flying insects in the nest of a hole-nesting bird. Oecologia 156:305-312

Tomás G, Merino S, Moreno J, Morales J (2007) Consequences of nest reuse for parasite burden and female health and condition in blue tits, *Cyanistes caeruleus*. Animal Behaviour 73:805-814

Tripet F, Glaser M, Richner H (2002a) Behavioural responses to ectoparasites: time-budget adjustments and what matters to Blue Tits *Parus caeruleus* infested by fleas. Ibis 144:461-469

Tripet F, Jacot A, Richner H (2002b) Larval competition affects the life histories and dispersal behavior of an avian ectoparasite. Ecology 83:935-945

Tripet F, Richner H (1997) The coevolutionary potential of a 'generalist' parasite, the hen flea *Ceratophyllus gallinae*. Parasitology 115:419-427

Waage JK (1979) Evolution of insect-vertebrate associations. Biological Journal of the Linnean Society 12:187-224

Waite JL, Henry AR, Clayton DH (2012) How effective is preening against mobile ectoparasites? An experimental test with pigeons and hippoboscid flies. International Journal for Parasitology 42:463-467

Welty JC (1982) The Life of Birds. Saunders College Publishing, Philadelphia

Wright J, Hinde C, Fazey I, Both C (2002) Begging signals more than just short-term need: cryptic effects of brood size in the pied flycatcher (*Ficedula hypoleuca*). Behavioral Ecology and Sociobiology 52:74-83