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Article

Experimental manipulation of temperature reduce ectoparasites in nests of blue tits *Cyanistes caeruleus*

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Several models predict changes in the distributions and incidences of diseases associated with climate change. However, studies that investigate how microclimatic changes may affect host–parasite relationships are scarce. Here, we experimentally increased the temperature in blue tit *Cyanistes caeruleus* nest boxes during their breeding season to determine its effects on the parasitic abundance (i.e. of nest-dwelling ectoparasites, blood-sucking flying insects and hemoparasites) in nests and the host condition of nestlings and adults. The temperature was increased using heat mats placed underneath the nest material, which resulted in an average temperature increase of 3ºC and a reduction in relative humidity of about six units. The abundance of mites *Dermanyssus gallinoides* and blowfly pupae *Protocalliphora azurea* was significantly reduced in heated nest boxes. Although not statistically significant, a lower prevalence of flea larvae *Ceratophyllus gallinae* was also found in heated nests. However, heat treatment did not affect hemoparasite infection of adult blue tits or the body condition of adult and nestling blue tits. In conclusion, heat treatment in blue tit nests reduced nest-dwelling ectoparasites yet without any apparent benefit for the host.

Keywords: blue tit, ectoparasites, mites, *Protocalliphora*, relative humidity, temperature.

Introduction

Our knowledge of the ecological responses to climate change in different organisms is still being developed (Teplitsky et al. 2008). In fact, only a few empirical studies have shown microevolutionary changes in birds and other vertebrates due to climate change (Sheldon 2010, Charmantier and Giennap 2013, Urban et al. 2014). Although there is clear evidence of global climate warming during the last century, data that support the effects of climate change favoring infectious diseases is lacking (Lafferty 2009). The effects of climate change and changes in the distribution and phenology of a variety of taxa imply that seasonal patterns of development and transmission of many

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pathogens would also be impacted by climate (Harvell et al. 2002, Dobson et al. 2003, Root et al. 2003, Kutz et al. 2005). Although climate change models predict that diseases will spread with increasing temperatures (Patz et al. 1996, Lafferty and Mordecai 2016, Wu et al. 2016), we still need more precise information on how increases in temperature could affect the presence of infections in wild animals. In the case of ectoparasites, which have free-living stages, abiotic factors may be especially important for their development and transmission.

In this sense, the effect of climate change on host–parasite interactions is difficult to assess. For example, decreasing bird populations may be due to changes in ecological factors, such as habitat loss, and not due to any direct effect of pathogens (Merino and Møller 2010). In fact, climate change has altered many natural ecosystems, thus affecting the organisms that live there (Rosenzweig et al. 2007). The first experiments to investigate the effect of temperature on bird reproduction were carried many years ago (Suomalainen 1937) and since then many other have been conducted (Wada et al. 1990, Wingfield et al. 1997). For example, it has been shown that the breeding success of the passerine blue tit *Cyanistes caeruleus* has increased over the last 20 years with increasing average temperatures (Potti 2009). Other studies of birds have shown that environmental temperatures can affect vital strategies such as phenology (Charmantier et al. 2008, Saino et al. 2009) and reproductive success (Visser et al. 2009, Durant et al. 2013). A more recent study has shown that higher temperatures during the incubation period in passerines can reduce long-term survival (Nord and Nilsson 2016).

However, few studies to date have investigated the effects of climate change on the prevalence and abundance of bird parasites, and their potential impact on host fitness (Merino and Møller 2010). Abiotic factors, such as temperature, rainfall or wind speed, are known to affect interactions between ectoparasites and hosts (Smith et al. 1998, Roulin 1999, Hubálek et al. 2003, Martínez de la Puente et al. 2009). Likewise, Dawson et al. (2005a) demonstrated that the experimental manipulation of temperature can vary the abundance of *Protocalliphora* larvae in tree swallow nests. Others studies have shown that the temperature during different seasonal periods influences the emergence and diapause termination of a hematophagous ectoparasite fly *Carnus hemapterus* in nests cavities (Calero-Torralbo 2011, Amat-Valero et al. 2013). Heeb et al. (2000) showed that relative humidity in passerine nests affects nest-dwelling ectoparasite abundance, while Moyer et al. (2002) found that feral pigeons *Columba livia* living in an environment with higher humidity had a higher abundance of lice (Insecta: Ischnocera). In contrast, wind speed has been shown to have a negative effect on the abundance of blackflies (*Simulium* spp.) in the nests of several hole-nesting birds (Martínez de la Puente et al. 2009). Weather conditions have also been shown to affect bird parasites. For example, Merino and Potti (1996) found that the prevalence and abundance of some ectoparasite species in bird nest boxes depends on weather conditions.

In this study, we experimentally assess if the prevalence and abundance of blue tit *Cyanistes caeruleus* ectoparasites and hemoparasites are affected by temperature and relative humidity in nest boxes. Moreover, we assess if the body condition of adults and nestlings is affected by an increase in temperature. We hypothesize that experimentally increasing the temperature inside of nest boxes will affect the abundances of ectoparasites (biting midges, black flies, blowflies, fleas and mites) infesting nestlings. On the one hand, higher temperatures and lower humidity levels within nests could be unfavorable for ectoparasite development. On the other hand, higher temperatures may increase the volatiles that some ectoparasites use to locate and infest nests. Hemoparasitic infections are chronic and, therefore, are typically maintained from year to year in birds. However, the prevalence or intensity of hemoparasites may increase in adult birds that have increasing numbers of ectoparasites as some may also transmit hemoparasitic diseases. On the contrary, a lower infestation of ectoparasites may be beneficial for nestlings, plus adult birds could reduce their breeding stress to better control blood parasite infections, thus reducing their presence in peripheral blood.

Material and methods

Study population

This study was carried out during the 2015 bird breeding season in a Pyrenean oak *Quercus pyrenaica* deciduous forest located in Valsaín (Segovia, central Spain, 40°53′74″N, 4°01′W, 1200 m a.s.l.). Blue tits *Cyanistes caeruleus* breeding in wooden nest boxes hanging from tree branches about 5 m above the ground have been studied in this area since 1991 (Sanz 2002, Tomás et al. 2006). Each breeding season, nest boxes are periodically inspected to determine reproductive parameters including laying date, clutch size and hatching date (Merino et al. 2000, Tomás et al. 2007).

Experimental design

In May 2015, we began an experiment that manipulated the temperature in nest boxes occupied by blue tits. Prior to experimental manipulation, nests were paired according to hatching date and number of nestlings, and each nest within a pair was randomly allocated to either the heat treatment or control group. A total of 40 nests (20 heated and 20 control) were matched. However, six desertions (five controls and one heated) occurred during the experimental period for unknown reasons. As a result, the overall sample size was reduced by 12 nests, including the desertions and their matches. In the end, we collected information from 28 nests (14 heated and 14 control nests).

Experimental nests were supplied with heat mats (102 × 107 mm, 4 W; HabiStat, Euro Rep Ltd, UK) for 12 days (from day 3 to 13 post-hatching). For each of these nests, a metal grid was placed directly underneath the nest

material followed by a heat mat. Heat mats were connected to 12 V batteries through a cord and a DC transformer (24-h autonomy). Batteries were replaced daily with fully charged ones. Metal grids and cords were also installed in control nest boxes, though heat mats and batteries were not. Control nests were visited with the same frequency as heated nests.

Nest boxes were fitted with sensors that register both temperature and humidity (Thermochron DS1923; 6 × 17 mm, temperature range: –20–85ºC; resolution 0.0625ºC; humidity range: 0–100% with a resolution of 0.04%; Maxim IC, USA). Temperature and relative humidity were recorded every 30 min during the experimental period. Once nestlings fledged (day 20 post-hatching) sensors were removed, and the nest material was collected in a sealed labeled plastic bag and transported to the laboratory to assess ectoparasite abundance. Nests were stored at 4ºC from 2 to 4 days and then defaunated using Berlese funnels. Nests were placed in funnels for 48 h, under conditions of constant temperature and light provided by lamps (60 W) placed 20 cm above the nest (Merino and Potti 1996). Infestation by *Protocalliphora* larvae was estimated by dismantling the nest and counting pupae. The abundance of other ectoparasites (mites and flea larvae) was estimated by counting the material obtained from the funnels under a magnifying glass microscope (Merino and Potti 1995).

In addition, we estimated the number of biting midges (*Culicoides* spp.) and black flies (Diptera: Simulidae) attracted to the blue tit nests using a simple trapping method. We placed a plastic petri dish (Ø 8.5 cm; 55.67 cm^2) containing a commercially available body oil gel (Johnson's baby oil gel with chamomile) on the ceiling of the nest box (Tomás et al. 2008). The petri dishes were placed in nests boxes on day 10 post-hatching and retrieved at day 13. Petri dishes were then observed under a magnifying glass to count the number of biting midges and black flies that had adhered to the gel.

The body mass of adults (captured at days 3 and 13 post-hatching) and nestlings aged 13 days was measured with an electronic balance to the nearest 0.1 g. Tarsus length was measured with a digital caliper to the nearest 0.1 mm. A body condition index was calculated as the residuals of mass on tarsus length. We also took blood sample from the brachial vein of adults when nestlings were 3 and 13 days of age. The blood was stored on FTA classic cards and later used to quantify hemoparasites (i.e. *Haemoproteus, Plasmodium, Leucocytozoon, Lankesterella* and *Trypanosoma*) using qPCR as previously described by Badás et al. (2015). Only four males were captured on both occasions (3 and 13 days of nestling age); thus, changes in measurements and infection were only analyzed for females.

Statistical analyses

For statistical analyses, we use parametric statistics (paired *t*-test and Pearson's correlation). If the data did not follow the normality assumptions, they were transformed logarithmically in order to use parametric tests (Zar 1996). If normality was still not attained, we used nonparametric tests (Spearman's correlation and Wilcoxon paired-test). Graphics and statistical analyses were performed with STATISTICA 7 (<www.statsoft.com>).

Data deposition

Data available from the Digital CSIC repository: <http://dx.doi.org/10.20350/digitalCSIC/8550> (Castaño-Vázquez et al. 2018).

Results

Effect of heat treatment on temperature and relative humidity

Significant differences in mean temperature were observed between heated and control nests (paired *t*-test: $t = -4.00$, $df = 13$, $p = 0.001$). In addition, mean relative humidity (log_{10}) was significantly lower inside of heated nests compared with control nests (paired *t*-test*:* $t = 2.796$, $df = 13$, $p = 0.015$).

Effect of heat treatment on ectoparasites and hemoparasites

The abundance of mites and blowfly pupae was significantly lower in heated nests (Wilcoxon matched-pairs test for mites: $Z = 2.60$, $p = 0.009$, Table 1, Fig. 1a; Wilcoxon matched-pairs test for blowfly pupae: $Z = 2.76$, $p = 0.005$, Table 1, Fig. 1b). However, the abundance of flea larvae, biting midges and black flies was not significantly different between nest groups (Table 1).

The prevalence of mites, blowfly pupae, biting midges and black flies was not significantly different between groups

Table 1. Prevalence (Prev.) as a percentage and mean ± SD abundance (MA) of different ectoparasites species in control and heated nest. Statistical tests show differences in abundances between experimental and control nests. p = significance value for abundance (MA); p* < 0.01.

	Control		Heated		Wilcoxon test	
Parasite species	Prev.	MA	Prev.	MA	ʒ	p
Ceratophylus gallinae	35.71	2.57 ± 5.44	7.14	0.21 ± 0.80	1.46	0.142
Dermanyssus gallinoides	100	1363.42 ± 1320.89	92.85	392.50 ± 569.72	2.60	0.009*
Protocalliphora azurea	92.85	26.14 ± 19.55	92.85	11.21 ± 12.15	2.76	0.005*
Culicoides spp.	100	56.07 ± 44.64	100	70.85 ± 95.29	0.56	0.576
Simulidae (Diptera)	84.61	1.92 ± 2.05	57.14	1.78 ± 2.60	0.17	0.858

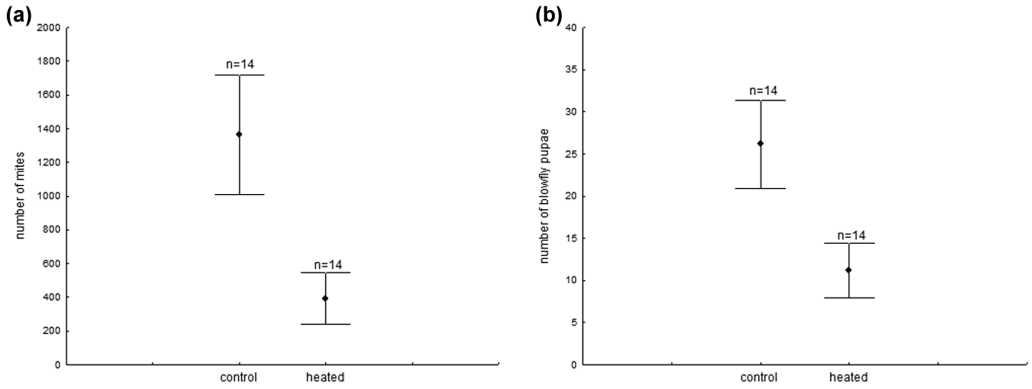

Figure 1. Mean ± SE number of mites (*Dermanyssus gallinoides*) (a) and of blowfly pupae *Protocalliphora azurea* (b) observed in control and heated nests of blue tits *Cyanistes caeruleus*. Sample size is indicated above the bars.

(Fisher's exact test: $p > 0.05$). However, heated nests tended to have a lower prevalence of flea larvae (Fischer's exact test: $p = 0.082$). Moreover, the abundance of flea larvae was positively correlated with the abundance of blowfly pupae (Spearman correlation, $n = 28$, $r_s = 0.399$, $p = 0.035$).

Initial parasitemia (i.e. before heat treatment) and the change in parasitemia (i.e. from initial to final capture of adult birds) of control and experimental female blue tits did not differ significantly for any of the tested hemoparasites (Wilcoxon matched-pairs test: $n = 14$, $p > 0.05$).

Effect of heat treatment on body condition, mass and nestling survival

The body condition and mass of female blue tits prior to heat treatment were not significantly different between the two nest groups (Wilcoxon matched-pairs test: $n = 14$, $Z = 0.72$, $p = 0.470$; paired *t*-test: $t = -0.21$, $df = 13$, $p = 0.833$, respectively). Likewise, significant differences between groups were not observed in the final body condition and mass of females (paired *t*-tests: $t = -1.36$, $df = 13$, $p = 0.194$ and $t = -1.56$, $df = 13$, $p = 0.140$, respectively). Moreover, the change in body condition (i.e. from initial to final capture of adult birds) of control and experimental female blue tits was not significant (Wilcoxon matched-pairs test: $n = 14$, $Z = 0.91$, $p = 0.362$).

Control and experimental nestlings also showed no significant differences in mean final body condition (paired *t*-test: $t = -1.09$, $df = 13$, $p = 0.292$). Nestling survival was higher in experimental nests, although differences were not statistically significant (Fisher's exact test: $p = 0.091$).

Discussion

In this study, we manipulated two associated abiotic factors, temperature and relative humidity, in blue tit nests during nestling development. To our knowledge, this study is the first to manipulate nest temperatures for such a duration (11 days) during nestling development to test whether these abiotic factors influence parasite load. Our results show that temperature manipulation negatively affected some parasite species including mites and blowfly pupae. Dawson et al. (2005a) and Chen and Mullens (2008) similarly reported lower survival of blowfly pupae *Protocalliphora azurea* and fowl mites, respectively, in nests that were subjected to an increase in temperature. Taken together, these results suggest that the development of these parasites is adapted to an optimum temperature–humidity range. We also found that the prevalence of flea larvae was lower in heated nests, although this difference was not significant ($p = 0.08$). Heeb et al. (2000) found that ectoparasites like fleas require high levels of humidity for their development. In natural conditions, nest material is mainly heated by nestlings. In our experimental design, we heated nests from the bottom of the box, which may account for the greater reduction in humidity in the experimental nests. Thus, the abundance or prevalence of some nest-dwelling ectoparasites might have been affected by changes in humidity associated with the increase in temperature compared with other parasites, such as biting midges (*Culicoides* spp.), which were not affected in our study. Alternatively, this result may reflect the fact that flying ectoparasites typically visit nests for a short period of time, long enough to obtain a blood meal before flying away. Thus, a nest's temperature and/or humidity may have less of an effect on these insects. Given that we did not detect a significant increase of midges in heated nests, the hypothesis that higher temperatures may facilitate better detection of nests by volatiles is not supported.

Despite the lower abundance of ectoparasites in heated nests, nestling condition was not affected. The beneficial effect of a lower ectoparasite abundance on nestlings might have been compensated by a negative effect of temperature on nestling development. However, Dawson et al. (2005b) showed that a temperature increase of about 5°C in nests were associated with benefits on mass and on ninth primary feather of passerine chicks. Thus, beneficial effects on nestlings may potentially be observed at higher experimental temperatures. Moreover, nest design and location are known

to influence microclimatic conditions within nests and affect nestling conditions (Mainwaring et al. 2014). In our population, nest boxes are of similar dimensions, but nest thickness varies from pair to pair (Tomás et al. 2006). This variation may differentially affect ectoparasite and nestling variables not measured in this study and should be explored in the future.

Alternatively, the lack of change in nestling condition could be related to parental care. Some studies have shown that parental effort varies in the presence of ectoparasites during nestling growth (Møller 1993, Merino et al. 1998, Avilés et al. 2009). Therefore, the deleterious effect of ectoparasites might have been buffered by the effort of parents. However, neither the condition nor the hemoparasitic infections of females were affected by treatment. Thus, the effects of parasites in this case do not appear to significantly affect nestlings or adult birds.

The effect that climate change can exert on parasitic interactions represents a multifactorial problem whose results are difficult to predict (Martinez and Merino 2011). The degree of adaptation of organisms to new environmental conditions is a key factor in ecological interactions, and experimental studies are necessary to better predict the effects that changing environmental conditions may exert on these interactions. In our experimental study, we showed that heat treatment 1) altered the microclimatic conditions of nests (i.e. temperature and relative humidity increased and decreased, respectively), and 2) significantly reduced the abundance of nest-dwelling ectoparasites, while parasites less linked to the nest environment (i.e. dipteran species) were not affected, and that 3) the body condition of blue tit nestlings and females were not affected by the increase in temperature or decrease in ectoparasite abundance.

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