

Nest-climatic factors affect the abundance of biting flies and their effects on nestling condition

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Abstract

The first step in the establishment of a host-biting fly relationship is host location. While a number of studies highlight the role of host emitted products as important cues affecting host location by biting flies, the role of host temperature is far from clear. We investigated the role of different nest microclimatic variables affecting the interaction between pied flycatchers and two biting flies: black flies and biting midges. Biting midge abundances increased with temperature inside the nest, supporting the potential importance of nest temperature as a cue used by insects to localize their hosts. The possibility that biting fly infestations were associated to ecological conditions in the vicinity of the nests is also discussed. Furthermore, we found a negative association between nestling weight (including tarsus length as a covariate in the analyses) and the interaction between the abundance of biting midges and the presence/absence of black flies in nests. The potential negative effect of these ectoparasites on nestling weight (condition index) and potential differences in the bird phenotypic/genetic quality associated with nest site choice and parasite infestations are considered.

1. Introduction

Location of the hosts by biting flies is a complex and active behavioural task that enhances the probability of contact of haematophagous insects with their hosts (Sutcliffe, 1986). This behaviour could be divided into three phases: (i) appetitive searching, (ii) activation and orientation and (iii) attraction (Lehane, 2005), although the definition of these phases could vary between authors (Kettle, 1995). In addition to vision and smell, other stimuli including heat and humidity become available to the insect in close proximity to their hosts and are also involved in the biting flies' host-seeking behaviour (Gatehouse and Lewis, 1973).

Biting insects may respond to the heat emitted by the host or to the thermal gradient between insects and hosts (Lehane, 2005). There are clear evidences for the role of thermoreceptors of blood-sucking arthropods in responses to thermal stimuli related to the presence of their hosts (Howlett, 1910; Nigam and Ward, 1991; Guerenstein and Lazzari, 2009). However, the importance of host temperature or humidity on host location is far from clear for the

vast majority of blood-sucking insects, in contrast to the well-known role of chemical cues (Guerenstein and Lazzari, 2009). In the case of biting flies attacking wild birds the difficulties for sampling insects in avian nests (but see two recent descriptions of easy sampling methods by Tomás et al., 2008a; Votýpka et al., 2009) do the research on these factors even more complicated.

Biting midges (genus *Culicoides*, Diptera: Ceratopogonidae) and black flies (Diptera: Simuliidae) are two of the main haematophagous insects attacking birds under natural conditions (Kettle, 1995; Malmqvist et al., 2004; Martínez-de la Puente et al., 2009a, 2009b; Votýpka et al., 2009). Among these insects only females feed on animal blood, affecting hosts directly by draining resources and causing physical harassment and indirectly as vectors of multiple pathogens (Bennett, 1961; Valkiūnas, 2005). In spite of the few studies on the effects of biting fly attacks on wild birds, there are clear evidences supporting the role of biting flies increasing mortality in immature birds (Hunter et al., 1997; Smith et al., 1998). These adverse effects on nestlings may be due to the direct impact of insect attacks but also by their effects affecting parental decisions, including nest desertion or reduction of parental provisioning rate (Bukaciński and Bukacińska, 2000).

Here, we investigated the effects of two microclimatic factors, nest temperature and humidity, potentially affecting the relationship between biting flies and avian hosts. To that end, we studied the interaction between pied flycatchers *Ficedula hypoleuca* and two

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different haematophagous insect groups, biting midges and black flies, after controlling for the potential effect of other variables such as brood size and hatching date (see [Martínez-de la Puente et al., 2009b](#)). Also, we investigated the potential negative effect of biting flies on nestling condition after controlling for the confounding effect of rearing conditions ([Dawson et al., 2005b](#); [Pérez et al., 2008](#)).

2. Methods

2.1. Host population

This study was conducted during the spring of 2008 in a population of pied flycatchers *F. hypoleuca* breeding in nest-boxes in a Pyrenean oak *Quercus pyrenaica* forest located in Valsaín (Central Spain, 40° 53' 74" N, 4° 01' 0" W, 1200 m.a.s.l.). All nest-boxes were hung from branches at about 4 m above the ground, thus, avoiding potential height differences in avian exposure to insect attacks ([Fallis and Smith, 1964](#)). Periodical visits in the course of the breeding season allowed us to record hatching date and brood size of birds at each nest.

2.2. Nestling measurements

At the age of 12 days, nestlings were weighed with a Pesola spring balance to the nearest 0.1 g and tarsus length was measured with a digital calliper to the nearest 0.01 mm. The average of nestling body mass and tarsus length per brood was calculated. Mass and tarsus length were not significantly correlated (Simple regression: $F_{1,44} \text{ } \frac{1}{4} 2.55$; $p \text{ } \frac{1}{4} 0.12$). Nestling mass was corrected by size by using tarsus length as a covariate in further analyses (see [Biard et al., 2005](#); [Suárez et al., 2005](#); [Shapiro et al., 2006](#) for a similar procedure).

2.3. Insect collection

In order to capture biting flies visiting nest-boxes, we put inside and close to the roof of each nest-box a plastic Petri dish (8.5 cm diameter; 56.7 cm²) with a thinly spread layer of body geleoil (Johnson's[®] baby chamomilla, Johnson & Johnson, Dusseldorf, Germany) when nestlings were 9 days old (see [Tomás et al., 2008a](#) for a validation and a thorough description of the method of insect capture). Petri dishes were collected 3 days later. Although daily changes of Petri dishes could provide data on daily variation in biting fly activity, this possibility was discarded to reduce avian disturbance during bird reproduction that could induce nest desertion. According to a previous study, nest-boxes without broods attract almost no biting flies ([Tomás et al., 2008a](#)). A stereoscopic microscope (Motic K700; 46.5x magnification) was used to quantify the abundance of black flies and biting midges collected at the bottom of each Petri dish.

2.4. Rearing conditions in the nests and weather data collection

A HOBO data logger (Onset Computer Corporation, Pocasset, Massachusetts, USA) with a flexible sensor was placed in each nest-box to record nest temperature (°C) and nest absolute humidity (gm/M3) with a frequency of 2 min during the 3-day period of insect capture. Data loggers were inserted between the nest-material and the inner wall of the nest-box, close to the chicks but avoiding any contact with them. The average of nest temperature and nest humidity for the 3 days of sampling was calculated.

The Spanish National Meteorological Institute provided daily maximum and minimum temperature data from the closest meteorological station located in Segovia, approximately 9 km from the study area. In previous studies, data from this meteorological station have been used to find clear effects of weather on several factors related to avian biology and bird-haematophagous insect interactions

carried out in the same area ([Sanz et al., 2003](#); [Lobato et al., 2006](#); [Martínez-de la Puente et al., 2009b](#)). Environmental temperature was then estimated by calculating the averages of the maximum and minimum temperatures respectively for each insect sampling period. Furthermore, the temperature gradient between the nest and the environment was estimated in two different ways. In the case of biting midges, the temperature gradient was measured as the difference between temperature in the nest and the minimum environmental temperature because most of the biting midges are considered crepuscular or nocturnal ([Lehane, 2005](#)) with a pronounced burst of activity at sunset during spring, summer and autumn ([Kettle et al., 1998](#)). However, in the case of black flies, the temperature gradient between the nest and the maximum environmental temperature was calculated because, contrary to biting midges, most adult black flies are essentially diurnal, usually showing a maximum activity in the early morning and afternoon ([Lehane, 2005](#); [McCreadie et al., 1985](#); [Grillet et al., 2005](#)). Although there are some reports of nocturnal black fly activity, the level of activity detected in these cases is low ([McCreadie et al., 1985](#)) and there are not evidences of black flies attacking avian hosts after dark ([Bennett, 1960](#)).

2.5. Statistical analysis

Statistical analyses were conducted using Statistica (version 6.0, StatSoft, Inc. 2001). In order to investigate the variables affecting the temperature in avian nests, the Akaike's second-order Information Criterion (AICc) was used for ranking the fit of models to the data ([Burnham and Anderson, 1998](#)) including hatching date, brood size and maximum and minimum external temperatures as continuous variables. The same procedure was employed to test for the effect of brood size, hatching date, nest temperature, nest humidity and the temperature gradient (the difference between the nest temperature and the environmental minimum temperature) on the abundance of biting midges. We also used AICc to identify the ranking of models to the presence/absence of black flies in avian nests, including brood size, hatching date, nest temperature, nest humidity and the temperature gradient (the difference between the nest temperature and the environmental maximum temperature) on the abundance of biting midges. We considered that models with a difference in AICc (ΔAICc) of less than two units ($\Delta\text{AICc} < 2$) were similarly supported by the data. In order to quantify the relative importance of individual variables we calculated variable weights by summing the Akaike weights for each model containing the variable of interest ([Johnson and Omland, 2004](#)). All models obtained in the analyses (including those with a difference in AICc higher than two units) were considered to get variable weights.

Finally, to investigate the variables affecting nestling mass, we included in a General Regression Model (GRM, lineal models) the presence/absence of black flies as a factor and hatching date, brood size, nest temperature, nest humidity, tarsus length, biting midge abundance and the interaction between the presence/absence of black flies and biting midge abundance as covariates. This analysis allows us to identify the role of biting midges and black flies as well as their potential joint effect in affecting nestling body mass after control for body size (including tarsus length as a covariate in our analyses). The abundance of biting midges was logarithmically ($\log_{10}(x + 1)$) transformed to normalize its distribution. Residuals of the model were tested for normality.

In this study, we include 21 control and 25 unmanipulated nests from several experiments carried out in the pied flycatcher population with known nest temperature and humidity. Nests treated as control were sprayed with water 3 times during the incubation period. Unmanipulated nests were visited in the same way as controls but they were not sprayed with any product. As expected, this treatment conducted during the incubation period did not modify significantly

Table 1

Models (within $\Delta\text{AICc} < 2$ units) explaining the abundance of biting midges in pied flycatcher nests using the Akaike's second-order Information Criterion (AICc). Variables included in each model were marked with "X". A total of 46 nests were included in this study.

Model	Variables					AICc	ΔAICc	Akaike weight
	Brood size	Nest humidity	Nest temperature	Hatching date	Temperature gradient			
1	X	X	X			61.55	0	0.24
2	X		X			62.14	0.59	0.18
3	X	X	X	X		63.36	1.81	0.10
4	X	X	X		X	63.53	1.98	0.09

nest temperature or humidity during the nestling period (at the age of nestlings of 9 days) when insects were captured (all $p > 0.15$) so we do not include this variable in further analyses. No experimental manipulations were conducted during the nestling period.

3. Results

Overall 1761 biting midges (mean abundance \pm SD \pm 38.3 \pm 52.4; prevalence \pm 0.91; range \pm 0e227) and 78 black flies (mean abundance \pm SD \pm 1.7 \pm 2.5 black flies; prevalence \pm 0.54; range \pm 0e10) were captured in 46 pied flycatcher nests. Among them, one nest was infested only by black flies, 18 nests were infested only by biting midges and in 24 nests we collected both biting midges and black flies. In three additional nests we did not collect biting flies.

We obtained 15 different models explaining the temperature inside nests, with one model within a difference of less than two units ($\Delta\text{AIC} < 2$) with respect to the best model. The model with the lowest AICc (AICc \pm 160.07; w \pm 0.40) included two variables, environmental minimum temperature and brood size. The other competing model (AICc \pm 160.72; w \pm 0.29) included environmental minimum temperature, brood size and environmental maximum temperature. Overall, environmental minimum temperature (w \pm 0.99) and brood size (w \pm 0.92) were the variables with higher weights explaining temperature inside nests, followed by environmental maximum temperature (w \pm 0.42) and hatching date (w \pm 0.24).

We found four ($\Delta\text{AIC} < 2$) competing models out of 31 models explaining the abundance of biting midges in pied flycatcher nests (Table 1). Nest temperature (w \pm 0.89) and brood size (w \pm 0.85) had the higher weights explaining the abundance of biting flies in avian nests followed by nest humidity (w \pm 0.58), the temperature gradient (the difference between the nest temperature and the environmental minimum temperature) (w \pm 0.34) and hatching date (w \pm 0.33). Higher abundances of biting midges were found in

warmer nests occupied by larger broods (Fig. 1). In addition, the abundance of biting midges was positively associated with nest humidity (Fig. 2). Furthermore, we obtained four competing models from the total of 31 models to explain the presence of black flies in pied flycatcher nests (Table 2). The variable with the highest weight was brood size (w \pm 0.99) followed by nest humidity (w \pm 0.39), hatching date (w \pm 0.38), nest temperature (w \pm 0.31) and temperature gradient (w \pm 0.26). Black flies were more frequently found in nests with larger broods.

Average nestling mass was significantly associated with the interaction between the abundance of biting midges and the presence/absence of black flies after control for the effect of different variables (Table 3). Nestling mass decreased as the abundance of biting midges increased, with a more pronounced effect on nestlings suffering also the attack of black flies (Fig. 3). In addition, nestling mass was significant and positively associated with hatching date and tarsus length and negatively with nest temperature (Table 3). We did not find any significant association between nestling mass and the rest of the variables included in the model (Table 3).

4. Discussion

Rearing conditions in the nest affect the interaction between avian hosts and nest-dwelling ectoparasites (Eeva et al., 1994; Heeb et al., 2000; Dawson et al., 2005a). However, conclusions from these studies are difficult to apply to the case of biting flies due to the higher mobility and independence of these insects from their hosts as compared to nest-dwelling ectoparasites. According to our correlative results, it is possible that microclimatic conditions were used directly as cues or indirectly as factors contributing to the spread of other host attractants used by biting flies to detect their hosts. Previously, Howlett (1910) reported that females of the mosquitoes *Culex fatigans* and *Stegomyia scutellaris* were attracted to heat even if

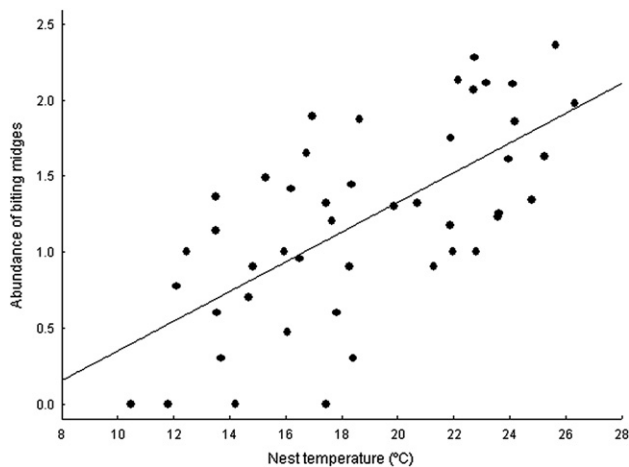


Fig. 1. Relationship between the abundance of *Culicoides* biting midges (log10 transformed) and the temperature inside pied flycatcher nests. Regression line is shown ($r^2 \pm 0.44$; $p < 0.001$; $y \pm 0.63 \pm 0.10^*x$).

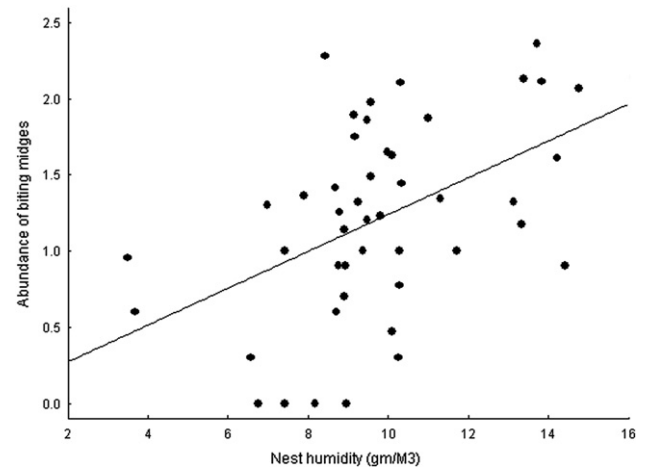


Fig. 2. Relationship between the abundance of *Culicoides* biting midges (log10 transformed) and humidity inside pied flycatcher nests. Regression line is shown ($r^2 \pm 0.22$; $p \pm 0.001$; $y \pm 0.03 \pm 0.12^*x$).

Table 2
Models (within $\Delta AICc < 2$ units) explaining the presence/absence of black flies in pied flycatcher nests using the Akaike's second-order Information Criterion (AICc). Variables included in each model were marked with "X". A total of 46 nests were included in this study.

Model	Variables					AICc	$\Delta AICc$	Akaike weight
	Brood size	Nest humidity	Nest temperature	Hatching date	Temperature gradient			
1	X					57.00	0	0.18
2	X	X				57.17	0.17	0.16
3	X			X		57.76	0.76	0.12
4	X		X	X		58.53	1.53	0.08

this stimulus was not accompanied by other cues. These conclusions were later supported by Marchand (1918) and more recently Kline and Lemire (1995) found also support for the role of heat affecting the host-seeking behaviour of different flies including the biting midge *Culicoides furens* Poey. According to our results nest humidity may also partially affect the abundance of biting midges in avian nests, although its effect is clearly much lower than the effect of nest temperature. Alternatively, it is possible that the association found between the abundance of biting midges and nests microclimatic conditions could be due to the ecological conditions in the vicinity of the nests and not directly to nest temperature or humidity. However, some important characteristics of nests location such as the distance to water sources do not affect the abundance of biting midges in blue tit nests (Tomás et al., 2008a). It is clear that host related factors, including brood size and hatching date, as well as weather conditions during the insect sampling period are of major importance to influence the abundance of biting flies in avian nests (Martínez-de la Puente et al., 2009a, 2009b). Furthermore, our results suggest that nest temperature did not affect significantly the presence/absence of black flies in avian nests. Contrary to biting midges, black flies have diurnal activity (Lehane, 2005) implying that host-seeking behaviour is activated when differences between environmental and nest temperatures are lower. Diurnal activity imply other advantages for detecting hosts such as the availability of visual cues, given that there are better light conditions to detect colours and higher host mobility during the day than at night (Lehane, 2005). In addition, because during sunny hours there are reduced background levels of atmospheric carbon dioxide (Lehane, 2005), it could be possible that chemicals also play a more important role in host detection by black flies than thermal cues. This conclusion is supported by the study of Fallis and Smith (1964), showing that the ornithophilic simuliid *Simulium rugglesi* was mainly attracted by an extract from birds plus CO_2 or CO_2 alone, but much less by heat.

We also found a significant negative association between body mass (including tarsus length as a covariate in the analyses in order to control for the effect of body size) and the interaction between the abundance of biting midges and the presence/absence of black flies. This association could be obviously a reflection of the effects of biting flies on nestlings, directly by draining resources and causing physical harassment but also indirectly as they are vectors of

Table 3
Results of a General Regression Model (lineal model) relating body mass of nestling pied flycatchers *Ficedula hypoleuca* to brood size, hatching date, nest temperature, nest humidity, tarsus length, the abundance of biting midges, the presence/absence of black flies and the interaction between the abundance of biting midges and the presence/absence of black flies (model: adjusted $r^2 = 0.41$, $p < 0.001$).

Variables	$F_{1,37}$	P
Brood size	1.22	0.28
Hatching date	4.75	0.04
Nest temperature	11.42	0.002
Nest humidity	1.03	0.32
Tarsus length	11.88	0.001
Biting midge abundance	0.22	0.64
Presence/absence of black flies	3.12	0.09
Biting midge abundance \times presence/absence of black flies	5.04	0.03

several pathogens. Overall, these effects may lead to anaemia and other physiological damages in nestlings, finally affecting their general body condition and survival probability (Hunter et al., 1997; Smith et al., 1998; Tomás et al., 2008b). The fact that, in presence of black flies and abundant biting midges the pathogenic effect on nestling weight was higher than in nests without black flies could be due to several reasons. For example, double infestations may increase the adverse effects suffered by birds because they were inoculated with, and need to respond against, a higher diversity of antigens contained in insect salivary solutions. In fact, insect saliva may affect the haemostatic, inflammatory and immune responses of vertebrate hosts in different ways, thus potentially affecting the expression of the major histocompatibility complex (MHC) (Currie and Hunter, 2008). Moreover, black fly saliva produces an

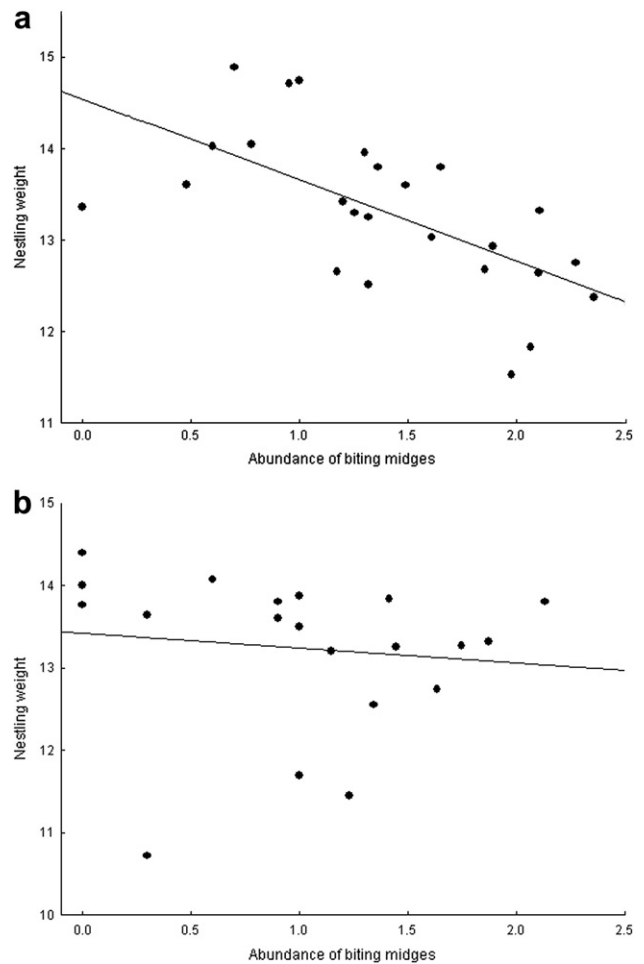


Fig. 3. Relationship between the average of nestling mass and the abundance of *Culicoides* (log10 transformed) in (a) nests with black flies ($y = 7.41 - 0.45x$) and (b) without black flies ($y = 2.05 - 0.08x$). Regression lines are shown. (Model: adjusted $r^2 = 0.601$).

inhibition of lymphocyte mitogenesis (see review by Andrade et al., 2005 and references therein) with potential effects on nestling condition. Additionally, double infestations by biting midges and black flies could increase multiple blood parasite infections because while biting midges are the main vectors of *Haemoproteus*, other parasites such as *Leucocytozoon* and *Trypanosoma* are mainly transmitted by black flies (Bennett, 1961; Valkiūnas, 2005). These blood parasites infect pied flycatcher nestlings in central Spain (Merino and Potti, 1995; Lobato et al., 2005) and multiple infections could be more virulent than single ones (Marzal et al., 2008; del Cerro et al., 2010). Obviously, the effects of the interaction between vectors and parasites on birds merit further studies. Alternatively, the association between fly ectoparasites and nestling mass could be due to other different factors. For example, it could be possible that low quality pairs (i.e. infected birds) rearing nestlings with lower body mass occupied nest-boxes around areas with higher abundances of biting midges and prevalences of black flies. In addition, it could be possible that these low quality birds suffering higher intensities of infection by parasites were more attractive to insect attacks. However, our previous results do not support this possibility, because the abundances of biting midges were negatively associated with the infection status of wild birds (Tomás et al., 2008b; Martínez-de la Puente et al., 2009b).

In conclusion, although correlative, our results support an association between nest temperature and the abundance of parasitic flying insects in avian nests. Furthermore, we found a negative association between nestling condition and biting midge abundance in nests also affected by black flies.

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